Research Article

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Nursing Personnel Services in Handling Tuberculosis Patients at Darul Imarah Health Center Aceh Besar District

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Abstract.

The quality of services of nursing staff in the management of tuberculosis (TB) patients at the Darul Imarah Community Health Center, Aceh Besar Regency due to the limited capacity of human resources in carrying out data collection is not yet known in reality. This research aims to describe (i) the services of nursing staff in treating tuberculosis patients and (ii) the supporting and inhibiting factors for nursing services in treating tuberculosis patients. A descriptive-qualitative approach was used through interviews, documentation, and observation techniques. The results show that to improve the quality of service at the Darul Imarah Community Health Center, Aceh Besar Regency, it is necessary to take steps to improve the quality of nursing staff services for TB patients.

Keywords: nursing personnel services, tuberculosis, health center

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1. Introduction

Public services such as the service of nursing staff for tuberculosis patients at the Darul Imarah sub-district level still encounter a number of people as sufferers, so the government needs to monitor the existence of the community according to individual specifications, regional and sufferer zones. In line with these developments, the service of nursing staff to tuberculosis patients is an activity that is carried out carefully in an effort to detect a disease that the government through medical and paramedical personnel has for a number of people who have every activity that benefits in a group or unit, and offers satisfaction even though the results not tied to a product physically, but as a real action in curing disease. In an effort to create a healthy society in the understanding that health is a human right, the 1945 Constitution of the Republic of Indonesia, which is described in article 28 H paragraph (1), states that “Health as a human right and at the same time as an investment, so it needs to be strived for, fought for and improved by each individual and by all components of the nation, so that people can enjoy a healthy life, and in the end can realize an optimal degree of public health”.

It is important to improve a healthy society, it has become a national policy, with the consideration of helping to encourage the growth of national people who have

resilience, fighting power, so as to be able to bond together the spirit of togetherness which is an asset in the life of the nation and state. According to Ngarawula Bonaventura et al [1], states that "strength in the spirit of unity and unity will be able to foster a spirit of nationalism so that it can make this nation richer and more competitive". In this case all layers of society, stakeholders and all components involved in health activities that need to be carried out to anticipate health failures, because health is not the sole responsibility of the government, but in general as a joint responsibility of the government and society, including the private sector.

Services for nursing staff for TB patients in stages and integrated are carried out through collaborative activities between the relevant programs and activities to be carried out as field actions, meaning that the services for nursing staff for tuberculosis patients are carried out in accordance with the principle of decentralization within the framework of regional autonomy with districts/cities as the focus of management. program, which includes: planning, implementation, monitoring and evaluation as well as ensuring the availability of resources (funds, personnel, facilities and infrastructure). Basically, in the management of each health center must establish a work unit (team) that is responsible for managing the nursing staff service program for tuberculosis patients. Work units must at least have health workers with competence in the field of public health and non-health workers with certain competencies. Health Centers must appoint trained doctors, nurses and laboratory analysts who are responsible for the implementation of the Nursing Staff Service program for Tuberculosis patients. Hospitals must establish a DOTS (Directly Observed Treatment Shortcourse) Team who are responsible for implementing the nursing staff service program for Tuberculosis patients in the regions and spread out to gam pongs.

The study aim of the research are 1) To describe the services of nursing staff in treating tuberculosis patients; 2) To describe the supporting and inhibiting factors for nursing services in treating tuberculosis patients.

2. Research Methods

2.1. Research approach

In this research approach was carried out by Nursing Service in the Handling of Tuberculosis (TB) Patients (Study of Health Policy Implementation Based on the Regulation of the

Minister of Health of the Republic of Indonesia Number 67 of 2016 [2] concerning Tuberculosis Control) at the Darul Imarah Health Center in Aceh District, which is oriented towards a descriptive qualitative approach. research), in order to be able to describe social phenomena that arise in people's lives, especially with regard to the examination and treatment of Tuberculosis (TB) patients by describing social phenomena that arise in people's lives, especially with regard to dealing with them in accordance with the Regulation of the Minister of Health of the Republic of Indonesia Number 67 of 2016 concerning Tuberculosis (TB) control.

2.2. Research focus

The focus of research, it can change by itself and develop according to conditions in the field through the identification and formulation of problems according to the dimensions and indicators of field research as follows:

1. Nursing Staff Services in Handling Tuberculosis (TB) Patients at the Darul Imarah Health Center, Aceh Besar District, with the following dimensions and indicators:

TABLE 1: Dimensions and indicators of nursing services.

No	Dimensions	Indicator
1	Health Promotion	Commitment of Policy Makers; Improving the Integration of Program Implementation; Empowering the community;
2	TB surveillance	Active Data; Passive Data;
3	Drug Administration and Prevention of TB	Children under 5 (five) years old who are in close contact with active TB patients; People with HIV and AIDS (ODHA) who are not diagnosed Other Certain Populations.

Source: Article 6 (a, b, f) Article 7 (1) Article 9 paragraph (1), Article 15 Permenkes No 67 of 2016

2. Supporting and Inhibiting Factors for Nursing Services in Handling Tuberculosis (TB) Patients at the Darul Imarah Health Center in Aceh Besar District, with the following dimensions and indicators:

2.3. Research informants

Interactive methods, researchers deliberately choose in-depth interviews (In-depth Interviewing) and direct observation (Passive Participation), while non-interactive methods researchers are more oriented towards the existence of documents and/or archives in

TABLE 2: Dimensions and Indicators of nursing capacity.

No.	Dimensions	Indicator
1	Human Resources	Health workers; Non-Health Workers;
2	Availability of drugs and health supplies	Anti Tuberculosis Drugs; Vaccines for immunity;
3	Technology	Diagnostic; Risk control;

Source: Article 17 paragraph (2) Article 18 (1) Article 21 (ad) Permenkes No 67 of 2016.

research locations, so as to be able to make informants as work partners who provide information, The informants selected in this study totaled 9 (four) people consisting of:

TABLE 3: Research informants.

No	Informant Name	Number of Informants
1	Head of Disease Prevention and Control	1 (one) person;
2	Head of Health Center	1 (one) person;
3	Administration	1 (one) person;
4	Police Nurse	2 (two) people;
5	Laboratory staff	1 (one) person;
6	TB patient	3 (three) people;
Amount		9 (nine) people;

Source: Data processed in 2020.

Thus the research informants who have been selected by the researcher are very dependent on the ability of the researcher to explore the needs of data and information as well as facts in the field which are needed as the perfection of scientific work in the form of an accurate thesis and can be used as a reference for further work.

2.4. Data analysis technique

Data were analyzed using several steps according to the theory of Miles, Huberman and Saldana [3], namely analyzing data in three steps: data condensation, presenting data (data display), and drawing conclusions or verification (conclusion drawing and verification). Data condensation refers to the process of selecting, focusing, simplifying, abstracting, and transforming data.

3. Results and Discussion

3.1. Nursing staff services in handing Tuberculosis (TB) patients at Darul Imarah Health Center, Aceh Besar District

3.1.1. Health promotion

Research on the service of nursing staff to Tuberculosis (TB) patients at the Darul Imarah Health Center in Aceh Besar District is important to evaluate and improve the quality of care provided to TB patients in the region. This research can provide deeper insight into the factors that influence the service of nursing staff, the challenges faced, and the efforts that can be made to improve services and treatment outcomes for TB patients.

Health promotion on the one hand is an effort to increase the commitment of policy makers in increasing the integration of program implementation and on the other hand the ability to empower the community, through the vision, mission and health promotion strategy in carrying out program activities of the institution. This activity is carried out in accordance with the direction and objectives of the prevention that will be achieved is inseparable from the vision of health development that is oriented towards Mubarak [4] states as follows: The process of enabling individuals and communities to increase control over determinants of health and there by improve their health.

Health promotion is a systematic effort to improve public health through various strategies, programs and activities. The goal of health promotion is to encourage individuals and communities to take active steps to improve their health, both physically, mentally and socially. Characteristics of nursing staff: In this study, we can discuss the characteristics of nursing staff involved in treating TB patients, such as their education, training, and work experience. It is important to evaluate the level of qualifications and competence of nursing staff in providing effective TB care.

In the concept of organizational commitment, health promotion related to tuberculosis is related to the level of involvement of people with the organization where they work and are interested in staying in the organization. This is as Wibowo [5] state that “Organizational commitment is a level where individuals identify and are involved with their organization and or do not want to leave it”.

Persuading promotional activities that are persuasive and encourage consumers to make purchases of the products offered, this is one pattern of promotion and many other ways that are done by creating a positive impression on the public so that promotions can affect the behavior of individuals and/or community groups in the long run. long.

Promotional activities are a means of communication between the government, the private sector/companies and consumers or potential customers.

Proposition 1 (one) is formulated as follows:

Proposition 1 : Health promotion designing and implementing well-targeted health education programs, including counseling, campaigns, and effective communication activities to increase public health knowledge and promote healthy behaviors. Build partnerships with local organizations, communities and other institutions involved in public health, such as educational institutions, non-governmental organizations and other government agencies, to increase the effectiveness of health promotion.

Practical implications, this helps Puskesmas in designing and implementing effective and sustainable health promotion programs. By adopting a collaborative approach, good health education, community outreach, ensuring the availability of resources, good monitoring and evaluation, and involving the community, health promotion efforts at the Darul Imarah Health Center in Aceh Besar District can have a positive impact on the health of the people served.

3.2. TB surveillance

Schematically it can be described the Health Surveillance network between the main units in the Ministry of Health and the Central Technical Implementation Unit (UPT Kemenkes), the research and development center (Puslitbang) and the data and information center, among the Darul Imarah Health Centers in Aceh Besar District government agencies in the Province responsible for the health sector) and the Darul Imarah Health Center in Aceh Besar District, and among the work units of the Darul Imarah Health Center in Aceh Besar District (government agency in the Regency/City that is responsible for the health sector).

Assessment of the attributes of the surveillance system shows that the system is not simple, inflexible, not acceptable, unstable, with poor data quality, even though the Predictive Positive Value is high and the timeliness of data collection is good. In line with the development of TB Surveillance, Article 9 of the Minister of Health Regulation Number 67 of 2016 concerning Tuberculosis (TB) Control states the following: In the implementation of TB Surveillance, active and passive data collection is carried out both manually and electronically; Active data collection as referred to in paragraph (1) is the collection of data obtained directly from the public or other data sources; Passive data

collection as referred to in paragraph (1) is the collection of data obtained from Health Service Facilities.

Health Surveillance (SK) is very important for decision makers in the health sector in the context of efforts to improve public health status as high as possible. The optimal implementation of Health Surveillance (SK) requires the participation of all sectors, especially all health service facilities belonging to the government or the community, health agencies both at the regional and central levels.

Proposition 2 (two) is formulated as follows:

Proposition 2 : TB surveillance is important in TB control efforts. Conducting regular TB surveillance and using the data obtained can help in better understanding the epidemiology of TB, identify high-risk areas or populations, and plan and implement appropriate interventions to reduce the burden of this disease.

Practical implications, TB surveillance is an effort carried out with the government and the community as well as stakeholders in the health sector in order to improve the ability to manage health data and information. decisions in health efforts, both local and national, and contribute to global commitments.

3.2.1. Drug administration prevention of TB

Prevention of tuberculosis (TB) in people's lives is carried out as an action through the availability of Health Service Facilities both in the form of places used to carry out health service efforts, or in supporting forms both promotive, preventive, curative and rehabilitative carried out by the Government, Regional Governments, private and/or or society. Provision of TB prevention drugs as according to Tjiptono [6] states that "Promotion is an element of the marketing mix that focuses on efforts to inform, persuade, and remind consumers and/or public about brands and products.

Early detection and treatment of active TB cases is very important to prevent the spread of the disease. People who have TB symptoms such as a cough that lasts more than two weeks, fever, weight loss, and ongoing fatigue should seek immediate medical care. People infected with active TB should follow preventive measures to prevent transmission to others. This involves keeping a distance from other people, covering your mouth when you cough or sneeze, and maintaining personal hygiene by washing your hands regularly. People who are at high risk of developing TB, for example

those who have direct exposure to people with active TB or those who test positive for tuberculin, can be given preventive therapy.

Proposition 3 (three) is formulated as follows:

Proposition 3 : The importance of adequate resources, competence of medical personnel, availability of drugs, prevention programs, external collaboration, and periodic monitoring and evaluation in TB control at the Darul Imarah Health Center in Aceh Besar District. By fulfilling this proposition, it is hoped that puskesmas can provide optimal services to TB patients and be able to reduce the burden of disease significantly.

Practical implications, It is important to ensure adequate accessibility of health services for individuals infected with TB. This can be done through approaches such as increasing the number and availability of community health centers or TB clinics, expanding the health service network in Darul Imarah District, Aceh Besar District or remote areas, and providing a mobile clinic approach to reach populations that are difficult to reach. These practical implications will help ensure that individuals infected with TB can easily access the diagnosis, treatment and care they need.

3.3. The capacity nursing staff in handing Tuberculosis (TB) patients at Darul Imarah Health Center, Aceh Besar District

3.3.1. Human resources

Skills in TB management. Nursing staff must have skills in carrying out physical examinations and laboratory tests related to tuberculosis. They must also be able to understand the results of the test and make the appropriate interpretation. Skills in providing comprehensive care to TB patients, including administering medication regularly, monitoring drug side effects, and supporting patients during treatment are also critical. Experience in treating TB patients: Experience of nursing staff in caring for TB patients is also an important factor. With sufficient experience, they will become better trained in recognizing symptoms, managing complications, and providing psychological support to patients and families.

Cross-sectoral health is a recognized relationship between a part or parts of different sectors, formed to take action on a problem so that results are achieved in a way that is more effective, sustainable or efficient than the health sector. effectively as according to Widodo [7] states the following: Training is a series of individual activities in systematically increasing skills and knowledge so that they are able to have professional

performance in their field. Training is a learning process that enables employees to carry out current work according to standards.

Human Resources (HR) in the management of Tuberculosis (TB) patients at the Darul Imarah Health Center in Aceh Besar District are an important element in providing health services to TB patients. Adequate nursing staff capacity is needed to improve the quality of care, supervision and treatment of TB patients. The following is an explanation of the HR Capacity of Nursing Staff in managing TB patients at the Darul Imarah Health Center, Aceh Besar District. By having adequate and trained human resources for nursing staff, the Darul Imarah Health Center can provide optimal service in the management of TB patients. It is important to continue to increase the capacity of the nursing workforce through continuing education, training and support. Proposition 4 (four) is formulated as follows:

Proposition 4 : Lack of Qualifications and Knowledge of TB. Nursing staff may have limitations in the qualifications and knowledge needed to effectively manage TB patients. They may not have adequate understanding of TB, including the treatment, monitoring and prevention of this disease. This can affect their ability to provide appropriate and up-to-date care to TB patients. The Capacity of Nursing Staff in the Management of Tuberculosis (TB) Patients at the Darul Imarah Health Center, Aceh Besar District.

The practical implication is that Puskesmas human resources must provide ongoing training and development for nursing staff involved in TB management. This includes initial training in TB, screening, monitoring, treatment, and prevention of TB. In addition, further training and regular knowledge updates also need to be provided so that nursing staff remain skilled and up-to-date with the latest developments in TB management. Use of Guidelines and Protocols: Puskesmas must ensure that nursing staff use the guidelines and protocols established in TB control.

3.3.2. Availability of drug and health supplies

In line with the national target for the Availability of Medicines and Health Supplies at the Darul Imarah Health Center in Aceh Besar District with due observance of the National Strategy. By understanding this structuring, the goal to be achieved is to protect public health from TB transmission so that morbidity, death and disability do not occur but under controlled conditions.

In general, carrying out health activities requires competence as a reactionary ability that a person has in carrying out, mastering and completing actions according to his field of work which is based on abilities and skills in a professional, effective and efficient manner. Proposition 5 (five) is formulated as follows:

Proposition 5 : Providing training and updating knowledge to nursing staff at the Darul Imarah Health Center on the use of anti-TB drugs, supply management, and infection prevention measures will enhance their competence in managing TB patients and managing health supplies. Improving accessibility to the tools and equipment needed in TB control, such as tuberculin gauges, sputum sampling kits, and consumables, will strengthen the ability of nursing staff to perform services safely and effectively.

The practical implication is that the Puskesmas can also collaborate with referral centers or other institutions that provide special drugs or equipment that are more specific in TB control. This collaboration ensures accessibility to medicines and equipment that may not be available directly at the Puskesmas.

3.3.3. Technology

Health diagnosis is an inseparable part of the health service program in the hospital. The hospital is a health service institution that has a direct relationship with patients so that hospitals must provide safe, quality, non-discriminatory and effective health services, according to a person's perception or understanding that can be influenced by knowledge, attitudes, actions and demands for rules or policies. supporting a particular activity. In the various alternatives developed by the government as an integrated discussion, the government remains consistent. The formation of the National Integrated Movement (Gerdunas) for TB is one proof of the strong political commitment to expand the implementation of the DOTS strategy with the participation of various related sectors in TB control.

The quality of health services can be seen from the availability of health services that care about the needs and expectations of patients. Patient value can be a benchmark for the provision of health services. Fitriani [8] states the following: Being one of the conditions that must be fulfilled, to realize a comprehensive public health degree, improving health in Indonesia is aimed at being able to carry out the vision of "Healthy Indonesia" which is the hope for the Indonesian nation in the future. future in order to live in a healthy environment. Thus the population has a clean and healthy lifestyle that

allows them to be able to reach quality, fair and equitable health services in achieving optimal health.

Patient monitoring technologies, such as remote monitoring devices or wearable sensors, can assist nursing staff in continuously monitoring patient conditions. The device can measure a patient's health parameters, such as body temperature, heart rate or oxygen level, and provide early warning of changes that require medical attention. The application of this health provisioning technology at the Darul Imarah Health Center can improve efficiency, accuracy, and quality of service in treating TB patients. However, it is also important to ensure that nursing personnel have sufficient training to use these technologies appropriately and effectively.

Proposition 6 : Puskesmas can adopt patient monitoring technology, such as remote monitoring devices or wearable sensors, to monitor the condition of TB patients in real-time. This technology will assist in monitoring health parameters, providing early warning of changes, and facilitating more regular interactions between patients and nursing staff. Puskesmas need to strengthen technology infrastructure, including stable internet access, adequate hardware, and regular maintenance. Adequate infrastructure will support the effective use of technology and overcome constraints associated with limited access.

Practical implications, Evaluation and Monitoring Community Health Centers need to conduct periodic evaluations and monitoring of the use of technology in TB control. This involves reviewing the performance of health information systems, the effectiveness of mobile applications, and the reliability of rapid diagnostic tools. This evaluation and monitoring will help identify problems or deficiencies in the use of technology and enable necessary improvements.

Based on propositions 1 (one) to 6 (six) as mentioned above, the major proposition is formulated as follows:

Major Proposition : Nursing Staff Services in Handling Tuberculosis (TB) Patients at the Darul Imarah Health Center in Aceh Besar District, based on a policy implementation study based on the Regulation of the Minister of Health of the Republic of Indonesia Number 67 of 2016 concerning Tuberculosis Control.

Practical Implications, By taking these steps, the Darul Imarah Health Center in Aceh Besar District can improve nursing staff services for TB patients in accordance with the Regulation of the Minister of Health of the Republic of Indonesia Number 67 of

2016. This will contribute to improving the quality of life of TB patients, effective disease management, and efforts to prevent the spread of TB in the community.

Prevent drug resistance and reduce the negative impact caused by Tuberculosis. Human Resources is also a factor that influences the development of an organization, a government institution can develop very rapidly if it has many competent human resources in their fields, the development of an organization and a government institution.

4. Conclusion and Suggestions

4.1. Conclusion

Based on the results of research and discussion regarding the Services of Nursing Staff in the Handling of Tuberculosis (TB) Patients at the Darul Imarah Health Center in Aceh Besar District, it can be concluded as follows:

TB patients often face social stigmatization and psychological pressure. Health promotion in the management of TB patients requires collaboration between various members of the health team, including nursing staff. Research shows that the services of nursing staff who provide emotional and social support can help reduce stigmatization, increase patient motivation in taking treatment, and improve the patient's quality of life. Health promotion also emphasizes the importance of monitoring and evaluating TB control programs. Nursing staff services can play a role in collecting data, tracking and monitoring patient progress, as well as reporting results and challenges faced in tackling tuberculosis at the Darul Imarah Health Center in Aceh Besar District.

Lack of Qualifications and Skills or Resources Nursing staff involved in TB control may not have sufficient qualifications and skills. They may not have sufficient understanding of TB, methods of monitoring, treatment, and prevention. This can hinder effective efforts to handle TB cases and reduce the quality of care provided.

It is important to carry out a thorough evaluation of human resources at the Darul Imarah Health Center in Aceh Besar District so that this evaluation can involve interviews, surveys, observations, and analysis of existing HR performance data. By identifying relevant HR issues, it is possible to plan and implement strategies to increase the capacity and quality of services in the management of TB patients.

Limited tools and equipment at the Puskesmas may not have the tools and equipment needed to treat TB patients. This could include shortages of tuberculin measuring

devices, sputum sampling kits, or consumables such as respiratory protective masks or medical gloves. Limited maintenance and replacement of equipment. Funding constraints or lack of proper maintenance and replacement of equipment can be a problem in keeping the required equipment available.

Inadequate system integration, if the technology systems used in the Puskesmas are not properly integrated, there may be obstacles in exchanging information between systems. This can hinder the efficiency and quality of services provided. Data privacy and security, privacy protection and patient data security are important in the use of health technology. If adequate measures are not in place to protect patient personal data, this can raise legal and ethical issues.

4.2. Suggestions

Based on the discussion above, the following suggestions are concluded:

Nursing Services in Handling Tuberculosis (TB) patients at the Darul Imarah Health Center in Aceh Besar District by means improve training and education, optimize healthcare team collaboration, improve monitoring and evaluation, improve patient care and education, use appropriate technology, encourage research and development, strengthen collaboration with communities

Nursing Capacity in Handling Tuberculosis (TB) patients at the Darul Imarah Health Center in Aceh Besar District by means continuous training and education, increasing knowledge about infection prevention and control, interprofessional collaboration, improving communication skills, forming networks, increasing access to resources, evaluation and feedback.

In the orientation of policy implementation is a crucial thing in the study of public policy because it contains philosophical, historical and sociological so that it requires a certain theory in accordance with the specifications of the policies made by the government. The primary targets of health promotion efforts are patients, healthy individuals and families (households) as components of society. They are expected to change their unclean and unhealthy living behavior into clean and healthy living behavior (PHBS).

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Research Article

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Empowerment Model for Persons with Disabilites: Application Village Inclusion for Incumbent Disability in the Village Mangliawan Pakis District, Malang Regency

Macaria Theresia Laiyan

Empowerment Model for Persons with Disabilites: Application Village Inclusion for Incumbent Disability in the Village Mangliawan Pakis District, Malang Regency

Abstract.
Disability is a condition that restricts an individual's ability to fully participate in social, economic, and political activities, whether physically, mentally, or sensorily. Disability groups, especially people with disabilities, are vulnerable to discrimination and marginalization due to a lack of support, facilities, and adequate opportunities. The empowerment model of people with disabilities is an approach aimed at enhancing their independence, social participation, and quality of life. This article discusses the empowerment model of people with disabilities, including the benefits and challenges in its implementation. Disability empowerment is focused on enabling them to reach their full potential and participate equally in society by overcoming everyday barriers. Mangliawan Village, a rural area in the Pakis District, Malang Regency, Indonesia, faces similar challenges. Despite the lack of resources and adequate support for people with disabilities, Mangliawan Village has taken significant steps by implementing the principles and goals of Inclusive Village. The implementation of the Inclusive Village in Mangliawan Village begins with an assessment of the needs and challenges of people with disabilities involving local stakeholders, including people with disabilities, families, community leaders, and government officials. The results of this assessment form the basis for developing a comprehensive plan to enhance accessibility and inclusion in Mangliawan Village. This article highlights Mangliawan Village's efforts to create a more inclusive environment and inspires the development of similar programs in other rural areas.

Keywords: empowerment, persons disabilities, social inclusion, participation, training skills

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Selection and Peer-review under the responsibility of the 4th ICONISS Conference Committee.

1. Introduction

1.1. Defining empowerment disability

Disability is a condition that hinders an individual's ability to fully participate in social, economic, and political activities. Disabilities can be physical, mental, or sensory, typically limiting an individual's material mobility, vision, hearing, or cognition [1,2].

People with disabilities, as a vulnerable group, often face discrimination and marginalization in society due to a lack of support, facilities, and adequate opportunities. The empowerment model of people with disabilities focuses on enhancing their independence, social participation, and quality of life. This article will discuss this model and explore the benefits and challenges associated with its implementation [3].

Disability empowerment refers to the process of enabling people with disabilities to reach their full potential and participate equally in society. This involves addressing the everyday barriers faced by people with disabilities and providing the skills and resources needed to overcome these challenges. However, on a global scale, the challenges faced by people with disabilities are not limited to the local level. Therefore, in this introduction, we will highlight crucial and fundamental issues related to disabilities, supported by references from relevant international and global journals. We will also detail previous research related to this topic by citing appropriate references. Finally, we will identify research gaps and explain the objectives of this article [1-4].

1.2. Importance inclusion in community

Community inclusive is those who recognize and appreciate diversity, and strive to create friendly and supportive environment for all its members . This includes disabled person disability, which is often face discrimination and exclusion in various aspect life. Inclusion important Because promote equality, cohesion social, and a sense of belonging. This also helps remove stereotypes and prejudices, and creates opportunity for disabled person disabilities to contribute to the community they with meaningful way [5-7].

1.3. Empowerment model incumbent disability

Empowerment model disabled person disability is something an approach aimed at improving independence, participation social, and quality life them. This approach assumes disabled person disability as an active and possessing subject the same rights with other people in terms of access to source power and opportunity. This model involves disabled person disability in taking decision and deliver support to develop potency them. Empowerment model disabled person disability covers a number of aspects, including:

1. Access to adequate facilities and services

Incumbent disability need access to adequate facilities and services, such as facility friendly transportation disability, accessibility buildings, and services appropriate health with need them. This will help them to feel more independent and capable participate in activity social and economic.

2. Enhancement skills and knowledge

Incumbent disability need support to upgrade skills and knowledge them, like Skills in manage finances, skills in communication, and knowledge about rights them. This will help them to be more independent and capable participate in a way active in activity social and economic.

3. Enhancement participation social and economic

Incumbent disability need encouraged to participate in activity social and economic. This can be done with give support to open business small or working on site friendly work disability. This support will help them to feel more independent and feeling more involved in activity social and economic.

1.3.1. Benefits of the empowerment model incumbent disability

1. Increase independence: Empowerment model disabled person disability can increase independence they with give support in develop skills and knowledge, so they can feel more independent and not dependent on others.

2. Increase participation social: With access to adequate facilities and services, as well support in increase skills and knowledge, people with disability can more easily involved in activity social and feeling more connected with environment approx.

3. Increase quality life: Empowerment model disabled person disability can help increase quality life they with provide access to service appropriate health with need them, as well support in increase skills and participation social.

1.3.2. Challenges of the empowerment model incumbent disability

1. Lack of access to adequate facilities and services: Lack of access to adequate facilities and services, such as friendly transportation disability and accessibility building, yes become obstacle for disabled person disability to feel independent and involved in activity social and economic.

2. Discrimination: Discrimination Still often happen to disabled person disability, both in terms of access to services and facilities, as well as in matter opportunity to work or participate in activity social .

3. Limitations financial: Indemnity disability often experience limitations financially, that can be become obstacle in access adequate services and facilities, as well in increase skills and participation social.

2. Method

This article employs a qualitative research approach to understand the empowerment methods of people with disabilities. Here are the detailed steps in the research method:

2.1. Research design

1. The applied research design is qualitative research.

2. Data is collected through observation, interviews, and directed group discussions.

3. This qualitative approach does not involve numerical or statistical data.

2.2. Case study

1. A case study approach is utilized, focusing on one community, namely the Inclusive Village for People with Disabilities in Mangliawan Village.

2. Data is gathered from individuals within the community and observations of their daily lives.

2.3. Data collection

1. Observations are conducted on the daily lives of people with disabilities in the Inclusive Village for People with Disabilities.

2. Focus is on their interactions with the environment, communication methods, and the execution of daily tasks.

2.4. Interviews

1. Interviews are conducted with people with disabilities, their families, and community members.

2. Aimed at gaining an understanding of the experience of living in the Inclusive Village for People with Disabilities and its impact on their lives.

2.5. Focused Group Discussions (FGD)

1. Focused group discussions involve people with disabilities, their families, and community members.

2. Focus on discussing challenges and successes in living in the Inclusive Village for People with Disabilities, as well as improvement strategies.

2.6. Data analysis

1. Thematic data analysis is employed.

2. Researchers search for patterns and themes in the collected data.

3. Identification of common experiences and challenges faced by people with disabilities in the Inclusive Village for People with Disabilities.

3. Results and Discussion

3.1. Overview of village inclusion: Empowerment model for people with disabilities

The article provides an overview of Inclusive Villages as a community development model aimed at empowering people with disabilities. This model emerged as a response to the lack of services and support for people with disabilities in various rural areas of Indonesia [8]. With a focus on the Inclusive Village for People with Disabilities in Mangliawan, Malang Regency, this article aims to understand the implementation and impact of the Empowerment Model for People with Disabilities.

3.2. Principles and main objectives of village inclusion

The main principles of Village Inclusion include community participation, partnership, and empowerment. Its primary objectives involve improving access to services and resources, promoting economic opportunities, increasing social participation, and enhancing the quality of life for people with disabilities and their families.

3.3. Implementation strategies in Mangliawan Village

Mangliawan Village employs several key strategies, such as developing easily accessible infrastructure, providing training and education to raise awareness, and creating economic opportunities through microprograms.

3.4. Stakeholder involvement and community participation

The success of Inclusive Village in Mangliawan is driven by the active involvement of the entire community, including people with disabilities, families, and other stakeholders. Regular meetings, dialogues, and community-based monitoring systems support the program's implementation.

3.5. Accessible infrastructure and transportation

Easily accessible infrastructure and transportation, such as wheelchairs and accessible toilets, are crucial elements in creating an inclusive and accessible environment. This ensures the participation of people with disabilities in their community life.

3.6. Education and training for awareness and skills

Education and training are key to increasing awareness, understanding rights, and developing skills for people with disabilities. The program includes vocational training and disability awareness for the wider community.

3.7. Employment and economic empowerment

Creating economic opportunities for people with disabilities is a crucial strategy to promote social inclusion and reduce poverty. The program involves establishing micro-businesses, vocational training, and inclusive recruitment policies.

3.8. Health and social services

Access to quality health and social services is a key factor in improving the well-being of people with disabilities. This includes providing assistive devices, rehabilitation services, and healing facilities.

3.9. Positive impact of village inclusion in Mangliawan

The article highlights the positive impact of Village Inclusion in Mangliawan, including improved quality of life, increased social participation, and economic stability for people with disabilities.

3.10. Challenges and opportunities for improving village inclusion programs and disability empowerment

The article identifies several challenges, such as the lack of comprehensive policies, effective resource allocation, and changes in societal attitudes. Overcoming these challenges requires continuous efforts in education, advocacy, and the development of inclusive policies.

4. Conclusion

In order to provide a profound understanding of the implementation and impact of the Empowerment Model for People with Disabilities in the Inclusive Village for People with Disabilities, this article outlines the general overview, principles, objectives, implementation strategies, and program impacts in Mangliawan Village, Malang Regency.

The Inclusive Village, as a community development model, aims to empower people with disabilities and create an inclusive environment. Implementation strategies in

Mangliawan include the development of easily accessible infrastructure, training, education, and microeconomic programs. The active involvement of the entire community, including people with disabilities and their families, supports the program's success through regular meetings, dialogues, and monitoring systems.

Accessible infrastructure and transportation are key to creating an inclusive environment, while education and training aim to enhance awareness and skills. Economic empowerment efforts, through the establishment of micro-businesses and inclusive recruitment policies, are identified as vital strategies.

The positive impact of Village Inclusion in Mangliawan is evident in the improved quality of life, increased social participation, and economic stability for people with disabilities. Nevertheless, challenges such as the lack of comprehensive policies, effective resource allocation, and changes in societal attitudes remain obstacles to expansion and further empowerment. Therefore, continuous efforts in education, advocacy, and the development of inclusive policies are necessary to overcome these challenges and enhance the sustainability of the program. This article makes a significant contribution to highlighting the role of the Inclusive Village in creating a more inclusive society and empowering people with disabilities.

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Research Article

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Halotherapy As Adjuvant Therapy for Respiratory Diseases: A Literature Review

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Abstract.
Halotherapy (HT) is part of salt therapy derived from speleotherapy/speleoclimate, which comes from using a micro size of dry salt in aerosol form (such as sodium, potassium, magnesium, calcium, and sodium chloride) with stable air temperature (18–24°C) and moderate to high humidity (40–60%) for inhalation inside a cave/imitation cave since the nineteenth century. The benefits of HT as an adjuvant may help patients with many medical conditions, especially respiratory and dermatology diseases. This therapy is believed to alleviate inflammation and the immune response and improve respiratory function, etc. The clinical benefits of HT are advocated, but the mechanisms still need to be explicitly elucidated. This study's main objective is to critically review and evaluate the evidence from existing literature of HT efficacy as an adjuvant therapy for respiratory disease in a narrative review. This review used a systematic approach and narrative synthesis. PubMed, Google Scholar, and Cochrane Central Register of Controlled Trials (CENTRAL) were searched. Based on preset selection criteria, two reviewers separately looked over abstracts and chose relevant papers. HT improves mucociliary elimination, diminishes airway inflammation, and improves pulmonary function. This adjuvant therapy is safe and does not cause serious adverse events. Therefore, this therapy should be considered an adjuvant therapy for respiratory diseases because of its potential effects. However, scientific evidence of the effectiveness of HT is limited. High-quality further research is required to ascertain the effectiveness of this treatment for respiratory conditions.

Keywords: adjuvant, halotherapy, respiratory diseases, salt therapy, therapy

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1. Introduction

Prolonged exposure to indoor air dampness microbiota could result in problems with the vocal cords, irritation of the mucosa, chronic rhinitis, cough, wheezing, dyspnea, recurrent infections of the upper and lower respiratory tract, exacerbation of existing asthma, and the commencement of a new asthmatic attack [1]. Numerous undiagnosed respiratory symptoms may represent undiagnosed allergic alveolitis or panbronchoalveolitis. Notably, the components of moist microbiota may inhibit the function of epithelial cilia cells, the first line of defense in innate immunity [2].

Halotherapy (HT) comes from speleotherapy/speleoclimate, which comes from using a micro size of dry salt (such as sodium, potassium, magnesium, calcium, and sodium chloride) for inhalation inside a cave/imitation cave [3]. The benefits of HT may help patients with many medical conditions, especially respiratory and dermatology diseases. This therapy is believed to alleviate inflammation and the immune response, improve respiratory function, and many more [4]. The clinical benefits of HT are advocated, but the mechanisms still need to be explicitly elucidated. The main objectives of this study are critically review and evaluate the evidence from existing literature of HT efficacy as adjuvant therapy for respiratory disease in a narrative review. HT may be adjuvant therapy for preventing severe exacerbations, thus reducing the need for frequent pharmacological set-up interventions, especially in the pediatric population [5].

Salt therapy in respiratory medicine began in the 19th century due to the many practical benefits of salt treatment to respiratory patients. This discovery established the groundwork for speleotherapy (aerosols therapy offered in naturally occurring salt environments, such as salt caves) and HT (aerosols therapy in any salt-enriched environment, such as home devices or surface halo chambers). In treating and preventing asthma, HT has been shown to have various beneficial effects [5]. HT could also diagnose asthma through a bronchial challenge analogous to physical activity. An opposing process accomplishes this. In addition, it has a bacteriostatic impact and a beneficial effect on the immune response of the immediate area. Even though it is ecological and kind to the environment, as well as the fact that there is new and recent evidence, including ongoing studies (NCT03556683) [5], no scientific study covering the topic of the benefits of HT in asthma has been published in the past two decades. In addition, the most recent guidelines for asthma sometimes reference HT, but solely as a bronchial challenge agent used to identify asthma. We aim to review all studies evaluating the effect of HT as adjuvant therapy for respiratory diseases.

2. Method

The Cochrane Central Register of PubMed, EAACI, MDPI, Liebert Pub, and Google Scholar were some sources used for study. Keywords associated with halotherapy/speleotherapy were employed in the search method: adjuvant, halotherapy, respiratory diseases, salt therapy, randomized controlled trials, both prospective and retrospective, comparing halotherapy or speleotherapy to a control group were taken into consideration. Full-text papers, and abstracts in languages other than English

were not included. Subsequently, full-text papers satisfying the inclusion criteria were acquired.

3. Results

3.1. Dose and duration of halotherapy

In this investigation, six publications examined the effect of HT on respiratory disorders. Two of the six journals examined in this study did not include the dosages used in their research, while the other four listed the doses. Diepeveen's research [6] and Rabbani et al. research [7] determined that the same dose of HT was administered to all intervention groups, regardless of the type of disease they were suffering from. In contrast, in the studies conducted by Chervinskaya and Zilber [8] and Sandell et al. [9], distinct concentrations of HT were administered based on the type of respiratory disease experienced [8] and the level of treatment dose.

All six journals reviewed for this study included the duration of HT treatment for patients with respiratory disorders. The duration of HT in the studies examined ranged from 1 to 8 weeks, with two studies providing HT in less than 2 weeks and four other studies providing HT for longer than 2 weeks.

3.2. Outcome of halotherapy management in patients with respiratory diseases

Most of the journals reviewed for this study demonstrated that HT treatment, when administered to patients suffering from respiratory disorders, had a beneficial influence (benefits) on their ability to overcome their conditions. Even at the cellular level, the treatment appeared to benefit patients who suffered from respiratory diseases. It was shown that administering HT treatment to rats with COPD led to a reduction in the amount of glandular hypertrophy, an improvement in lumen stenosis, and a reduction in the infiltration of surrounding inflammatory cells. It was discovered that HT might boost lung function indices while simultaneously decreasing antioxidant levels in rats [10]. It was also shown that rats with asthma who received HT had improved cell morphological characteristics compared to those who did not.

It was discovered that HT had a positive impact and was beneficial to individuals who suffered from bronchial blockage. This research looked at a total of two previous studies.

It concluded that HT compounds' antibacterial and antibacterial-suppressing properties could influence the microbiota in the respiratory system. As a result, these chemicals could help reduce inflammation and boost phagocytosis. It has also been discovered that HT could enhance the quality of life of people with respiratory disorders. However, this research includes two publications that suggest that HT does not substantially influence the management of individuals with respiratory disorders.

4. Discussion

4.1. Halotherapy

Halotherapy (HT; "halos" is the Greek word for salt) looks to be a potential alternative therapy that aims to introduce salt particles into the upper and lower airways. Pavel Gorbenko used the term "halotherapy" in 1985 to characterize methods of using halite in the form of dried aerosols above ground [11]. The most rational is using dried aerosols of rock salt (haloaerosols) with specific concentration and dispersion characteristics. Summary of the benefits of halotherapy for respiratory diseases can be seen in Table 1.

People have visited natural salt caverns for centuries, particularly in Eastern Europe, for the medicinal properties of the air. The microclimate within the caves is distinguished by its stable air temperature, moderate to high humidity, fine aerosol elements (sodium, potassium, magnesium, and calcium), and absence of airborne contaminants and pollens [13].

Halos contain regulated environments in which tiny salt particles are inhaled [14]. The chamber's architecture aims to replicate the natural salt cave's environment. By distributing salt particles inside a regulated airspace, HT mimics the environment of a natural salt cave. While comparable in concept to hypertonic saline, HT delivers dry aerosol microparticles of salt (1-5m) instead of a moist solution. In most cases, a person spends 30 to 60 minutes reading or doing other calming activities at a facility that provides HT services. Nasal and oral respiration allow medical practitioners to target the upper and lower airways [15].

Asthma, cystic fibrosis, chronic obstructive pulmonary disease (COPD), eczema, and dermatitis have been associated with HT treatment [13]. Many clinical investigations have assessed the effectiveness of HT, despite it being regarded as a spa therapy. 139 individuals with respiratory conditions, five of whom had cystic fibrosis, were examined for HT. After 10 to 20 sessions, plethysmography indicated lower bronchial resistance

TABLE 1: Summary of the benefits of halotherapy for respiratory diseases.

Study	Dose and Duration	Another drug-induced in the regimen	Outcome
Zhang et al. [10]	Dose: N/A Duration: 1 month	Terbutaline, Caspase-1 inhibitor	The coat color gradually returned to normal after 1 month, the degree of glandular hypertrophy in COPD rats was reduced, lumen stenosis was improved, and the infiltration of surrounding inflammatory cells were reduced; the rats' TV, EV, EE, and pulmonary function were improved, and COPD rats in the HT group showed decreased NO levels.
Chervinskaya & Zilber [8]	Dose: Bronchial asthma: 0,5-2 mg/m3 Chronic obstructive bronchitis: 0,5-1 mg/m3 Chronic non-obstructive bronchitis: 3-5 mg/m3 Bronchiectasis FEV<60%: 1-2 mg/m3 Bronchiectasis FEV>60%: 7-9-mg/m3 Cystic fibrosis: 3-5 mg/m3 Duration: Bronchial asthma (allergic): 12-14 days Bronchial asthma (infection-dependent), chronic, and non-chronic obstructive bronchitis: 18-21 days Bronchiectasis and cystic fibrosis: 21- 25 days	Beta- agonist, theophyllin e, cromoglyca te nitric, corticostero id	HT significantly improved bronchial patency, started on the 7th day, persisted until the course's end, and gradually positively influenced bronchial obstruction. Sodium chloride aerosol causes bactericidal and bacteriostatic effects on the respiratory airway microflora and prevents the development of inflammatory processes.
Sandell et al. [9]	Dose: Placebo: 0,3 mg/m3 Low salt: 6,6 mg/m3 High salt: 10,8 mg/m3 Duration: 2 weeks	N/A	The treatment did not affect lung function and eosinophilic markers.
Simionca & Mirescu [12]	Dose: N/A Duration: 7 days	HAM-F12, Penicillin, streptomyci n, neomycin, fetal calf serum	The culture of the 7 days skin cells acquired from rats with injuries and burns of Calcica and Dej's study showed a microscopically visible improvement of morphologic parameters, lung fibroblast in the 7 days culture acquired from ovalbumin- sensitized rats showed an improvement of morphologic parameters of cells.
Diepeveen [6]	Dose: 0,5 – 10,8 mg/m3 Duration: 2– 8 weeks	N/A	Two studies showed increased quality of life; Lazarescu et al. found that HT increases phagocytosis processes and non-specific anti-inflammatory resistance. The high salt group showed a decrease in the ECP values directly after treatment, and it continued until four weeks after treatment.
Rabbani et al. [7]	Dose: 70- gram crystal salt Duration: 2 months	N/A	No statistically significant difference was detected in spirometry results and the 6-minute walk test, and it caused no change in any of the patients' SF- 36 quality of life questionnaire scales.

and improved flow volume loop parameters [15]. CF patients were reported to respond similarly to the treatment. Six patients with cystic fibrosis demonstrated an improvement in respiratory function and sputum secretion after only five sessions of HT [6].

A recent study of patients with bronchiectasis found that HT was of limited advantage. Vital Signs on Cable Network News and other TV networks including the British Broadcasting Corporation have also covered HT extensively. In Australia, HT has been covered by Channel 9's A Current Affair and Channel 7's Today Tonight.

4.2. Mechanism action of halotherapy

HT is an aerosol therapy procedure instead of speleotherapy, which relies on complex natural factors for its therapeutic effect [16]. medium spread by air that has been saturated with dry sodium chloride aerosol at mass concentrations between 5.5 and 10.3 mg/m3 and particle sizes between 1 and 5 mkm provide therapeutic action; these specifications were taken from several physiotherapy establishments. The major negative charge of haloaerosol particles is 6–10 nK/m3 [11]. The temperature and relative humidity of the air are both comfortably high (18–24 degrees Celsius and 40–60 percent). Normal bronchial ciliated epithelium functioning requires sodium chloride, in contrast, bronchial secretions from individuals suffering from chronic pulmonary disease had a lower salt chloride level. In addition to producing anti-inflammatory effects, aerosolized sodium chloride exerts bactericidal and bacteriostatic effects on respiratory tract microflora and increases alveolar macrophage responsiveness by promoting the growth of phagocytic elements and their phagocytic activity [17]. Haloaerosol particles have a significant negative charge (6-10 nK/m^3). Furthermore useful for therapy, a large negative charge also makes the aerosol more stable. The physical characteristics of sodium chloride aerosol are just as important to the HT technique as its biological features. All parts of the respiratory system, including the deepest ones, can be reached by haloaerosol [18].

The method's fundamental characteristic is the administration of dried sodium chloride aerosol [14]. The basis of haloaerosol influence is a hyperosmolar stimulus, primarily manifested by enhancing the rheological properties of sputum and stimulating the ciliated epithelium, resulting in enhanced mucociliary clearance and bronchial discharge function. When equal dispersion was studied, the respiratory tract's ability to absorb dry and droplet sodium chloride aerosols showed that the dry aerosol caused the greatest amount of particle delay [11]. This meant that lesser dosages may be given and negative side effects might be avoided by applying a dry, widely diffused aerosol.

At the level of the pulmonary membranes and the mucociliary system, the action mechanisms of the dried NaCl nanoparticles are direct and inverse osmosis and an ionic change process, with a revitalizing and detoxifying effect. Dried aerosol optimizes the camera's temperature and humidity parameters [8]. This makes it possible to prevent the bronchial spasm and mucosal edema in the respiratory tract, which are common responses in individuals exposed to wet aerosols. The additional effects of HT could be explained by the patient's exposure to hypoallergenic, antimicrobial, noiseless, and psychologically comfortable air.

HT is an all-natural, risk-free therapy with no serious adverse effects [14]. This method is extremely beneficial for an individual's overall health because it strengthens respiratory system function, gets rid of harmful elements, and promotes promotes immunity, eases stress, and improves the look and function of the epidermis [19].

4.3. Benefit of halotherapy

The effectiveness of HT is primarily attributable to three factors. First, salt has antibacterial, antimycotic, and anti- inflammatory properties. Chervinskaya and Zilber (1995) also found that HT acts on the pulmonary airway microbiota, bactericidally and bacteriostatically, to prevent the development of inflammatory processes [8]. Salt rooms are three times more hygienic than the best operating room, according to research [14].

Second, salt naturally emits negative ions, which neutralize a positive charge. The salt particles have a considerable negative charge (6-10 nK/m^3), and the surface of the airways has a slight positive electric charge; as a result, the salt could enter the respiratory system and "bind" to the airway epithelium. This contributes to the increase in the organism's resistance to respiratory infections and has desensitizing effects on allergens [12]. Third, dried salt has an incredible absorption capacity. The salt itself is inherently absorbent, and according to one theory, it may collect and dehydrate any infectious droplets before they become airborne [20].

Most respiratory ailments, including tonsillitis [21], pharyngitis [16], sinusitis [15], rhinitis [21], asthma [5], allergies [18], and chronic bronchitis [18], as well as respiratory tract infections [8], recurring colds [5], and pneumonia following an acute phase [16], can be treated with HT. It has been demonstrated that this is a highly effective method of positively influencing a variety of respiratory diseases with a rapid resolution of symptoms, enhancement of pulmonary ventilation and tolerance of physical exertion, and an increase in the body's immunity and resistance [14].

Salt therapy is a complementary treatment for a few pediatric conditions [22]. It is risk-free, noninvasive, and without adverse effects or potential health hazards. Clinical studies have demonstrated that adolescents react more rapidly and vigorously than adults (Larson). Data suggests that HT is very effective as a preventative measure in children who are sick often [14], additionally to the prospect of using it to treat acute respiratory illnesses in kids who suffer from long-term ENT, respiratory, and skin conditions. This therapy is readily applicable to minors. Toys and work equipment are placed in dedicated salt rooms, which helps kids feel comfortable and calm [23].

4.4. Future prospective of halotherapy as adjuvant therapy

Although HT has been utilized for centuries in various cultures, its modern application as a complementary therapy for respiratory diseases has attracted considerable attention. Several studies have demonstrated its efficacy to be promising. In asthma and COPD patients, HT has been demonstrated to enhance lung function, reduce respiratory symptoms, and lessen the need for conventional medications, according to clinical trials [1]. In addition, it has been reported that HT improves the quality of life and general health of individuals with respiratory conditions [24].

As interest in HT increases, scientific studies will likely examine its mechanisms of action, effectiveness, and safety. Future research may focus on determining how salt particles specifically interact with the respiratory system and how they modulate inflammatory responses. Additionally, research efforts may be focused on optimizing HT protocols, such as determining the optimal treatment duration and frequency for various respiratory conditions.

As interest in HT grows, scientific studies examining its mechanisms of action, efficacy, and safety will likely increase. Future research may investigate how salt particles interact specifically with the respiratory system and how they modulate inflammatory responses. Additionally, research may concentrate on optimizing HT protocols, such as determining the optimal treatment duration and frequency for different respiratory conditions.

Future expansion of telemedicine and remote healthcare services could include HT. Patients may benefit from HT without physically visiting salt chambers if virtual or at-home HT options become available. Incorporating HT into telemedicine platforms may increase accessibility and convenience for patients, especially those with limited mobility or who reside in remote areas.

As HT grows in popularity as a complementary treatment for respiratory diseases, it may contribute to a closer working relationship between halotherapists and respiratory specialists. This approach could facilitate knowledge sharing, research collaboration, and the development of evidence-based guidelines for incorporating HT into managing respiratory diseases. HT may have applications in preventive care and its prospective function in treating respiratory diseases. Regular HT sessions may aid in maintaining respiratory health, bolstering the immune system, and decreasing the risk of respiratory infections and exacerbations in susceptible individuals.

5. Conclusion

Future research will likely unearth additional therapeutic advantages and refine the clinical application of HT as an adjunctive treatment for respiratory diseases. HT could enhance the quality of life for people with respiratory conditions by reducing inflammation, enhancing mucous clearance, and alleviating respiratory symptoms. As medical technology advances, HT may become integral to holistic treatment plans, complementing conventional therapies to offer patients a more comprehensive and individualized approach to respiratory disease management. However, rigorous research must continue to establish its efficacy, safety, and optimal implementation in clinical practice.

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Research Article

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Comparison of the Antioxidant Effect of Robusta and Arabica Coffee Ethanol Extracts on Oxidative Stress in Diabetic Wounds

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Abstract.

Impaired wound healing in diabetes mellitus is caused by metabolic disorders due to hyperglycemia. Hyperglycemia causes an auto-oxidation reaction of glucose, which synthesizes the formation of reactive oxygen species (ROS). Robusta and Arabica coffee contain chlorogenic acid, caffeine, and trigonelline, which can prevent oxidative stress. This study aimed to analyze the antioxidant effect of the ethanol extract ointment of robusta coffee and arabica coffee on oxidative stress in diabetic wounds by measuring the decrease in plasma malondialdehyde (MDA) and leukocytes. This study used 24 experimental animals consisting of a positive control group, a negative control group, a group of rats with diabetic wounds who were given a 15% concentration of Robusta coffee ethanol extract ointment, and a group of rats with diabetic wounds who were given a 15% concentration of Arabica coffee ethanol extract ointment. After eight days of treatment, plasma MDA and leukocyte counts were examined. The results showed that plasma MDA levels and leukocytes did not differ between the diabetic wound groups that were given a 15% concentration of Robusta coffee extract ointment and a 15% concentration of Arabica coffee extract ointment. Robusta coffee and Arabica coffee ethanol extract ointments have the same effect on reducing plasma MDA levels and leukocytes.

Keywords: arabica, diabetic wound, malondialdehyde, robusta

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1. Introduction

Hyperglycemia can reduce the function of the immune system in defending itself from being infected with microorganisms that attack people with diabetes mellitus (DM). In hyperglycemia, autonomic neuropathy can occur, which causes a loss of ability to moisturize the surface of the skin due to decreased secretory function of the sebaceous glands and sweat glands, so that the skin of the feet becomes dry and facilitates the spread of infection [1–4]. Hyperglycemia conditions can also cause a decrease in the number of basal cells and impaired proliferation. Keratinocytes and skin barrier function, thus interfering with the wound healing process [1,4,5]. Hyperglycemia causes an auto-oxidation reaction of glucose, which can trigger free radicals, namely reactive oxygen

species (ROS) [1,6]. Oxidant compounds can affect the level of production of ROS and inflammatory mediators, so the higher the production of ROS due to decreased antioxidants, the higher the free radicals, resulting in oxidative stress [6].

Wounds that occur in conditions of hyperglycemia can cause oxidative stress, thereby increasing plasma MDA levels. The increase in MDA is caused by several proinflammatory mediators resulting from skin injury. As is well known, hyperglycemia will also induce an inflammatory response, oxidative stress, and an increase in the number of free radicals, which in turn will increase plasma MDA levels and reduce antioxidants [3,7].

Indonesian people believe that coffee beans are an ethnomedicine because they have many benefits and are easily available to the public. Coffee beans contain polyphenolic compounds such as chlorogenic acid, ferulic acid, caffeine, and trigonelline, which have antioxidant properties to neutralize the effects of oxidative damage to the skin and also have antibacterial, antiviral, and anti-inflammatory properties [8–10]. The administration of antioxidants can suppress the effects of free radicals that arise from the inflammatory process due to diabetic wounds [10,11]. Administration of a 15% concentration of robusta coffee ethanol extract ointment has been known to increase fibroblasts, resulting in diabetic wound healing [11]. Other studies also state that a 30% concentration of robusta coffee extract ointment is capable of repairing diabetic wounds [12]. However, there is still not much data explaining the effectiveness comparison between Robusta and Arabica coffee ethanol extract ointments for healing diabetic wounds. This study aimed to analyze the antioxidant effect of the ethanol extract ointment of robusta coffee and arabica coffee on oxidative stress due to diabetic wounds by measuring the decrease in plasma malondialdehyde (MDA) and leukocytes in hyperglycemic rats.

2. Methods

This research is a laboratory experimental study with a post-test-only control group design. The research subjects were white rats (Rattus norvegicus) of the Wistar strain, which had been acclimatized for seven days. The rats were divided into four groups, consisting of a positive control group, a negative control group, a group of hyperglycemic rats that experienced incisions and were given a 15% concentration of robusta coffee ethanol extract ointment, and a group of hyperglycemic rats that experienced incisions and were given arabica coffee ethanol extract ointment. concentration 15%.

2.1. Diabetic wounds

Induction of hyperglycemia in experimental animals by giving alloxan 200 mg/kg BW intraperitoneally. Diabetic wounds in rats are made by making an incision in the skin. Before the incision, the rats were given intraperitoneal general anesthesia. The rat skin was disinfected with 10% betadine before the incision. Making an incision 2 cm long using a scalpel with a depth of 0.2 cm to the hypodermis layer, the incision is then sutured and covered with sterile gauze.

2.2. Preparation of robusta coffee and arabica coffee ethanol extract ointment

In this study, an ointment made from the ethanol extract of Robusta coffee and Arabica coffee was used. The ointment used is fat-based, using Vaseline. The standard ointment base formula used is 100 grams of Vaseline. The ointment used in this study has a concentration of 15%.

2.3. Examination of plasma malondialdehyde levels with the TBARs method

Animal blood was taken from the retroorbital vein in as much as 3 mL. Then the blood was centrifuged, and then the plasma was added with Na2EDTA, 300 µl of aqua dest, 200 µl of SDS solution, 50 µl of BHT solution, 50 µl of EDTA solution, 1500 µl of acetic acid solution, and 1500 µl of TBA solution. The mixture was heated for 45 minutes in boiling water (100^{o} C), and then immersed in a bath filled with ice water. The sample was then centrifuged, and then read using a spectrophotometer at a wavelength of 532 nm.

3. Analysis

Data analysis to determine the comparative effect of Robusta Coffee and Arabica Coffee ethanol extract ointment on blood sugar levels used the Kruskall-Wallis test and continued with the Mann-Whitney post-hoc test, while for the comparative effect on MDA levels and leukocyte counts used the one-way ANOVA and Tukey post-hoc test.

3.1. Aspects of research ethics

This research has obtained permission from the Health Research Ethics Commission, Faculty of Medicine, Universitas Jenderal Achmad Yani No: 019/UH.1.11/2021.

4. Results

After the rats were induced with alloxan, there was an increase in blood glucose levels in the positive control (KP), whereas in K1 (ethanol extract of Robusta coffee concentration 15%) and K2 (ethanol extract of Arabica coffee concentration 15%), blood glucose levels were not higher when compared to KP. Blood glucose examination results can be seen in Table 1.

The results of the analysis with Kruskal-Wallis revealed that the blood glucose levels between the treatment groups differed significantly with a p-value <0.05. Furthermore, the Mann-Whitney test was carried out between groups. The results of the analysis showed that blood glucose levels were significantly different between the KP groups compared to the KN, KI, and K2 groups.

Table 1: Blood glucose levels, MDA plasma, leukocytes.

Groups	Mean ± SD		
	Glucose	MDA	Leukocytes
KN	77.6±16.3	0.83±0.3	9.7±1.7
KP	299±53.8	17.0±17.6	14.8±3.0
K1	90.8± 13.1	1.5±1.3	7.1±2.7
K2	108±29.8	1.7±0.6	8.6±1.6
p-value	0.000	0.000	0.000

Note: Kruskal Wallis p value <0.05 for blood glucose. One-way ANOVA ; *)p <0.05 for MDA and leukocyte count. KN = negative control, KP = positive control, K1 = 15% concentration of Robusta coffee ethanol extract, K2 = 15% concentration of Arabica coffee ethanol extract.

The plasma MDA levels of rats from each group are presented in Table 1. The highest mean plasma MDA levels were shown in the KP group, and the lowest average plasma MDA levels were shown in the KN group. Increased plasma MDA levels in KP could be due to incisions and hyperglycemia in rats, resulting in the production of several free radicals and ultimately an increase in plasma MDA levels.

Based on these data, it is known that the highest leukocyte count was in the KP group compared to the other groups. Incision wounds that occur in hyperglycemic rats cause an inflammatory reaction, increasing the number of leukocytes. In Table 1, it is known that there are significant differences in plasma MDA levels between groups with a p-value <0.05. Furthermore, from these data, a post-hoc Tukey test was carried out, with the result that there was a significant difference between KP and KN, K1, and K2. While the KN group, compared to K1 and K2, showed no significant difference in plasma MDA levels. Robusta coffee and arabica coffee ethanol extract ointments have the same effectiveness in reducing plasma MDA in hyperglycemic rats that have had incisions.

5. Discussion

Hyperglycemia affects keratinocyte and fibroblast activity, which induces protein synthesis, proliferation, and migration and increases oxidative stress [3].

Antioxidants such as chlorogenic acid, caffeine, and trigonelline, which are found in robusta and arabica coffee, can suppress the appearance of free radicals resulting from the inflammatory process. A decrease in plasma MDA levels may reflect the ability of antioxidants to suppress oxidative stress. Antioxidants in coffee can accelerate wound healing, increase hydroxyproline, reduce malondialdehyde and nitric oxide, and increase glutathione reductase [3,5].

Malondialdehyde is a compound produced from plasma lipid peroxidation by several free radicals such as ROS. Reactive oxygen species (ROS) and nitric oxide appear due to an inflammatory process due to incision wounds and hyperglycemia conditions. Increased antioxidant defense against free radicals causes a decrease in plasma malondialdehyde levels and accelerates wound healing [12–14].

Another indicator to determine the effect of the ethanol extract ointment on Robusta coffee and Arabica coffee is by measuring leukocyte levels. The number of leukocytes between the treatment groups was shown to be different based on the data in Table The results of Tukey's post hoc test analysis showed that the number of KP leukocytes was significantly different from KN, K1, and K2, with a p-value <0.05. Impaired wound healing in diabetes is associated with reduced levels of nitric oxide [6]. Nitric oxide (NO) plays an important role in the inflammatory process because it has a vasodilator, an antimicrobial effect, prevents platelet aggregation, and increases vascular permeability.

NO is responsible for upregulation and downregulation in the inflammatory phase of wound healing [6,15].

Hyperglycemia stimulates leukocytes to release a number of proinflammatory mediators in response to increased glucose. In diabetes, there is an imbalance between pro- and anti-inflammatory cytokines. Neutrophils as the first line of defense against inflammation show decreased functional activity and cause susceptibility and severity of infection.[2] Chlorogenic acid, caffeine, and trigonelline can accelerate wound healing because they provide anti-inflammatory and antibacterial effects [2,4,11].

6. Conclusion

The antioxidants of robusta and arabica coffee ethanol extracts have the same effect on oxidative stress in diabetic wounds, reducing plasma MDA (malondialdehyde) levels and leukocyte count.

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Research Article

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The Effect of Cinnamomum Burmanii Ethanol Extract on Isoniazid-induced Serum Levels of Serum Glutamate Piruvate Transaminase (SGPT) Wistar Strain Male Rats

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Abstract.
Hepatotoxicity can be caused by excessive drug use, triggered by increased oxidative stress which is considered as the initial mechanism of Drug Induced Liver Injury (DILI). One of the causes of DILI is Isoniazid. One of the plants acting as a hepatoprotector in protecting liver cells is cinnamon (Cinamommum burmanii). This study aims to determine the effect of 96% ethanolic cinnamon extract as a hepatoprotector against an increase in Alanine Transaminase (ALT) levels induced by isoniazid. The parameters used to assess liver damage were rat plasma ALT. The sample used in this study was rat plasma, which was taken through the retro orbital sinus. This research is an experimental study with a sample of 25 experimental animals divided into five groups, namely negative control (K1), positive control I (K2), and experimental group with cinnamon ethanolic extract at a dose of 100 mg/kgbw (K3), 200 mg/kgbw(K4), and 400 mg/kgbw(K5). The research used a post-test-only control group design. The results were analyzed using the One-way ANOVA test followed by the post hoc Tukey test. The results proved that cinnamon ethanolic extract at doses of 100 mg/kgbw (P = 0.029), 200 mg/kgbw (P = 0.001), and 400 mg/kgbw (P = 0.000) was effective in reducing plasma SGPT levels in isoniazid-induced rats when compared with the positive control group (K2). The most effective dose was at 200 mg/kgbw (K4). Thus, this proves that all of the doses in experimental groups have a hepatoprotective effect against isoniazid-induced liver damage.

Keywords: Alanine Transaminase (ALT), cinnamon ethanol extract, Drug Induced Liver Injury (DILI), isoniazid

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1. Introduction

Hepatotoxicity can be caused by excessive drug use and drug abuse [1]. When exposed to hepatotoxic substances, the liver tends to be susceptible to damage to necrosis due to oxidative stress [2]. Oxidative stress in hepatocytes is the initial mechanism for drug induced liver injury (DILI) [3]. DILI is a condition of liver injury due to the use of drugs, herbs or other toxic substancessuch as in tuberculosis therapy namely rifampicin and isoniazid [4]. Various kinds of TB drugs that must be consumed by patients include a fixed dose anti-tuberculosis drug package (OAT-KDT) which contains isoniazid, rifampicin, pyrazinamide, and ethambutol [5].

Tuberculosis (TB) is a chronic disease that has become a global problem [6]. Circulating global issues have been supported by the fact that in 2019, Indonesia is ranked third with the highest number of TB sufferers in the world [7]. One of the first-line drugs for TB therapy is isoniazid [8,9]. The incidence of liver damage induced by the TB drug isoniazid (INH) is reported to be the highest and is ranked second in the United States [10]. The result of isoniazid metabolism which produces free radicals has an effectvarious natural products such as cinnamon [11]. Cinnamon has secondary metabolites such as flavonoids, alkaloids, tannins, and essential oils which can act asantioxidants [11,12]. The success of the mechanism of action of cinnamon as a hepatoprotector is illustrated by a decrease in serum glutamic pyruvate transaminase (SGPT) levels which has increased due to DILI by isoniazid [13]. Selection of SGPT is an indicator that can increase faster than other enzymes and is the most common enzyme found in the liver so that it is considered the best indicator to assess liver damage. The novelty of this research can be assessed from the use of cinnamon as an ingredient that is often used as a daily things. However, no one has discussed the hepatoprotective effect on DILI cases [14].

2. Materials and Methods

Research begins in July 2022 and ends in January 2023. Cinnamon ethanol extract was made at the FMIPA Laboratory and the Biochemistry Laboratory, Faculty of Medicine, Jenderal Achmad Yani University. The preparation and treatment of experimental animals was carried out at the Animal Laboratory of the Faculty of Medicine, Jenderal Achmad Yani University. After that, SGPT measurements were carried out by the Pharmacology Laboratory of Padjadjaran University. This research was approved by the

Ethics Commission for Health Research, Faculty of Medicine, Jenderal Achmad Yani University and received ethical approval with letter number 021/UH2.10/2022.

2.1. Research design

The type of research used is experimental in nature with the post test only control group design method using a completely randomized design technique (CRD).

2.2. Research subject

This study used 25 male Wistar rats (Rattus norvegicus) obtained commercially from Biofarma which were divided into 5 groups, namely the negative control group (K1), the positive control group (K2), cinnamon ethanol extract at a dose of 100 mg. /kgBB, 200 mg/kgBB, and 400 mg/kgBB.

2.3. Object of research

The object of this study used cinnamon (Cinnamomum burmanii) obtained from the Manoko Experimental Garden in Lembang, West Bandung.

2.4. Research preparation

This study was initiated by preparing 25 male Wistar rats for 5 experimental groups. All animals with homogeneity tried to be acclimatized. for 7 days in the biopharma laboratory. By also preparing cinnamon ethanol extract in three doses consisting of 100 mg/kgbb, 200 mg/kgbb, and 400 mg/kgbb and isoniazid 200 mg/kgbb which has been dissolved to be induced through male rats of the Wistar strain.The procedure for acclimatizing experimental animals at the Animal Laboratory of FK Unjani is according to operational standards. Wistar rats in this study were required to go through an acclimatization period of 7 days at room temperature 26 – 28°C, the cage consisted of 5 rats which were given standard feed of 20-25 g/head/day and drank from a bottle ad libitum. The cage used is 60 x 40 cm in size with a height of 60 cm. High wood shavings±3 cm is needed for the bottom of the cage which will be changed 3 times a day [15].

2.5. The making of cinnamon ethanol extract

Making the extraction in this study using the maceration method. Cinnamon bark as much as 2.5 Kg which is washed thoroughly with water, then cut into small pieces so that it can be dried in an oven at 60°C for 3 days at the Biochemistry Laboratory, FK Unjani. Then it was weighed again to determine the weight by continuing the process of chopping and refining the wood using a milling machine at the Chemistry Laboratory, Faculty of Science and Informatics, Jenderal Achmad Yani University. Furthermore, cinnamon bark powder as much as 1 Kg. In this study, 300 grams will be put into three Erlenmeyer with 100 grams each added with 900 ml of 96% ethanol solvent, so a comparison between cinnamon bark powder and 96% wood ethanol extract will be obtained, namely 1: 3. The maceration process is carried out for 3 – 4 days with stirring, then followed by filtration or separating the solution using filter paper. The maserate that has been formed is then evaporated through a rotary evaporator at 90 °C, so that a thick extract is obtained [16].

2.6. Experimental animal treatment

The number of groups in this study were 5 groups, each group containing 5 rats so that the number of animals in this study used 25 male Wistar rats. Each group was adapted for 7 days and given standard feed. Furthermore, body weight was measured to ensure that the rats met the inclusion criteria. After weighing, cinnamon ethanol extract was given with three different dose variants after 1 hour the rats experienced physiological gastric emptying, then induction of isoniazid with a toxic dose of 200 mg/kgbb [13]. Treatment for the next 14 days in each group including group 1 as a negative control group which was only given aquabidest (K1), positive control group which was only given a toxic dose of isoniazid (K2), treatment group 1 by giving a dose of 100 mg cinnamon ethanol extract /kgBB (K3), treatment group 2 with a dose of 200 mg/kgBB (K4), and treatment 3 with a dose of 400 mg/kgBB (K5). Taking blood samples for measuring SGPT levels after treatment. Then, the rats were destroyed through inhalation technique with carbon dioxide gas (CO2) and determined using an incinerator based on the AVMA Guidelines for the Euthanasia of Animals [17].

2.7. Measurement of SGPT levels

Measurements using the IFCC method using the ASAT reagent. The method of measurement is that 1 ml of blood is taken from the retro-orbital sinus and put in an Eppendorf tube. It takes ASAT reagent with a ratio of 1: 4 to be a monoreagent. Monoreagent taken 1000μL is then mixed with control serum or Trulab-N as much as 100μL, then homogenized and allowed to stand for 1 minute. Followed by measuring the solution with a Rayto 1904c photometer at a wavelength of 340 nm and a temperature of 37°C, after that calculate the difference in absorption per minute (ΔA/min). Then enter into the SGPT range that has been set. In this study, SGPT measurements will be carried out at the Pharmacology Laboratory of Padjadjaran University (UNPAD) [13].

2.8. Data analysis

Data analysis using IBM SPSS application. The analysis begins with a normality test using the Shapiro-Wilk test. This test is used to determine the normality of data if sig > 0.05. Then, Levene's test is used to determine the homogeneity of the variance of the data obtained if sig > 0.05. Next, the One-Way Anova Test will be used to prove that there is a difference in the control group with the treatment. If the One-Way Annova or Kruskal-Wallis tests show a p value <0.05 which indicates a significant difference, then it is continued with Tukey's Post Hoc Test analysis to determine the significance of each group treatment [18].

3. Results and Discussion

3.1. Results of rat plasma SGPT measurements

In assessing the effect of administration of cinnamon ethanol extract from three treatments on increasing plasma SGPT levels caused by isoniazid induction. This can be explained in Table 1.

Table 1 shows an overview of rat plasma SGPT levels from each experimental group. The highest SGPT level was shown by the positive control group (K2) which was induced by isoniazid, which means that this group had liver damage. The lowest SGPT level was shown by treatment group 3 (K5) which was given cinnamon ethanol extract at a dose of 400 mg/kg BW. The results of the descriptive calculations meant that the greater the

TABLE 1: Test results of rat plasma SGPT average levels.

Group	N	Average (U/L) ± sd
K1	5	63.00 ± 5.15
K2	5	85.60 ± 3.98
K3	5	75.40 ± 5.41
K4	5	71.20 ± 4.60
K5	5	61.20 ± 5.45

Description : Shapiro Wilk Test ; p>0.05 (normally distributed)
Levene Statistics ;p>0.05 (homogeneous data)
K1 = Negative Control
K2 = Positive Control
K3 = Administration of cinnamon ethanol extract 100 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K4 = Administration of cinnamon ethanol extract 200 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K5 = Administration of cinnamon ethanol extract 400 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH.

dose of cinnamon ethanol extract, the greater the decrease in plasma SGPT levels in rats induced by isoniazid.

This correlates with the theory that the antioxidant content of cinnamon can inhibit the increase in free radicals or prevent liver damage or necrosis [19,20].

3.2. Effectiveness of cinnamon ethanol extract on isoniazid-induced increases in SGPT levels in male wistar rats

Prior to statistical analysis, for the type of numerical data obtained from the research, a normality test was carried out using the Shapiro Wilk Test to see the distribution of the data. The results of the normality test can be explained in Table 2.

The results of the data normality test using the Shapiro Wilks Test in Table 2 show that rat plasma SGPT levels are normally distributed in all treatment groups (p>0.05). However, to perform data analysis using One Way ANOVA, the data must first be tested for homogeneity of variance. data using levene. After testing the homogeneity of the variance, it turned out that the results obtained were p> 0.05, which means that the data can be stated as homogeneous. Followed by the One Way ANOVA test described in Table 3.

Table 3 shows the results of a comparison of SGPT levels in male rats of the Wistar strain obtained from rat plasma, then analyzed using One Way Anova showing that

Table 2: The normality test results for rat plasma SGPT levels.

Group	Normality Test p*) Value	Homogeneity Test
K1	0.497	0.949
K2	0.911	
K3	0.966	
K4	0.992	
K5	0.671	

Description : Shapiro Wilk Test ; p>0.05 (normally distributed)
Levene Statistics ;p>0.05 (homogeneous data)
K1 = Negative Control
K2 = Positive Control
K3 = Administration of cinnamon ethanol extract 100 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K4 = Administration of cinnamon ethanol extract 200 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K5 = Administration of cinnamon ethanol extract 400 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH

Table 3: The results of the One Way ANOVA test in rat plasma SGPT levels.

Group	Average (U/L) ± SD	P-value*)
K1	63.00 ± 5.15	0.000*
K2	85.60 ± 3.98	
K3	75.40 ± 5.41	
K4	71.20 ± 4.60	
K5	61.20 ± 5.45	

Description : Shapiro Wilk Test ; p>0.05 (normally distributed)
Levene Statistics ;p>0.05 (homogeneous data)
K1 = Negative Control
K2 = Positive Control
K3 = Administration of cinnamon ethanol extract 100 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K4 = Administration of cinnamon ethanol extract 200 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K5 = Administration of cinnamon ethanol extract 400 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH

there was a significant difference in SGPT plasma rat levels in at least two experimental groups (p=0.000).

3.3. Effective dose of ethanol extract of cinnamon (Cinnamommum burmanii) against isoniazid induced SGPT levels of male wistar rats

To find out which treatment group is better so as to produce an effective dose in reducing SGPT levels in rats experiencing hepatotoxicity, further tests will be carried out using the Post Hoc Tukey test. Post Hoc Tukey test results can be seen in Table 4.

TABLE 4: Tukey Post Hoc test results rat plasma SGPT levels.

Group		Post Hoc Tukey Test	
		P-values	Conclusion
K1	K2	0.000	Different meaning
	K3	0.006	Different meaning
	K4	0.104	Meaningless
	K5	0.977	Meaningless
K2	K3	0.029	Different meaning
	K4	0.001	Different meaning
	K5	0.000	Different meaning
K3	K4	0.039	Different meaning
	K5	0.093	Meaningless
K4	K5	0.699	Meaningless

Description: Post Hoc Tukey ;*) $p<0.05$ (there is a significant difference)
K1 = Negative Control
K2 = Positive Control
K3 = Administration of cinnamon ethanol extract 100 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K4 = Administration of cinnamon ethanol extract 200 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K5 = Administration of cinnamon ethanol extract 400 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH

Based on the results of the Post Hoc Tukey Test described in Table 4, it can be seen that the ethanol extract of cinnamon at doses of 100 mg/kgBW (p=0.029), 200 mg/kgBW (p=0.001), and 400 mg/kgBW (p=0.000) proved effective in reducing plasma SGPT levels in isoniazid-induced rats when compared to the positive control (K2) group. The most effective dose was assessed in treatment group 2 (K4), which was 200 mg/kgBW.

4. Conclusion

Based on the results of this study, it can be concluded that administration of cinnamon ethanol extract at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg has a hepatoprotective effect on decreasing SGPT levels in isoniazid-induced male wistar rats. The effective dose of cinnamon ethanol extract as a hepatoprotector against isoniazid-induced Wistar strain rats was 100 mg/kg, 200 mg/kg, and 400 mg/kg. However, the most effective dose was in treatment group 2 (K4), which was 200 mg/kgbb.

Conflict of Interest

There is no conflict of interest in writing this research.

Closing

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Research Article

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The Effect of the Mediterranean Diet Components on Blood Pressure, Mean Arterial Pressure, and Pulse Pressure in Hypertensive Patients

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Abstract.
According to the 2018 Basic Health Research Data (RISKESDAS), the prevalence of hypertension in West Java was 39.6%, while in Cimahi City it was 41.83%. The composition of the Mediterranean diet contains a high proportion of MUFA and PUFA which act as anti-inflammatory, antioxidant, and cardioprotective with a potential to lower blood pressure. The aim of this study is to determine the impact of Mediterranean diet components on blood pressure in patients with hypertension. The study uses observational analytic methods as the design and also employs a prospective cohort sample of 36 patients with hypertension for three months, using a systematic random sampling technique. The research subjects were checked for blood pressure in the first month (T1) and the last month of the study (T2). The relative Mediterranean Diet (rMED) was used as a source of data on component intakes by administering the Food Frequency Questionnaire (FFQ) 12 times within three months. The differences in Mediterranean diet composition and blood pressure in all Mediterranean diet adherence groups were examined using the ANOVA test and Tuckey's post-hoc test, as well as to test comparisons of proportions between rMED adherence groups. The impact of each component of the Mediterranean Diet on blood pressure was evaluated using a linear regression analysis test. The results showed that the average age of hypertensive patients who underwent the Mediterranean diet was 57.94 years and 69.44% were female. The components of fruit and nuts, vegetables, and fish have a significant effect on systolic blood pressure. Fish components have a significant effect on diastolic blood pressure. The components of vegetables and fish have a significant effect on the mean arterial pressure (MAP). Fruit and nut components have a significant effect on pulse pressure. The limitations of this study are the short research time, the absence of salt consumption restrictions on the Mediterranean diet, and the presence of smoking and physical activity as confounding activities.

Keywords: blood pressure, composition, Mediterranean diet, mean arterial pressure, pulse pressure

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1. Introduction

Hypertension (high blood pressure) is an elevation in blood pressure in the arteries. Hypertension is diagnosed if the systolic blood pressure have reach 140 mmHg and/or diastolic blood pressure have reach 90 mmHg or more during a blood pressure check at a health facility [1–3]. The 2018 Basic Health Research (RISKESDAS) revealed that the prevalence of hypertension in West Java was 39.6%, while in Cimahi City was 41.83%. In these measurements, it was found that the prevalence of people with hypertension differed in each age group of the population, with each percentage of hypertension being 18 years old (34.1%), 31-44 years old. (31.6%), aged 45-54 years (45.3%), and aged 55- 64 years (55.2%). The data concludes that the percentage of hypertension prevalence is higher with increasing age [4,5].

Over the past decades, the Mediterranean diet has been shown to have cardiovascular protection. This is due to lower rates of atherosclerosis in people who adopt a Mediterranean diet. Through several trials, it has been shown that the Mediterranean diet reduces the incidence of metabolic syndrome, obesity, and type 2 diabetes mellitus which counts as cardiovascular disease risk factors [6,7]. The difference and advantages of the Mediterranean Diet compared to DASH is how the Mediterranean Diet is also various other beneficial effects in preventing hypertension and lowering blood pressure both directly and indirectly, and not only focused on a low sodium diet, but there are also various other beneficial [7–9].

The food components on the Mediterranean diet include having antioxidant potential contained in the Mediterranean diet have beneficial effects of improve blood pressure in hypertension.

Olive oil that has been known contains MUFA (monounsaturated fatty acids) and PUFA (polyunsaturated fatty acids) can act as an anti-inflammatory. Olive oil which contained in the Mediterranean diet can improve systolic blood pressure and diastolic blood pressure in hypertension [6,7].

Research on the impact of each component contained in the Mediterranean diet on blood pressure has so far not been carried out in Indonesia, based on data from various literature and research, many beneficial effects that can be utilized from the Mediterranean diet. On the other hand, until now the prevalence of hypertension continues to rise and remains as the major risk factors for other cardiovascular diseases which has a fairly high mortality. Therefore, by seeing the opportunities that can be utilized from the various components contained in the Mediterranean diet for hypertension,

the researchers are interested in conducting research on the effect on blood pressure, MAP, and pulse pressure in hypertensive patients, by consuming the composition of the Mediterranean diet.

2. Methods

The method employed in this research is an analytic observation design in the form of a prospective cohort. The data were collected from patients with chronic disease management program at a clinic in the city of Bandung 12 times for three months who filled out the Mediterranean diet questionnaire using the Food Frequency Questionnaire (FFQ).

Examination using a digital sphygmomanometer twice, namely in the first month of the study (T1) and in the third month of the study (T2) was used to check blood pressure, pulse pressure, and mean arterial pressure (MAP). FFQ data were presented in the form of rMED, then an ANOVA test was performed to determine the variations in the amount of each composition of the Mediterranean diet at each level of adherence, then a Tuckey posthoc test was carried out to determine the different adherence groups to each component of the Mediterranean diet. Then for each of these components, a linear regression test was carried out on all the variables of this study including blood pressure, mean arterial pressure (MAP), and pulse pressure. The data from the linear regression can then be used as a reference to see which components have the most influence on each of the variables in this study.

The study population consisted of hypertensive patients, both men and women, that aged ≥40 years, who received education regarding the application of the Mediterranean diet from the clinic and joined the Chronic Disease Management program at a clinic in the city of Bandung. The inclusion criteria in this study were Hypertensive patients that have ≥140 mmHg systolic blood pressure and/or ≥90 mmHg diastolic blood pressure, also hypertensive patients who are on regular treatment and are willing to become research subjects. This study excluded subjects who unwilling to follow all research procedures.

In this study, the composition of the Mediterranean diet was an independent variables, and systolic blood pressure, diastolic blood pressure, and mean arterial pressure (MAP) and pulse pressure was an dependent variables.

3. Results

The characteristics of hypertensive patients based on age are summarized in Table 1 below. Table 1 shows mean age of 57.94 years with median of 58.50 years and standard deviation of 10.84 years. In this study, the youngest and youngest hypertensive patients in this study were 30 years, whereas the oldest patients was 86 years.

TABLE 1: Characteristics of hypertensive patients based on age.

Variable	Mean	Median	SD	Min- max	95% CI
Age	57,94	58,50	10,84	30 – 86	54,27-61-61

Characteristics of hypertensive patients based on gender was shown in Table 2, it was found that the majority of hypertensive patients who underwent the Mediterranean diet in this study were female, namely 25 people (69.44%) and as many as 11 people (30.56%) were diabetic. male genital.

TABLE 2: Characteristics of hypertensive patients by sex.

Sex	N	Percentage
Male	11	30.56
Female	25	69.44
Total	36	100.00

Characteristics of hypertensive patients based on blood pressure, mean arterial pressure (MAP), and pulse pressure showed that the systolic blood pressure of hypertensive subjects who followed the Mediterranean diet had an average value of 135.75 mmHg with a median of 136 mmHg and a standard deviation of 15.73 mmHg. The diastolic blood pressure of hypertensive subjects who followed the Mediterranean diet had an average value of 85.97 mmHg with a median of 86.50 mmHg and a standard deviation of 8.57 mmHg. The mean arterial pressure (MAP) of hypertensive subjects who followed the Mediterranean diet had an average value of 102.48 mmHg with a median of 104.15 mmHg and a standard deviation of 9.99 mmHg. Pulse pressure of hypertensive subjects who followed the Mediterranean diet had a mean value of 0.502 mmHg with a median of 51 mmHg and a standard deviation of 12.45 mmHg.

3.1. Distribution of consumption of food components based on rMED compliance group

The distribution of consumption of food components can be seen in Table which shows that the highest average consumption of fruit and nut components was found in the hypertensive patient group with high adherence to the Mediterranean diet, namely 4030.33 grams. The highest average consumption of vegetable components was found in the hypertensive patient group with adherence to the high Mediterranean diet, namely 4406.83 grams. The highest average consumption of legume components was found in the hypertensive patient group with high adherence to the Mediterranean diet, namely 659.67 grams. The highest average consumption of whole grain components was found in the hypertensive patient group with high adherence to the Mediterranean diet, namely 473.17 grams. The highest average consumption of fish components was found in the hypertensive patient group with moderate adherence to the Mediterranean diet, namely 472.50 grams. The highest average consumption of olive oil components was found in the group of hypertensive subjects with high obedience to the Mediterranean diet, namely 28.83 grams. The highest average consumption of meat components was found in the hypertensive patient group with moderate adherence to the Mediterranean diet, namely 442.50 grams, and the highest average consumption of product components was found in the hypertensive patient group with high obedience to the Mediterranean diet, namely 375 grams with a standard deviation of 23 grams.

The distribution of consumption of food components presented in Table 3 also states that there are significant differences between the Mediterranean diet adherence groups in the amount of fruit and nut component consumption, and also vegetables, legumes, whole grains, fish and meat consumption. The relationship between the Mediterranean diet adherence group in the amount of consumption of components of olive oil and dairy products was not significant.

3.2. Effects of mediterranean diet food components on systolic blood pressure, diastolic blood pressure, mean arterial pressure, and pulse pressure

The effect of the Mediterranean diet food components on systolic blood pressure, diastolic blood pressure, mean arterial pressure, and pulse pressure can be seen in Table 3 below. Table 3 shows that the effect between the components of fruit and nuts,

vegetables, fish on systolic blood pressure is significant. The effect between the fish component on diastolic blood pressure is also significant. The effect of components of vegetables and fish on the mean arterial pressure (MAP) is significant. It also has been stated that the effect between fruit and nut components on pulse pressure is significant.

TABLE 3: Effects of mediterranean diet food components on systolic blood pressure, diastolic blood pressure, Mean Arterial Pressure (MAP), and pulse pressure.

	p-value			
	Systolic BP	**Diastolic BP**	**MAP**	**Pulse Pressure**
Fruit & Nuts	0,037	0,762	0,241	0,009
Vegetables	0,03	0,059	0,03	0,063
Legumes	0,952	0,441	0,711	0,594
Grains	0,403	0,133	0,211	0,864
Fish	0,037	0,017	0,018	0,536
Olive Oil	0,189	0,422	0,425	0,670
Meat	0,495	0,067	0,094	0,670
Diary product	0,344	0,187	0,226	0,648

4. Discussion

The effect of age on hypertension is thought to be through several mechanisms including inflammation, oxidative stress, and endothelial dysfunction that occur due to aging. In addition, changes in the nature of arteries in the aging process can lead to atherosclerosis which can lead to elevated blood pressure and become risk of cardiovascular disease [10–13]. Other mechanisms that are also thought to be related, namely chronic inflammation which increases with increasing age are thought to have a close relationship with increased activity. Sympathetic co-morbid diseases that are common in old age such as obesity and autoimmune diseases also have a role on the process in increasing the risk of developing hypertension [13,14].

Gender differences are mainly related to the occurrence of hypertension, which is influenced by interactions between various hormones, in addition there are also different lifestyle as risk factors between men and women including physical activity, smoking, also body mass index which result in complex factors that affect related to gender other than biological factors. Basically, this difference is based on the two most influential hormones, namely androgen in males and estrogen in females. Androgen hormones are able to increase sympathetic activity which can increase levels of angiotensin II and

aldosterone in the body which causes an increase in renal vasoconstriction and sodium reabsorption so that it occurs increased blood pressure [15,16].

In the Mediterranean diet, fruits have rich flavonoids as antioxidants which can prevent endothelial dysfunction, and besides that, water-soluble dietary fiber such as fructans or inulin reduces the absorption of cholesterol and bile acids in the small intestine, resulting in increased absorption of LDL C by the liveras, and the effect is lowering blood pressure. High intake of fruits have an important role in lowering cholesterol levels by competing with intestinal cholesterol absorption, thus reducing atherosclerosis which causes a decrease in blood pressure, as the effect of phytosterols contained in the fruits [17,18].

Nuts have many benefits in lowering blood pressure, including having a role in reducing levels of the ET-1 vasoconstrictor which act as an important role in the endothelial dysfunction occurrence, besides that the unsaturated fatty acids contained in nuts act as anti-inflammatory. . Nuts are a component of the Mediterranean diet as an important source of tocopherols and phenolic compounds which act as antioxidants, so that the anti-inflammatory and anti-oxidative roles present in nuts can play a role in inhibiting atherosclerosis [19–21].

Phenolic compounds in the components of the Mediterranean was sourced from the vegetables, especially phenolics from the flavonoid group, that have a role as antioxidants that can prevent endothelial dysfunction. In addition, vegetables also contain phytosterols which can compete with the absorption of cholesterol in the intestine and reduce cholesterol levels [17,22,23]

There was no significant effect between the components of the leguminous diet on systolic blood pressure, diastolic blood pressure, mean arterial pressure (MAP), and pulse pressure. This is due to the consumption of the Mediterranean diet with a size below the recommended consumption of legumes per day according to research conducted by Perera, T in 2020, namely 37.5–87.49 g/day, so this results in no effect between the consumption of legumes and blood pressure [24].

The effect between the components of whole grain foods on systolic blood pressure, diastolic blood pressure, mean arterial pressure, and pulse pressure is not significant. This is possible because the content of biologically active compounds in grain components is lost during processing [17,24].

Fish components have an effect on reducing blood pressure by inhibiting atherosclerosis through their rich content of omega-3 which can act as an anti-inflammatory to

prevent endothelial dysfunction, besides that omega-3 also act in lowering LDL levels in the body, so all of these mechanisms can prevent the occurrence atherosclerosis which can indirectly reduce peripheral resistance in the arteries with the impact of a decrease in blood pressure [17,19].

There is no significant effect between the food components of olive oil on systolic blood pressure, diastolic blood pressure, mean arterial pressure (MAP), and pulse pressure. this is possibly due to the linolenic acid content contained in the olive oil, especially in the type of Olive Pomace Oil which is the cheapest and easy to obtain olive oil. The linolenic acid compound is thought to be able to reduce eNOS expression which then causes a decrease in NO levels in the body, this causes a tendency for blood vessels to vasoconstrict, resulting in an increase in peripheral resistance which leads to an increase in blood pressure [17,25].

There was no significant effect between the components of the meat diet on systolic blood pressure, diastolic blood pressure, mean arterial pressure (MAP), and pulse pressure. This is caused by 2 main mechanisms including sodium levels in meat which can cause fluid retention in the body, thus causing an increase in preload. there was no significant effect between the components of the meat diet on systolic blood pressure, diastolic blood pressure, mean arterial pressure (MAP), and pulse pressure. This is caused by 2 main mechanisms including sodium levels in meat which can cause fluid retention in the body, thus causing an increase in preload. Carnitine content can increase peripheral resistance due to blood vessel proliferation triggered by oxidative stress for a long time, causing an increase in blood pressure [26–28].

There is no significant effect between the components of dairy products on systolic blood pressure, diastolic blood pressure, mean arterial pressure (MAP), and pulse pressure. This is related to the relationship between the components of milk with blood pressure, presumably this difference mainly occurs between low-fat and milk whole fat milk, according to several studies it is said that whole-fat milk is able to increase levels of saturated fatty acids in the body so that it will cause an increase in atherosclerotic plaques which is the main pathophysiological basis of hypertension through the mechanism of increasing peripheral resistance [26,27].

5. Conclusion

According to the results of the study above, we can conclude that hypertensive patients who followed the Mediterranean diet had an average age of 57.94 years and 69.44% were female. The effect between the components of fruit and nuts, vegetables, fish on systolic blood pressure is significant. The effect between fish components on diastolic blood pressure is also significant. There is a significant effect between the components of vegetables and fish on the mean arterial pressure (MAP). This study also conclude that the effect between fruit and nut components on pulse pressure is significant. It is better if the research is carried out for more than 3 months, this aims to see the consistency of the impact of the Mediterranean diet components on lowering blood pressure so the effect can be seen even more clearly. factors of physical activity and smoking in the sample, so as not to be a confounding factor in the study.

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Research Article

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The Relationship Between Work Position Risk and the Level of Musculoskeletal Disorders in Private Limited Company Personnel

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Abstract.
Musculoskeletal disorders (MSDs) are abnormalities that affect the musculoskeletalsystem, triggered by various risk factors in the work environment. Commonly, MSDs occur due to non-ergonomic working conditions. It arises from chronic exposure to risk factors that can cause injury to the musculoskeletalsystem, this condition will impact on the social and economic aspects of employees. This research was conducted to determine the characteristics of employees, description of the region and the level of musculoskeletal complaints experienced, risk of work positions based on RULA assessment, and the relationship between risk of work positions and the level of musculoskeletal complaints with an analytic cross-sectional design. The study sample was 33 employees of the Engineering Section of private limited company personnel. The research instrument used was a questionnaire. The results show that the majority of the Engineering Department employees at private limited company personnel were 23 years old (36.4%) and had worked for two to three years (33.3%). As many as 48.5% of employees experienced moderate levels of musculoskeletal complaints. Of the 33 respondents, the majority of employees (63.6%) had a low-risk position, nine people (27.3%) had moderate risk, and three (9.1%) had high risk. There is a significant relationship between the risk of work position and the level of musculoskeletal complaints ($P < 0.05$). There is a significant relationship between the risk of working position and the level of musculoskeletal complaints in personnel of limited liability companies.

Keywords: employee health, musculoskeletal disorders (MSDs), level of occupational risk

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1. Introduction

Employee health problems have existed since the industrial revolution in the 18th century along with the development of the industry. One of the diseases that arise as a result of work is musculoskeletal disorders [1]. Musculoskeletal disorders (MSDs) occur as a result of work and workplace conditions that are not ergonomic [1]. MSDs are defined as disorders that affect the normal functioning of the musculoskeletal system due to repeated exposure to various risk factors in the workplace. MSDs occur as a result of the accumulation of injuries that occur continuously in the musculoskeletal system over a long period of time, also causing social problems such as increased compensation for health costs, decreased productivity, and low quality of life for workers [2]. In the 2015 Rovanaya study aimed at workers welding unit private limited company personnel. states that work postures and work positions that are not ergonomic have an impact on decreasing work productivity and work performance which can affect the health of workers with marked emergence of musculoskeletal complaints. In the results of this study there were 15 people (68%) who had a moderate risk of working positions and experienced moderate musculoskeletal complaints. This proves that non-ergonomic work positions can affect the level of musculoskeletal complaints in workers [3].

Prevalence of MSDs in 2018, based on the age of the worker, the lowest was 15 years old 1.2% and the highest was aged over 60 years 18.9% based on the type of work are farmers 9.9%, fishermen 7.40%, and laborers or housemaids 6.10% [4]. Based on Rachman's research in 2019 it stated that working period under 6 years has the most distribution, namely 25 respondents with a percentage (41.7%) and the least, namely 6-10 years of service as many as 14 respondents with a percentage (23.0%) [5]. The level of complaints assessed starts from discomfort (slight pain), pain to very pain. The NBM method uses a worksheet in the form of a body map. This body map includes twenty seven muscle regions in the human body. Interpretation of the level of complaints of MSDs is based on the total score obtained, and can be categorized into three levels of complaints, namely low, medium, high and very high. Static positions for long periods of time at work are thought to be vulnerable to the onset of MSDs in personnel. Therefore, the purpose of this study was to determine the relationship between the risk of work positions and the level of musculoskeletal disorders in private limited company personnel.

2. Materials and Methods

This study used an analytic method with a cross-sectional design, to see the relationship between the risk of work positions and musculoskeletal complaints in Engineering employees at private limited company personnel. The research population is 50 people who are employees of the Engineering division of private limited company. The research sample was taken through simple random sampling technique and obtained as many as 33 employees were measured age, years of service, level of work position risk and level of musculoskeletal complaints. Samples study were calculated using a two-proportion hypothesis test formula and formula according to the type of research, namely cross-sectional. The dependent variable in the study is the level of musculoskeletal disorders while the independent variable is the level of work position. The data used in this study are primary data using questionnaires that have previously been tested for validity and reliability before being distributed. Some of the components contained in the questionnaire are: respondent characteristic data instrument, RULA instrument for measuring human body posture, Nordic Body MAP (NBM) questionnaire instrument. Research data analysis used univariate analysis and bivariate analysis using the Statistical Product and Service Solution (SPSS) program for windows version 26.

3. Results and Discussion

The results of the research come from employees of the Engineering section of private limited company personnel totaled 33 people who met the inclusion criteria with a working duration of 6-8 hours/day.

The indicators observed by the researchers were age, years of service, level of risk in work positions and level of musculoskeletal complaints. This study used univariate analysis with the aim of knowing the characteristics of the respondents consisting of age, years of service, work position and musculoskeletal complaints, as well as bivariate analysis to examine the relationship between the level of risk in work positions and the level of musculoskeletal complaints by using the Chi-square statistical test. Univariate analysis of respondents characteristics based on age and years of service is shown in the following Table 1.

The results of the analysis in Table 1 show that the employees of the Engineering section of private limited company personnel based on age, most of them are 23 years old, namely 12 people (36.4%) and based on years of service, most of them work

TABLE 1: Frequency distribution of respondent characteristics.

Characteristic	Frequency (N)	Percentage
Age		
20 years	1	3.0
21 years	2	6.1
22 years	9	27.3
23 years	12	36.4
24 years	4	12.1
25 years	2	6.1
26 years	3	9.1
Work Periode		
1-2 years	8	24.2
2-3 years	11	33.3
3-4 years	8	24.2
4-5 years	4	12.1
>5 years	2	6.1
1-2 years	8	24.2

between 2-3 years as many as 11 people (33.3%). Based on the research results, it can be seen that the Engineering staff at private limited company personnel with an age range of 20-26 years, and most were 23 years old (36.4%). This result is in line with Rovanaya's 2015 study which stated that the majority of respondents were less than 35 years old with an age range between 20 years and 34 years [3]. The results showed that the employees of the Engineering section of private limited company personnel mostly worked between 2-3 years (33.3%). This result is in line with Tiara Devi T's research in 2017 which stated that a rice transport company had a working period of under 5 years [6].

TABLE 2: Frequency distribution of work position risk level.

Level of Risk	Frequency (N)	Percentage
Low	21	63.6
Medium	9	27.3
High	3	9.1
Total	**33**	**100.0**

The results of data analysis in Table 2 regarding the risk level of work positions show that most of the Engineering Department employees at private limited company personnel is at the low risk level criteria, namely 21 people (63.6%). Evaluation of the

respondent's posture in the assessment of work position using the Rapid Upper Limb Assessment method is a method that identifies the risk of posture, especially in the musculoskeletal system.

Table 3: Distribution of work position risk level based on length of service.

Variable	RULA						
Working Period	Low		Medium		High		Total
	N	%	N	%	N	%	
1-2 Years	7	87.5	1	12.5	0	0.0	8
2-3 years	7	63.6	3	27.3	1	9.1	11
3-4 years	2	25.0	4	50.0	2	25.0	8
4-5 years	3	75.0	1	25.0	0	0.0	4
>5 years	2	100.0	0	0.0	0	0.0	2
Total	21	63.6	9	27.3	3	9.1	33

The results of the analysis based on Table 3 can be seen based on a working period of 3-4 years on employees of the Engineering Section of PT X with a high level of risk of work position there are 2 people (25%) out of 8 people, 4 people (50%) with a medium risk level and 2 people (25%) others have a low risk level. In the results of the analysis using the RULA worksheet for workers with a working period of 3-4 years, workers have a high risk level because the workstation is not ergonomic, has no support for the feet, hands, neck. The cause of unergonomic body posture often comes from individuals and work tools and work platforms that are not suitable. employees do their work with raised hands, backs and necks that are too bent, heads raised so that, the further the position of body gravity, the greater the risk of MSDs complaints [7].

Table 4: Frequency distribution of musculoskeletal complaint levels.

Level of Complaints	Frequency (N)	Percentage (%)
Low	13	39.4
Medium	16	48.5
High	4	12.1
Total	**33**	**100.0**

The results of data analysis in Table 4 show that the level of musculoskeletal complaints of employees of the Engineering section of PT X is mostly in the criteria for moderate levels of complaints, namely as many as 16 people (48.5%). The results of this study indicate that the level of musculoskeletal complaints in engineering employees of PT X is mostly in the criteria for moderate levels of complaints, namely as many as

16 people (48.5%), 13 people (39.4%) low levels of complaints and 4 people (12.1%) high levels of complaints. Musculoskeletal complaints are complaints that are in the skeletal muscles or skeletal muscles felt by someone ranging from very mild to very painful complaints, but most often are moderate. These results are in line with Rovanaya's research in 2015 which states that the results of research on workers at the Welding Unit of PT A Bekasi regarding musculoskeletal complaints can be seen that the majority of respondents have moderate musculoskeletal complaints [3].

Bivariate analysis using the chi square test to determine the relationship between the level of risk of work position and the level of musculoskeletal complaints in Engineering Employees of PT X.

Table 5: Relationship between the level of risk of work position and the level of musculoskeletal complaints.

		NBM								
No.	**Level of Risk**	**Low**		**Medium**		**High**		**Total**		***P-value**
		N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	
1	Low	8	38.10	11	52.38	2	9.52	21	100.00	0.031
2	Medium	5	55.56	4	44.44	0	0.00	9	100.00	
3	High	0	0.00	1	33.33	2	66.67	3	100.00	
Total		**13**	**39.39**	**16**	**48.48**	**4**	**12.12**	**33**	**100.00**	

The results of the study in Table 5 show that of the 21 respondents with a low risk level, most had a moderate level of musculoskeletal complaints as many as 11 people (52.38%), of the 9 respondents with a medium risk level, most had a low level of musculoskeletal complaints as many as 5 people (55.56%) and of the 3 respondents with a high risk level, most had a high level of musculoskeletal complaints as many as 3 people (66.67%). The results of the study obtained a p value = 0.031 ($p < 0.05$) which means that there is a significant relationship between the level of risk of work position and the level of musculoskeletal complaints in engineering workers of private limited company personnel.

This is in accordance with the results of research from Ayu and Ratriwardhani there is a noteworthy correlation between job position and the occurrence of Musculoskeletal Disorders (MSDs) among workers in the Kerupuk Industry sector. This arises from inadequacies in work equipment, leading to an impact on the ergonomics of work

positions for employees, consequently influencing musculoskeletal issues. It is recognized that reducing biomechanical workloads increases the likelihood of recovering from musculoskeletal symptoms [8,9].

4. Conclusion

Based on the results of research conducted by researchers, it can be concluded that based on the research obtained results that most employees of the Engineering section of private limited company personnel, have an age of 23 years, namely as many as 36.4% (12 people) with a working period of 2-3 years, namely as many as 33.3% (11 people). The majority of musculoskeletal complaints are at the level of moderate complaints, namely as many as 48.5% (16 people). The risk level of the work position of employees in the Engineering section of PT X is mostly at a low risk level criteria as many as 21 people (63.6%) of 33 respondents, 9 people (27.3%) have medium risk level criteria, while 3 people (9.1%) others have a high risk level. In this study, there was also a significant relationship between the risk of work position and the level of musculoskeletal complaints in engineering employees of PT X with a value of ($p=0.03<0.05$).

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Research Article

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Effect of Katuk Leaves Ethanol Extract Gel As an Antibacterial and Antifungal in Orthodontic Treatment

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Abstract.
Fixed orthodontic treatment is one option for treating malocclusion. The intricate design makes it difficult for patients to clean their teeth and can cause the emergence of microorganisms in the oral cavity. Katuk plant (Sauropus androgynus (L.) Merr.) is commonly used as herbal medicine. Flavonoids in katuk leaves have antimicrobial properties. The purpose of this study was to determine the effect of katuk leaf ethanol extract gel as an antimicrobial by inhibiting the growth of bacteria and fungi involved in orthodontic treatment. The study was an experimental study with a post-test-only control group using katuk leaves ethanol extract gel formulation as treatment, aquadest gel as a negative control, ketoconazole as a positive control against Candida albicans, and amoxicillin against Streptococcus mutans. Data were analyzed using the Kruskall–Wallis and Mann–Whitney tests ($P < 0.05$). The results show that katuk leaves have inhibition on Streptococcus mutans ($P = 0.000$) growth but have no inhibition on Candida albicans ($P = 1.00$) growth. The conclusion of this study is that the gel of katuk leaves ethanol extract (S. androgynus (L.) merr.) has antibacterial activity against S. mutans in the oral cavity but no antifungal effect on C. albicans.

Keywords: Candida albicans, fixed orthodontics, katuk leaves, Streptococcus mutans

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1. Introduction

Malocclusion is a condition in which the relationship of a tooth to another tooth deviates from the normal relationship [1]. The goal of orthodontic treatment is to improve oral function, but, orthodontic treatment still has a negative impact [2]. The oral cavity is in direct contact with fixed orthodontic appliances such as power chains or elastomer chains, brackets, bands, and wires, which are connected together, and the complex design can cause the emergence of microorganisms in the oral cavity because the patients is difficult to clean their teeth [3].

In the oral cavity, bacteria classified as normal flora were found, including Streptococcus mutans and Candida albicans. Normal flora, under certain circumstances, can be a problem [4]. This can occur when the amount of normal flora exceeds the normal amount or when the individual's immune system is unable to prevent normal flora from growing excessively. The most common problem caused by microorganisms in the oral cavity is canker sores, dental caries, and candida fungal infections [5,6].

Saliva contains protein, which will cause a selective protein absorption process that will then produce a pellicle layer. Streptococcus mutans is a bacterium that has a major role in causing dental caries by live in colonies encountered within the first 4 hours of the biofilm and can increase in the presence of carbohydrates. Acid causes tooth demineralization, and carbohydrates are substrates that are fermented by bacteria so that they get energy. If carbohydrates are consumed in excess accompanied by poor oral hygiene, it can have the potential for caries, especially in fixed orthodontic users. So, it is recommended that patients who are undergoing orthodontic treatment maintain healthy teeth to reduce bacteria in the oral cavity [7].

Candida albicans is a normal flora of the human oral cavity. If fixed orthodontic users don't pay attention to oral hygiene, it can cause candidiasis. In a study conducted by Grzegocka et al., 10 out of 17 (59%) patients who used fixed orthodontic treatment experienced an increase in the growth of Candida albicans fungus after orthodontic use [8].

Since ancient times, Indonesian people have used plants as ingredients for traditional medicine. Katuk leaves (Sauropus androgynus (L.) Merr) are potential alternative plants in traditional medicine because they have active compounds that include carbohydrates, triterpenoids, proteins, glycosides, saponins, tannins, flavonoids, steroids, and alkaloids. Flavonoids are often researched and believed to have antimicrobial activity [9]. One of the modern products is a gel preparation made from medicinal plant ingredients. Gels are preparations with a semi-solid consistency consisting of small and large molecules made into a kind of jelly with a gelling agent. The advantages of gel preparations are that they are non-sticky, have little forming material, and have no change in viscosity at storage temperature. Selection in gel dosage form has good potential as a topical medicinal ingredient compared to ointments due to the non-adhesive nature of the gel, a stable formulation, and better aesthetics [10].

Based on the description above, this study aims to determine the effect of katuk leaf ethanol extract gel (Sauropus androgynus (L.) Merr.) as an antimicrobal by inhibiting the

growth of Candida albicans and Streptococcus mutans bacteria involved in orthodontic treatment.

2. Experimental Details

2.1. Materials and procedures

There were two research subjects used in this study: Candida albicans, cultured in Saboraud Dextrose Agar (SDA) media, and Streptococcus mutans, cultured in Mueller Hinton Agar (MHA) media. The number of SDAs used was 8 each for 32 samples using the Federer formula. Each petri dish is filled with four samples.The object of this study was katuk leaves obtained from the Cisarua Lembang garden and made into a gel. The place to do the research is the Biochemistry Laboratory and Microbiology Laboratory, Faculty of Medicine, Jenderal Achmad Yani University, Cimahi. The materials used in this study include materials for making katuk leaf extract, including 70% ethanol and katuk leaves. The ingredients for making katuk leaf ethanol extract gel include carbopol, TEA, and distilled water. Ingredients for rejuvenation and treatment of fungi and bacteria are Candida albicans, Streptococcus mutans bacteria, Saboraud Dextrose Agar (SDA), Mueller Hinton Agar (MHA), ketoconazole, distilled water, aquades gel base, KOH, and katuk leaf ethanol extract gel. The katuk leaves used are green katuk leaves taken from the Manoko Experimental Garden, Lembang, West Java. Katuk leaf extract with ethanol solvent obtained by the maceration method.

2.2. The preparation for making katuk leaf ethanol extract gel

As much as 100 grammes of katuk leaf simplicia powder are put in a glass container, soaked in 1 litre of 70% ethanol solution, covered with aluminium foil, and left for 5 days with occasional stirring. After 5 days, the soaked samples were filtered through filter paper. Then the solvent evaporation was carried out with a rotary evaporator to obtain a thick extract. The preparation of gel preparations with a carbopol base is carried out by heating water to a temperature of 70–80 °C. The hot water used is stirred with the carbopol until it expands and a gel is formed for approximately 30 minutes, then TEA is added to the gel base and can be stirred until the gel base thickens and is homogeneous. Then the extract can be added with propylene glycol and methyl

paraben so that it dissolves. The dissolved extract can be transferred to the base and stirred until it is homogeneous.

2.3. Inhibition test of katuk leaf ethanol extract gel

First, the tools and materials were sterilised in an autoclave. Prepare 4 petri dishes given MHA added with 2 µL of Streptococcus mutans suspension and 4 more pieces poured with 2 ml of SDA each and added with 2 µL of Candida albicans suspension. The method used in this research is the well method, which involves making 4 well holes in each agar medium. After the holes were formed, the gel preparations of ethanol extract of katuk leaves, gel base, and amoxicillin were put in the wells and then observed for 4 days. After that, the clear zone was measured using a vernier calliper around the well. In the measurement, the outer zone is used from around the well to the outer boundary of the clear zone to determine its size.

2.4. Data analysis

Analysis of the test results was carried out by SPSS calculations using the normality test with the Shapiro-Wilk test. The Kruskall-Wallis test if the data is not normally distributed ($p<0.05$) and the Mann Whitney test if the data is not normally distributed ($p<0.05$) aim to find out whether there is effectiveness of katuk leaf ethanol extract gel from within in inhibiting bacterial growth in orthodontic treatment.

2.5. Research ethics

This research has risks for researchers, but these risks can be overcome by the use of Personal Protective Equipment (PPE). The PPE used in this study included a laboratory coat, gloves, and closed shoes, and masks to protect researchers from fungus contamination. The research was carried out after obtaining approval from the Research Ethics Commission (REC) of Jenderal Achmad Yani University (Number 029/UH3.12/2022 and 009/UH3.01/2023). Approval was obtained after the researchers sent the files and thesis proposals to the Ethics Commission.

3. Results and Discussion

3.1. Results

3.1.1. Streptococcus mutans

This study was conducted to determine the effect of ethanol extract gel of katuk leaves (Sauropus androgynus (L.) Merr.) as an antibacterial for Streptococcus mutans in orthodontic treatment.

TABLE 1: Test the diameter of the clear zone for each group Streptococcus mutans bacteria.

Group	Means	p.s	Interpretation
Day 1			
Group 1 (-)	0.00	0.000	There is a difference
Group 2 (test)	8.94		
Group 3 (+)	30.49		
Day 2			
Group 1 (-)	0.00	0.000	There is a difference
Group 2 (test)	8.89		
Group 3 (+)	31.70		
Day 3			
Group 1 (-)	0.00	0.000	There is a difference
Group 2 (test)	8.54		
Group 3 (+)	30.27		
Day 4			
Group 1 (-)	0.00	0.000	There is a difference
Group 2 (test)	8.25		
Group 3 (+)	30.96		

**) Kruskall Wallis, $p \leq 0.05$ (There is a significant difference).

The results of the research show that the calculation of the 1st, 2nd, 3rd, and 4th days shows a significant difference between each treatment group.

To find out more about the comparison between groups, further tests were carried out, namely pairwise comparisons, and the results can be seen in Table 2.

In the comparison test between groups on day 1, the results showed that there were significant differences between Group 1 (-), group 2 (test), and group 3 (+). But, there was no significant difference between group 2 (test) and group 3 (+).

TABLE 2: Comparison test of clear zone diameter on day 1 Streptococcus mutans bacteria.

Comparison between groups	P-Value	Interpretation
Group 1 (-) with group 2 (test)	0.000	There is a difference
Group 1 (-) with group 3 (+)	0.000	There is a difference
Group 2 (test) to group 3 (+)	0.102	No difference

Mann-Whitney test, $p<0.05$ (there is a significant difference).

TABLE 3: Comparison test of the diameter of the clear zone on the second day Streptococcus mutans bacteria.

Comparison between groups	P-Value	Interpretation
Group 1 (-) with group 2 (test)	0.000	There is a difference
Group 1 (-) with group 3 (+)	0.000	There is a difference
Group 2 (test) to group 3+	0.102	No difference

* Mann Whitney test , $p<0.05$ (there is a significant difference)

In the comparison test between groups on day 2, the results showed that there were significant differences between groups. Group 1 (-), group 2 (test), and group 3 (+) There was no significant difference between group 2 (test) and group 3 (+).

TABLE 4: Comparison test of clear zone diameter on day 3 Streptococcus mutans bacteria.

Comparison between groups	P-Value	Interpretation
Group 1 (-) with group 2 (test)	0.000	There is a difference
Group 1 (-) with group 3 (+)	0.000	There is a difference
Group 2 (test) to group 3 (+)	0.102	No difference

Mann Whitney test, $p<0.05$ (there is a significant difference)

In the comparison test between groups on day 3, the results showed that there were significant differences between groups. Group 1 (-), group 2 (test), and group 3 (+) There was no significant difference between group 2 (test) and group 3 (+).

TABLE 5: Comparison test of the diameter of the clear zone on the 4th day Streptococcus mutans bacteria.

Comparison between groups	P-Value	Interpretation
Group 1 (-) with group 2 (test)	0.000	There is a difference
Group 1 (-) with group 3 (+)	0.000	There is a difference
Group 2 (test) to group 3 (+)	0.102	No difference

*Mann Whitney test , $p<0.05$ (there is a significant difference)

Prior to statistical analysis, a normality test was performed using the Shapiro-Wilk test and a data homogeneity test with the Levene test. The results of the normality test

Figure 1: V +Source: Personal documentation. (a) Katuk leaf ethanol extract gel; (b) Base gel; (c) Amoxicillin.

showed that the p-value could not be obtained because the results of the study on all samples in the treatment and negative control groups showed the number 0.

In this study, it was continued with the Kruskall-Wallis test to determine the differences between the three groups.

Hypothesis:

Ho: a = 0 (antibacterial inhibition between the two groups showed no difference on days 1, 2, 3, and 4).

Ha: a 0 (the antifungal inhibition between the two groups was different on days 1, 2, 3, and 4).

3.1.2. Candida albicans

In the comparison test between groups on day 4, the results showed that there were significant differences between groups. Group 1 (-), group 2 (test), and group 3 (+) There was no significant difference between group 2 (test) and group 3 (+).

This research was conducted to determine the effect of katuk leaf ethanol extract gel (Sauropus androgynus (L.) Merr.) as an antifungal against Candida albicans fungus in orthodontic treatment. For the negative control treatment group (group 1) and the katuk leaf ethanol extract gel test group (group 2), there were 16 samples each. For the positive

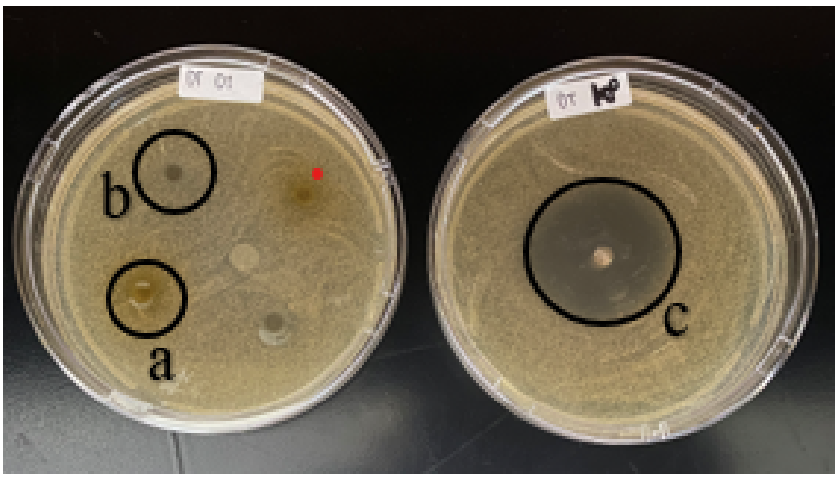

Figure 2: Source: Personal Documentacion. (a) Katuk leaf ethanol extract gel (b) base gel (c) Ketoconazole.

TABLE 6: Test the effectiveness of katuk leaf ethanol extract gel on Candida albicans fungi.

	Means	p.s	Interpretation
Day 1			
Group 1 (-)	0.0	0.000	There is a difference
Group 2 (test)	0.0		
Group 3 (+)	34.81		
Day 2			
Group 1 (-)	0.0	0.000	There is a difference
Group 2 (test)	0.0		
Group 3 (+)	34.55		
Day 3			
Group 1 (-)	0.0	0.000	There is a difference
Group 2 (test)	0.0		
Group 3 (+)	33.02		
Day 4			
Group 1 (-)	0.0	0.000	There is a difference
Group 2 (test)	0.0		
Group 3 (+)	33.02		

**) Kruskall Wallis, $p \leq 0.05$ (There is a significant difference)

group (group 3), there is only one sample. Group 1 (negative control): Candida Albicans mushroom culture in SDA media given an aquadest gel base. Group 2 (test): Candida Albicans mushroom culture in SDA media given the ethanol extract gel of katuk leaves (Sauropus androgynus (L.) Merr.). Group 3 (positive control): Candida Albicans cultured in SDA media given ketoconazole

Based on the results of measurements of the Minimum Inhibitory Concentration (MIC) on days 1 to 4, the diameter of the clear zone on the growth of Candida albicans using the well method, the ethanol extract gel of katuk leaves (Sauropus androgynus (L.) Merr.) did not show inhibition of the growth of the fungus Candida albicans. Table 1 shows that in the treatment group given katuk leaf ethanol extract gel on days 1, 2, day 3, and day 4, there was no change, while the positive control had effectiveness in inhibiting the growth of the Candida albicans fungus. The size of the diameter of the clear zone in the positive control was greatest on day 1, day 2, and so on, decreasing until 4^{th} day.

3.2. Discussion

This study used katuk leaves to determine the effectiveness of katuk leaf ethanol extract gel against the growth of Streptococcus mutans and Candida albicans in orthodontic treatment. Orthodontic treatment is a treatment performed to correct the abnormal position of teeth and jaws which divided into two categories: fixed orthodontic treatment and removable orthodontics [11]. A combination of brackets, wire, and orthodontic rubber is needed as a series of interconnected devices make a complex structure in the human oral cavity, and it will come into contact with saliva. Saliva contains protein, which will cause a selective protein absorption process that will then produce a pellicle layer. The pellicle is a thin layer of salivary, colorless glycoproteins that can come into direct contact with the surface of teeth and components in the oral cavity, including orthodontic devices. Within 2 hours of contact, the composition and activity in the oral cavity against the complex components of fixed orthodontic devices will form pellicles, which become the initial medium for the attachment of oral microorganisms such as bacteria and fungi, including Streptococcus mutans and Candida albicans [12,13].

Katuk (Sauropus androgynus (L.) Merr) beslongs to the Euphorbiaceae family. The dark green leaves are usually used as a potential treatment alternative, as they have many vitamins and nutrients. Active compounds that can be found in katuk leaves are saponins, tannins, flavonoids, steroids, and alkaloids, which have properties as antidiabetic, antiobesity, antioxidants, and antimicrobial [14].

In the oral cavity, humans have a wide variety of commensal bacterial species [15]. One of them is the bacterium Streptococcus mutans that play a very important role in the occurrence of dental caries. Streptococcus mutans is a facultative anaerobic, gram-positive bacterium belonging to the Streptococcus viridans group [16,17]. Streptococcus mutans plays a major role in the occurrence of caries because it can produce acid from

carbohydrate fermentation metabolism, which makes the oral cavity area acidic and increases the risk of caries. This acid causes demineralization, and carbohydrates are a substrate from which fermented bacteria get energy. If carbohydrates are consumed in excess, they can increase the incidence of caries, especially in patients using fixed orthodontics [7].

The study was conducted to test the antibacterial The results of the antibacterial effectiveness test in this study showed that the ethanol extract gel of katuk leaves could inhibit the growth of Streptococcus mutans bacteria. The result shows, each replicate didn't show too great a difference, so clear zone measurements were carried out with a calliper in order to find out the average diameter of the clear zone and the difference between treatments. The inhibition zone occurs because katuk leaves have flavonoid compounds that work to inhibit the function of cell membranes by forming complex compounds with extracellular and dissolved proteins that can damage the bacterial cell membrane and followed by the release of intracellular compounds [18,19]. It is proven that the antibacterial content of katuk leaves can inhibit growth of Streptococcus mutans bacteria used as a supportive therapy that can prevent of caries in orthodontic treatment. In the oral cavity, in addition to Streptococcus mutans bacteria, there are also other microbes in the form of fungi called Candida albicans fungi, which can be pathogenic as well.

Candida albicans is one of the microorganisms common in the human mouth, but it can become pathogenic if the amount is excessive [20]. Prevalence of Candida albicans as part of the normal flora of the gastrointestinal tract, upper respiratory tract, and genital mucosa in mammals Research conducted says that, on average, 35% are obtained in the oral cavity [20-22]. The pathogenesis of Candida albicans can be triggered by the use orthodontics applicance. The main factor causing the fungus Candida albicans is the physiological status of the host itself, which favors the virulence factor of the fungus [23].

Katuk leaves have flavonoid-active compounds [24]. Flavonoids inhibit fungal growth by various fungistatic mechanisms, such as disrupting plasma membrane formation, inducing mitochondrial dysfunction, inhibiting cell wall formation, inhibiting cell division, and inhibiting RNA and protein synthesis [25]. Fungistatic is the workings of antifungal compounds that can inhibit fungal growth but not kill it, characterized by turbid inhibitory zones in agar media [19]. Flavonoid fungstatic activity, among themdisrupts plasma membrane induction, inhibiting cell wall formation, induces mitochondrial dysfunction, inhibition of cell division, inhibition of the efflux pump, RNA/DNA inhibition and protein

synthesis [18]. Katuk leaves have a direct antibacterial role by disrupting the function of microorganisms such as bacteria or viruses and can be used to increase the body's immunity. Katuk (Sauropus androgynus (L.) Merr.) is a plant that contains chemical compounds, namely flavonoids, proteins, papaverine alkaloids, saponins, tannins, and fats [25].

Flavonoids can be obtained from a plant through the extraction process. Maceration is one of the most common extraction methods. In research conducted by Kusumawardhani et al. in 2020, it was stated that the content of flavonoid secondary metabolites in the best katuk leaves is best obtained by means of maceration using 70% ethanol extract with an average of 0.425mgQE/gr; therefore, researchers used 70% ethanol as a solvent in extraction in order to obtain antifungal power [26].

Based on the result of our research, there is no antifungals for Candida albicans. It was found that there was no inhibition zone around the katuk leaf ethanol extract gel treatment group and also the gel base, but a large inhibition zone was found around the wells given a positive control, namely ketoconazole. This difference can be due to several factors. According to research on plant extracts by Gunawan et al 2016 and Makhfirah et al 2020, metabolite compounds such as flavonoids, tannins, saponins, alkaloids, polyphenols, triterpenoids can be effective as antioxidants, anti-inflammatory, anticancer, antimicrobials such as antibacterial and antifungal, but the study did not mention the minimum amount of a secondary metabolite compound to inhibit Candida albicans [25,27]. A similar study on katuk leaf extract (Sauropus Andogynus (L.) Merr) was also conducted by Japar et al, 2021 which stated that there was no effectiveness of 96% katuk leaf ethanol extract against the growth of Candida albicans using a concentration of 5 extracts, namely 20%, 40%, 60%, 80%, and 100% [28]. This can state that ethanol solvents with a concentration of 96% or a concentration of 70% are not able to inhibit the growth of Candida albicans fungi. When compared to testing on other microorganisms, testing the activity of an extract as an antifungal, especially Candida albicans, requires more specific secondary metabolite compounds. Research conducted by Saleh et al. states that the content of specific flavonoids such as isoflavones, daidzein, genistein, favonol, flavones, flavans, and others plays a role in the death process of Candida albicans fungi [25,29]. Another study conducted by Adamu et al, 2021 about Pegangan leaves mentions the specific value of flavonoids to inhibit parasitic Aspergillus bacterial microorganisms, which is as much as 140 mg/g GAE [30]. This indicates the need for specific levels of flavonoids in plants to determine antifungal activity.

Another possible cause is that the ethanol extract of katuk leaves (Sauropus androgunys (L.) Merr) had no effect on fungi used in studies such as Candida albicans. Research by Kusumanegara in 2020 compared rose petal extract and katuk leaf extract, with the results showing that both inhibit the growth of Candida albicans fungus by the infusion method [9]. This study tested the antifungal activity of katuk leaf ethanol extract gel against the growth of Candida albicans fungus as an initial research project or is still a preliminary test, using different extraction methods that allowed this study to not succeed in obtaining good results. positive in having an inhibitory zone against the growth of the fungus Candida albicans [31].

4. Conclusion

Based on the results of the study, it can be concluded that administration of ethanol extract gel of katuk leaves (S. androgynus (L.) Merr) using the well method has an effect as an antibacterial on Streptoccus mutans bacteria but has no effect as an antifungal on the Candida albicans fungus present in the oral cavity of orthodontic users.

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Research Article

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Proteomic Profile of Blood Plasma of Mus Musculus in the Acute Toxicity Test of Single Black Garlic (Allium Sativum)

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Abstract.
This study aims to obtain overall information about all proteins formed in body fluids, cells, or tissues at a certain time and to analyze the mechanism of cell responses to various types of stress and drugs. Single black garlic (SBG) has the potential to be a pharmacological agent. However, the safety of using single black garlic is still unknown, so a material toxicity test is needed. This research is a laboratory experimental research with a post-test-only control group design. An oral acute toxicity test was used. Observation time was 14 days and consisted of four treatments, negative control, SBG dose 2000 mg/kgbw (P1), 3000 mg/kgbw (P2), and 5000 mg/kgbw (P3). Post-treatment protein analysis was done using the SDS-PAGE electrophoresis method. Observations of clinical symptoms of SBG at doses of 2000 mg/kgbw, 3000 mg/kgbw, and 5000 mg/kgbw showed no clinical symptoms in mice, both motor activity and pupils were still normal, no convulsions (seizures), lacrimation, paralysis or death were seen in mice. Observations on plasma proteins showed differences in protein between the treatments at the SBG dose of 2000 mg/kgbw with the SBG dose of 3000 mg/kgbw and 5000 mg/kgbw, but the four treatments have the same type of protein in the protein band with a molecular weight of 25 kDa. So, it can be concluded that SBG doses of 2000 mg/kgbw, 3000 mg/kgbw, and 5000 mg/kgbw are not toxic to mice but can stimulate the expression of certain proteins.

Keywords: Single Black garlic (SBG), oral acute toxicity test, proteomic analysis, SDS-PAGE

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1. Introduction

Proteomic analysis is a method to separate, identify and quantification of the entire complement proteins expressed by the genome, cell, or tissue. 1 This analysis is useful to get all the information of all proteins formed in body fluids, cells or tissues certain time as well as to analyse cell response mechanisms to various types of stress and drugs [1].

Majority proteomic analysis use plasma proteins blood to determine activity, distribution, rate of excretion or metabolism, and the toxicity of many pharmaceutical agents in body, where this is due to existence albumin protein which acts as receptors. 2 In addition, the use of the method protein separation in the form of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE) be most protein separation method widely used in analysis proteomics due to its reproducibility good at determining molecular weight protein. 3 The pharmaceutical agent in focus The mainstay of medical research is plants herbs, such as single black garlic [2].

Single black garlic is Garlic fermented product (Allium sativum) obtained through Maillard reaction in the form of heating at high temperature with a temperature range between 60-90°C and 70-90% humidity during 10-14 days. During the fermentation process, compounds in garlic which causes a sense of smell the sting will be changed naturally into a stable and safe compound, which will then produce a garlic with sweet taste and sour, more tender and has chewy texture like jelly [3,4].

Recent studies show that single black garlic (SBG) and compounds It has various bioactive activities more biological and pharmacological properties either among other things able to improve antibacterial activity so it can prevent bacterial infection, able to improve blood circulation in patients heart disease through increase antioxidant activity, enhance the activity of the antioxidant compounds able to inhibit the accumulation of compounds Advanced glycation end products (AGEP) which causes diabetes. as well as able Protects the liver by reducing levels Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and Lactate Dehydrogenase (LDH) in the blood [3].

Safe use of SBG as medicine or seasoning cooking is still little known, so there is an important reason to test the toxicity of this single black garlic. Test Acute toxicity was used to test toxicity in experimental animals within 24 hours. Symptoms of acute toxicity of an pharmaceutical ingredients, can be known based on clinical symptoms that occur

over time certain time after induction, such as effect tremors, lacrimation, paralysis, and diarrhea, based on changes in body weight as well histopathological picture [5].

Based on research by Marie and Wijayanti who used the extract black garlic from 0-2000 µg/mL indicates that garlicfresh and single black onion (SBG) did not show toxicity to cells Vero at all concentrations. However, research conducted by Permatasari et al stated that otherwise single single black garlic potentially decrease cell viability and able induces apoptosis of T47D cells despite the highest concentration range reach more than 2000 g/ml Study another conducted by Rumaseuw et al indicates that the provision of onions single black up to a dose of 2000 mg/kgbw does not cause toxic symptoms and there is no change in the behavior of the test animals [6].

Based on the data above, researchers are interested in doing the test SBG acute toxicity using the toxicity test method acute oral and proteomic analysis with using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE).

2. Methods

The research design is experimental laboratory with Post Test Only with Control Group. In this research focused on testing for single acute toxicity of black garlic (Allium sativum) and proteomic profile analysis against Mus musculus.

The Twenty four of Mice were divided into 4 groups given the following treatment Group 1 (KN): as a group negative control, not given any treatment except water and pellets. Group 2 (P1): given the extract black garlic dose of 2000 mg/kgbw. Group 3 (P2): given the extract black garlic dose of 3000 mg/kgbw. Group 4 (P3): given the extract black garlic dose of 5000 mg/kgbw. Mice were fasted before administration of SBG extract for 3-4 hours. Maximum volume administration of the test is 1 ml for mice. After 30 minutes post induction observed for clinical signs resulting intoxication motor activity, state of pupils, convulsions (seizures), lacrimation, paralysis, and death. Then observed periodically every 4 hours for 24 hours up to the 14th day in each group of animals [5].

On the 14th day, carried out blood sampling. The blood obtained is in the form of centrifuged blood plasma beforehand, then the blood plasma is tested protein profile. The proteomic profile is determined by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis method (SDS PAGE), through several stages namely protein sample

preparation, gel preparation polyacrylamide, chamber assembly and glass plate, protein sample induction, process running Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE), and the coloring process as well polyacrylamide gel wash [2].

Data analysis was carried out by descriptive in the form of a table of results observation of clinical signs of intoxication such as motor activity, pupillary state, convulsions (seizures), lacrimation, paralysis, and death and protein molecular weight of induced mouse blood plasma SBG through analysis SDS-PAGE.

This research got approval ethics committee from the Ethics Committee of the Faculty of Medicine General Achmad Yani University with ethics number 019/UH1.10/2022.

3. Results and discussions

SBG acute toxicity test single dose, performed several types Observations include 6 categories of symptoms clinical, including motor activity, state pupils, convulsions (seizures), lacrimation, paralysis, and death, were observed in the first 30 minutes post induction to 14 days post induction.

Mice Motor Activity 30 minutes until the 14th day after Onion Induction Black Single indicates that overall onion extract treatment single black given orally in mice do not cause symptoms intoxication on the motor activity of mice. This is proven through movement active mice after 30 minutes post induction to day 14 post induction without discovering behavior animals such as walking backwards and walking use stomach.

This also applies to parameters other clinical symptoms, where at 30 minutes until the 14th day after SBG induction, the condition of the pupils of mice still normal size without occurrence narrowing or widening pupil, mice that are still active without the occurrence of body movements that do not controlled (convulsions), the eyes of mice that still clear without the occurrence of irritation or red eyes accompanied by watery discharge eyes (lacrimation), can still move the extremities (not experiencing paralysis) and not found dead mice.

Based on the observation of symptoms clinical post-induction SBG prove that there is none active compound in it which can cause damage to nervous system and digestive system of mice. The results of this study prove statement by Sembiring and Iskandar which states that black onions alone has a beneficial effect on memory and the nervous system through effects anti amnesic, increased interference cognitive function, and

prevention of inflammation nerves and neurotoxicity and not have a toxic effect on consumer [7].

In addition, the research by Rumaseuw et al also stated that onion extract single black does not cause symptoms toxic and no change in behavior of animals is good from observation central nervous system and nervous system autonomous [8].

Blood plasma protein profile overview Oral induction of onion extract mice single black with various concentrations can be seen by the blue bands formed from an SDS-PAGE check. An SDS-PAGE check is performed using plasma samples mice taken on day 14 post induction of SBG extract. SDS PAGE results are displayed on Figures 1 and 2.

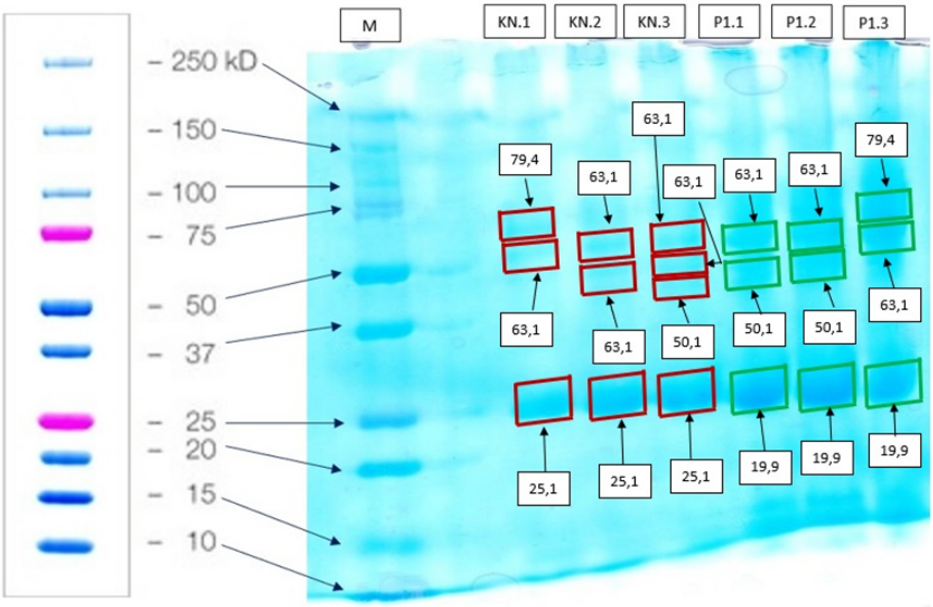

Figure 1: Protein bands of single black garlic extract. Description: KN : Negative Control Group, P1 : Black Garlic Extract Induction treatment Group Single dose of 2000 mg/kgbw.

Based on Figures 1 and 2 shows that the majority of the picture the pattern of protein bands almost have the same that is, it has a pattern of parallel thick bands with the 7th marker tape by weight 25 kDa molecule. According to Cahyarini et al. in Nugraha et al, the difference between thick and due to the thinness of the band formed by different numbers of migrated molecules, thick bands is the fixation of several bands.

Ribbons that have ionic strength larger ones will migrate further than bands of low ionic strength. In addition, there are other factors that differentiate the intensity of the band thickness proteins including: (1) there is a difference genetically between these proteins; (2) There are differences in protein concentrations in blood plasma; and (3)

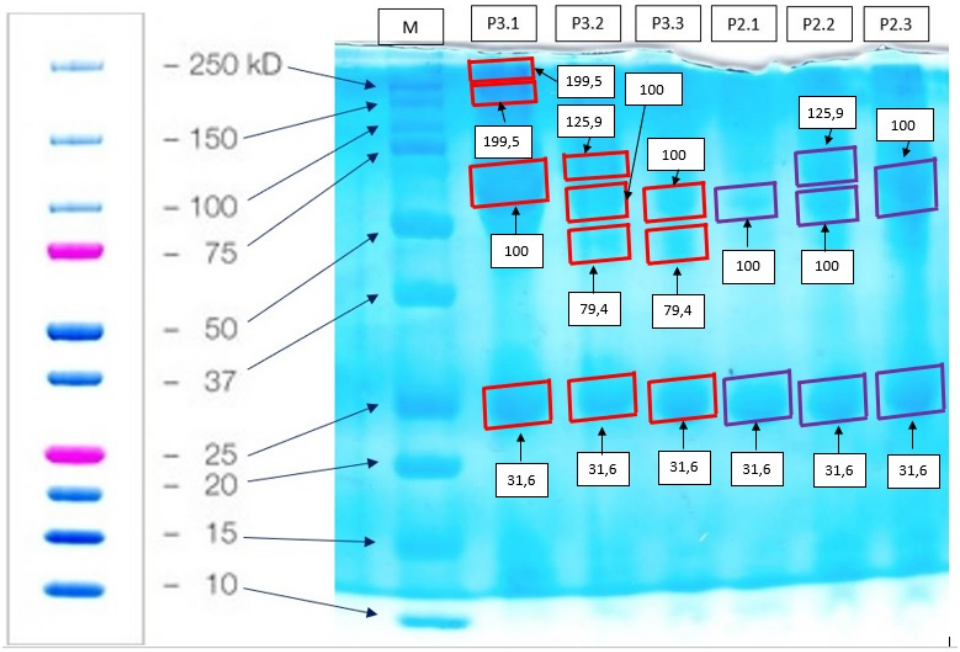

Figure 2: Protein bands of single black garlic extract. Description: P2 : Black Garlic Extract Induction Treatment Group Single dose of 3000 mg/kgbw, P3:Single Black Garlic Extract Induction Treatment Group dose of 5000 mg/kgbw, M : marker (standard protein).

the thinness of the ribbon due to less optimal process separation of proteins so that they have bands with a BM of less than 20 kDa.

According to Roy and Kumar, appearance and thickness of protein bands depending on the molecular weight of the protein acrylamide concentration used to make a gel, a voltage is applied on the gel and the length of time the voltage is applied [9].

In addition, Figures 1 and 2 as well shows that the whole treatment have protein bands parallel to 25 kDa BM marker tape. This matter prove that the protein is 25 kDa it remains in the blood plasma regardless undergo induced changes single black garlic extract. Protein identification results with The molecular weight of 25 kDa is estimated to belong to globulin proteins. Globulins work as a carrier of fats, vitamins, hormones and minerals. Blood plasma contains gamma globulin, i.e. immunoglobulin plays a role in immune system. Gamma-globulin function as an antibody. Globulins used to form fibrinogen musculin, crystallin, and antibodies. In addition, it is also estimated at negative control treatment and treatment 1 albumin was found in plasma blood with a molecular weight of 63.1. Albumin is one of the main proteins in the blood which has many functions is also a part important in the structure of blood plasma. Albumin functions include guarding fluid balance in the body, as intercellular bond forming agent its presence is required in the process cell regeneration and repair, binding all parts

of the cell with water so form blood fluids as well as transport various nutrients and hormones. Blood plasma besides containing the proteins albumin and globulin are also present other proteins such as fibrinogen, glycoproteins, haptoglobulins, and 10 lipoprotein, which was not identified in SDS-PAGE [10].

That treatment P1 differ in one type of protein with the KN group namely emergence a new protein band with a BM of 19.9 kDa. In addition, P1 has the same protein with the KN treatment of 63.1 kDa, 79.4 kDa and 50.1 kDa. Treatment P2 and P3 has the same protein with an average molecular weight obtained, namely 31.6 kDa, 100 kDa and 125 kDa. However, on P3 treatment had protein that did not owned by other treatments viz protein with a molecular weight of 199.5 kDa. From these results can be drawn the conclusion that when mice induced with SBG extract Black Single 2000 mg/kgbw, average The protein in plasma is still the same with the control group (KN), but additional protein expression occurs has a BM of 19.9 kDa. However, when mice induced with the extract Single Black Garlic 3000 mg/kgbw and 5000 mg/kgbw, obtained the type of protein completely different treatment SBG extract 2000 mg/kgbw and control. It shows that SBG extract 3000 mg/kgbw and 5000 mg/kgbw can stimulates the expression of new proteins that different from control mice.

Protein yield obtained showed that SBG extract can stimulate expression protein in the body of mice that have not can be identified. So that it can concluded that at a dose of 2000 mg/kgbw, 3000 mg/kgbw and 5000 mg/kgbw is not toxic in mice but can stimulate protein expression which is estimated to have a positive role for mice.

4. Conclusion

Based on the results of this study, it was concluded that SBG at a dose of 2000 mg/kgbw, 3000 mg/kgbw, 5000 mg/kgbw no show clinical signs of intoxication on Mus musculus such as motor activity and pupillary state normal, no convulsions (seizures), lacrimation, paralysis or mortality in mice and protein profile SBG parallel to 25 kDa BM marker tape.

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Research Article

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Potency of Red Guava Fruitghurt As an Antioxidant

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Abstract.
Antioxidants are compounds needed by the body to protect body cells from damage caused by free radicals. Free radicals can damage macromolecules such as cell membrane lipids, DNA, and proteins, and cause cell oxidative stress. Red Guava Fruitghurt is a probiotic with the basic ingredients of fruit. The purpose of this study is to determine the effect of fruitghurt on the MDA levels in the blood of mice induced by CCl4. This was an experimental study conducted using mice (Mus musculus) which were divided into three treatment groups – the negative control group (that were not induced and were not given fruitghurt), the positive control group (that were induced with CCl4 but not given fruitghurt), and the treatment group (that were induced with CCl4 and given fruitghurt). The data obtained in the study were analyzed using the one-way ANOVA test, and the results showed significant differences between the treatment groups. Giving fruitghurt to the P1 group had a significant effect on reducing malondialdehyde levels compared to the positive control group, with a P-value of 0.03. Lactobacillus acidophilus bacteria found in this fruitghurt can reduce oxidative stress by inhibiting ascorbic acid oxidation, reducing activity and capturing radial superoxide anions, hydrogen peroxide, and free radicals, and inhibiting lipid oxidation. So this fruitghurt has antioxidant properties. This is also strengthened by the content of red guava which is rich in phenolics, flavonoids, and vitamin C that have good free radical scavenging activity, so guava fruit is a natural source of antioxidants.

Keywords: antioxidants, fruitghurt, malon dialdehyde, probiotics, red guava

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1. Introduction

A free radical is an atom or molecule that has one or more unpaired electrons. Due to these unpaired electrons, free radicals tend to be highly reactive and can interact with other molecules in an effort to acquire additional electron pairs [1,2]. Free radicals can form naturally in the human body as a result of normal chemical reactions, but they can also be generated by external factors such as air pollution, excessive sunlight exposure, smoking, or exposure to hazardous chemicals [3]. And the habit of consuming unbalanced fast food meals, lacking in proper proportions of protein, carbohydrates,

and fats [4]. Free radicals can interact with various molecules within cells, including proteins, lipids, and DNA, thereby causing oxidative damage to cells and tissues, and contributing to the development of various diseases such as cardiovascular disease, cancer, and aging and other degenerative diseases [3]. This is because the antioxidants in the body are unable to balance the influx of oxidants entering the body [1].

Our bodies have a natural defense mechanism to combat free radicals, known as the antioxidant system. Antioxidants are compounds that can provide additional electrons to free radicals, thereby helping to neutralize them and protect cells from damage [5].

Our bodies can produce endogenous antioxidants, including glutathione peroxidase, superoxide dismutase (SOD), catalase, and glutathione S-transferase [6]. However, endogenous antioxidants within the body may not necessarily protect cells as a whole against external oxidants. Therefore, the body requires exogenous antioxidants, which come from outside the body [1]. External antioxidants can come in the form of foods or beverages that we consume, including Vitamin C, Vitamin E, beta-carotene, selenium, and various phytochemicals found in fruits, vegetables, grains, and spices [3,7].

Fruitghurt is a beverage produced from fruit juice that is fermented by lactic acid bacteria such as Lactobacillus acidophilus. Lactose from the fruit juice can be converted into lactic acid by lactic acid bacteria (LAB) [8].

Red guava Fruitghurt utilizes red guava as its main ingredient. Red guava is a fruit that has a sweet and sour taste, and it is rich in vitamin C, fiber, which is important for the immune system and antioxidants that protect cells from oxidative damage. Additionally, it also contains protein, calcium, and probiotics that are beneficial for health [8,9].

The LAB bacteria found in Fruitghurt, such as Lactobacillus acidophilus, also exhibit high antioxidant activity. Previous studies have found that lactobacillus bacteria in Fruitghurt have high antioxidant activity, thereby providing benefits by supplying safe and effective natural antioxidants to consumers [10,11]. In addition, Fruitghurt has an advantage over regular yogurt as it can be consumed by individuals with cow's milk allergy (lactose intolerance) [?].

Red guava (Psidium guajava L) is one of the tropical fruits characterized by its bright red color and sweet taste with a hint of acidity. Red guava can be utilized as a functional food component due to its beneficial effects on human health. It contains relatively high levels of vitamin C and is rich in carbohydrates, iron, phosphorus, calcium, vitamin A, and beta-carotene [?] These components can limit the development of free radicals in the body or act as antioxidants. These antioxidants can prevent and repair liver cell

damage caused by free radicals from used cooking oil by inhibiting the formation of free radicals at the initiation stage or limiting the continuation of chain reactions at the propagation stage [14,15]. In addition, red guava is rich in phenolic compounds and flavonoids, which can reduce oxidative stress by binding to free radicals. Flavonoids, in particular, contribute to 51.28% of the radical scavenging activity [4]. Flavonoids function as antioxidants by interrupting or trapping the chain reactions of free radicals' oxidation. As a result, free radicals are unable to react with biological components [?].

Red guava Fruitghurt is a probiotic beverage made from red guava as the main ingredient. The combination of these two ingredients is expected to provide greater health benefits, including its antioxidant properties in combating free radicals in the body.

Malondialdehyde (MDA) is a biomarker or laboratory parameter commonly used as an indicator of oxidative stress or free radicals [12,14]. Malondialdehyde is a highly reactive organic compound produced during lipid peroxidation, which is the oxidative degradation process of lipids. It is a byproduct of lipid peroxidation. When cells are exposed to toxins or free radicals, the lipids in cell membranes can become damaged, leading to the production of reactive oxygen species (ROS), including MDA [2]. MDA can be found in lipid peroxidation plasma, serum, and urine [?].

Carbon tetrachloride (CCl4) is a xenobiotic commonly used to induce and poison lipid peroxidation. The enzyme cytochrome P450 converts the CCl4 molecule into the free radical CCl3, which is responsible for the toxicity of CCl4 [2].

Assessing the potential health effects of regular consumption of red guava fruit yogurt on the body's antioxidant levels is crucial as it's highly associated with various conditions and diseases related to oxidative stress, such as cardiovascular diseases, diabetes, or cancer. Regular consumption of red guava fruit yogurt is expected to increase antioxidant substances in the body, which can counteract the dangers caused by oxidative stress.

Therefore, this research was conducted to determine the effectiveness of red guava Fruitghurt as an antioxidant in reducing MDA levels in the blood of mice induced by CCl4.

2. Materials and Methods

This study was a laboratory experiment using an experimental approach to evaluate the changes that occurred after the intervention.

The study subjects used were mice (Mus musculus), which had to meet inclusion criteria including being active and mobile (to ensure the mice were in a healthy condition), aged 10-14 weeks, and weighing between 20-40 grams. The exclusion criterion was a weight loss of more than 10%. Significant weight loss could indicate health problems or stress that might affect the research outcomes.

The object of this research is red guava Fruitghurt, which is made from red guava fruit fermented by Lactobacillus acidophilus. The inclusion criteria include the use of Lactobacillus acidophilus ATCC 4356 bacteria, a minimum bacterial count of 107 CFU/ml for Fruitghurt production, and the exclusion criteria are Fruitghurt with a foul odor and Fruitghurt contaminated with other substances.

The research subjects consisted of 27 mice, divided into 3 treatment groups: negative control group (NC), in which mice were not induced with Carbon Tetrachloride (CCl4) and were not given Fruitghurt; positive control group (PC), in which mice were induced with Carbon Tetrachloride (CCl4) and were not given Fruitghurt; and treatment group (T1), in which mice were induced with Carbon Tetrachloride (CCl4) and were given Fruitghurt.

Ethical approval was obtained from the Ethics Committee of Universitas Jenderal Achmad Yani with Approval No. 26/UH1.11/2022.

The research procedure began with the preparation of materials, tools, and bacteria. Then, Fruitghurt was prepared by adding 10% sucrose. Subsequently, the mice were acclimatized for 7 days in the Animal Laboratory of the Faculty of Medicine, Universitas Jenderal Achmad Yani. Blood samples were collected from the mice to examine the MDA levels, and then the mice were induced with CCl4, followed by the measurement of MDA levels in the mice. After 24 hours, Red Guava Fruitghurt was administered to the mice for 7 days, and then MDA levels were measured in the mice again (see Table 1).

The data testing began with the normality test using the Shapiro-Wilk test since the data sample size is less than 50. Then, data analysis was conducted using one-way analysis of variance (ANOVA) to assess the differences in antioxidant effects among

TABLE 1: Research groups.

No	Groups	Standard diet	Carbon Tetra-clorida (CCl4)	Fruitghurt	Duration of treatment
1	NC	+	-	-	16 days
2	PC	+	0,5ml/gr bw	-	16 days
3	P1	+	0,5ml/gr bw	1 ml/20gr bw	16 days

Description:
KN: Negative control group of mice not induced with Carbon Tetrachloride (CCl4) and not given Fruitghurt.
KP: Positive control group of mice induced with Carbon Tetrachloride (CCl4) and not given Fruitghurt.
P1: Treatment group of mice induced with Carbon Tetrachloride (CCl4) and then given Fruitghurt

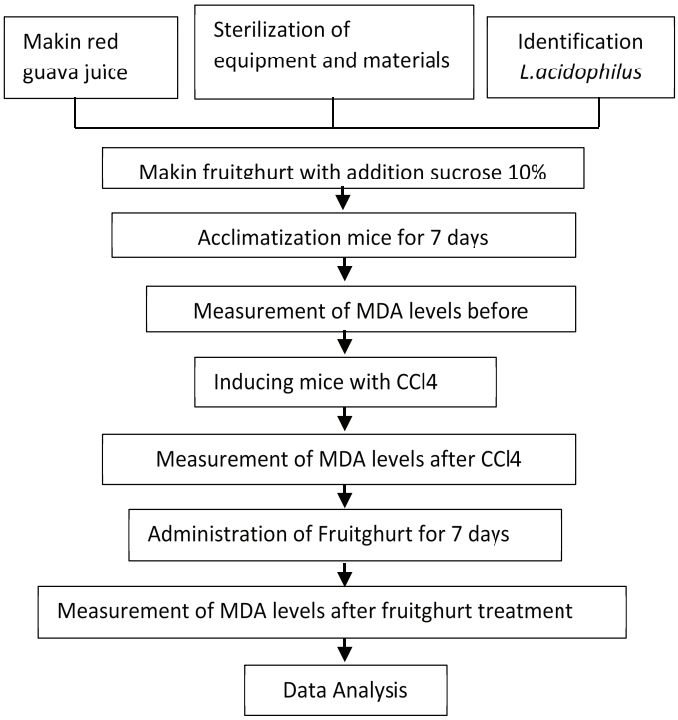

Figure 1: Research flow diagram.

the groups. Post hoc Tukey test was performed to evaluate the observed differences between the groups.

3. Results and Discussion

The measurement of MDA levels was first conducted on the 8th day after the mice were given standard pellet feed and ad libitum drinking water, and before the induction of Carbon Tetrachloride (CCl4) at a dosage of 0.5ml/20g bw for the treatment group.

The effect of administering fermented red guava fruitghurt with Lactobacillus acidophilus as an antioxidant on the reduction of Malondialdehyde (MDA) levels is presented in Table 2.

TABLE 2: Presents the mean values of MDA levels in mice (Mus musculus) after the treatments.

Groups	Mean value of MDA (mg/dl)	Standar Deviasi (SD)	Min-Max
Negative control (NC)	1.096	0.275	0.81 - 1.36
Positive control (PC)	1.683	1.459	0.24 - 2.59
Treatment Group (P1)	0.996	0.267	0.69 - 1.18

Note:
KN: Negative control group of mice not induced with Carbon Tetrachloride (CCl4) and not given Fruitghurt.
KP: Positive control group of mice induced with Carbon Tetrachloride (CCl4) and not given Fruitghurt.
P1: Treatment group of mice induced with Carbon Tetrachloride (CCl4) and then given Fruitghurt

MDA levels are used as an indicator of oxidative stress or oxidative damage in experimental animals. The higher the MDA levels, the higher the level of oxidative stress. In this study, the mean MDA values were used to compare the levels of oxidative stress between the treatment groups.

In the negative control group, the mean MDA value was 1.096 mg/dL. The relatively low standard deviation indicates consistency in the MDA values within this group. The observed range of MDA values ranged from 0.81 mg/dL to 1.36 mg/dL.

In the positive control group, the mean MDA value was 1.683 mg/dL. The high standard deviation indicates a large variation within this group. The observed range of MDA values ranged from 0.24 mg/dL to 2.59 mg/dL. The high results in the positive control group compared to the negative control group indicate an increase in oxidative stress due to CCl4 induction.

In the treatment group, the mean value of MDA is 0.996 mg/dl. The low standard deviation indicates consistency in MDA values in this group. The observed range of MDA values is between 0.69 mg/dl and 1.18 mg/dl. The lower results compared to the positive control group indicate that the administration of fruitghurt to CCl4-induced mice can reduce the level of oxidative stress.

Table 3 presents the results of the normality test on the MDA levels in mice for each treatment group using the Shapiro-Wilk test. From the normality test results, it can be concluded that the MDA levels in each treatment group follow a normal distribution ($p > 0.05$). This indicates that the assumption of normal distribution is fulfilled, allowing

Table 3: Results of Normality Test for MDA Levels in Mice (Mus musculus) After Treatment.

Groups	Normality testfor MDA levels		
	P value	Distribution of data	Significant value
Negative control (NC)	0.860	Normal	0.033
Positive control (PC)	0.085	Normal	
Treatment Group (P1)	0.215	Normal	

Note: Shapiro-Wilk Test: p-value > 0.05 indicates normal distribution of data.
One-Way ANOVA Test: p-value < 0.05 indicates a significant difference

for further analysis, in this case, using the one-way ANOVA test to compare significant differences among the treatment groups.

The one-way ANOVA test in Table 2 shows a p-value of 0.033. The obtained p-value is smaller than the predetermined significance level (0.05), indicating that there is a significant difference among the treatment groups in this study. Thus, based on the one-way ANOVA test, it can be concluded that the administration of fruitghurt derived from fermented red guava with Lactobacillus acidophilus as an antioxidant has a significant effect on reducing MDA levels in the experimental animals.

Table 4: Comparison of mean MDA levels among groups of mice.

	Groups	comparator	p-value
MDA	NC	PC	.058
		P1	.962
	PC	NC	.058
		P1	.042*
	P1	NC	.962
		PC	.042*

Note : The Tukey post hoc test results showed that the p-value was <0.05.
The asterisk (*) indicates statistical significance

In Table 4, a post hoc Tukey test was conducted to compare the differences in mean MDA levels between treatment groups. The comparison between the negative control group and the positive control group yielded a p-value of 0.058, which is greater than 0.05. Therefore, it can be concluded that there is no significant difference in MDA levels between these two groups..

The comparison between the negative control group and the treatment group resulted in a p-value of 0.962. This value is greater than 0.05, indicating that there is no significant difference in MDA levels between these two groups. Similarly, when

comparing the positive control group (PC) with the treatment group (P1), the obtained p-value is 0.058, which is greater than 0.05, indicating no significant difference between the treatment groups.

However, there is a significant difference in MDA levels between the treatment group and the positive control, as well as between the treatment group and the negative control, with a p-value of 0.042, which is smaller than 0.05. Therefore, based on the post hoc Tukey test, it can be concluded that there is a significant difference in MDA levels between the treatment group and both the positive and negative controls.

In a previous study conducted by Trisnowati, it was found that red guava fruit contains beneficial antioxidants for the body. Antioxidants can inhibit and repair liver cell damage caused by free radicals by either inhibiting the formation of free radicals in the initiation stage or inhibiting the continuation of chain reactions in the propagation stage [?].

Red guava fruit possesses functional properties, particularly its high content of vitamin C, which can act as an antioxidant. Vitamin C in red guava fruit plays a crucial role as an electron donor (reducing agent) by transferring one electron to metal compounds such as Cu, thus stabilizing reactive oxygen compounds. Vitamin C donates electrons as part of hydrogen atoms and captures free radicals. Research conducted by Norazmir indicates that administering red guava fruit at doses ranging from 0.5 to 2.0 g/kg body weight of the test animals can reduce free radicals. This is because red guava fruit can enhance the activity of antioxidant enzymes in the blood of the test animals [?]. In addition, red guava fruit contains high levels of flavonoids. Flavonoids bind to peroxyl radicals formed during the chain reaction of lipid peroxidation and donate an unpaired electron to the free radicals, thereby breaking the chain. Flavonoids contribute approximately 51.28% of the radical scavenging activity [?].

In this study, the preparation of fruitghurt involved the addition of 10% sucrose. Lactic acid bacteria, including Lactobacillus acidophilus, are capable of breaking down glucose into lactic acid and other sugars such as lactose, galactose, fructose, sucrose, and maltose during the fermentation process. Lactic acid bacteria utilize fructose and glucose in the fruit extract present in the probiotic beverage as a carbon and nitrogen source for their growth during fermentation. As a result, the growth of lactic acid bacteria in this probiotic beverage will increase [?]. During fermentation, lactic acid bacteria (LAB) convert sugars into lactic acid through a series of chemical reactions. These bacteria utilize sugars as substrates to produce energy through their metabolic pathways. The process involves the breakdown of sugars into simpler components

and their utilization within the bacterial metabolic pathways, ultimately resulting in the production of lactic acid as the end product [19,20].

4. Conclusion

Based on the conducted research, it can be concluded that the highest mean MDA level was observed in the Positive Control (KP) group, which was induced with Carbon Tetrachloride (CCl4) and not given fruitghurt. Furthermore, there was a significant difference between the positive control group and the treatment group, indicating that fruitghurt has a significant effect in reducing MDA levels.

Declaration of interests

The author hereby declares that there is no conflict of interest in the scientific articles that we write

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Research Article

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The Impact of Exercise on DNA Damage in Athletes: Causes, Mechanisms, and Case Studies

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Abstract.
Although physical activity is important for the athlete's health and performance, it may also lead to Deoxyribonucleic Acid (DNA) damage, which may have long-term health consequences and affect performance. This article summarizes different types of DNA damage that may occur in athletes, including their potential risk causes, such as mechanical stress, heat stress, and oxidative stress. Furthermore, several mechanisms by which physical activity might lead to DNA damage and affect health and performance are also discussed. Various strategies such as diet and nutrition, antioxidant supplements, cooling strategies, and recovery modalities are also presented to mitigate, prevent, and minimize the potential DNA damage upon physical activities. This article also highlights case studies of athletes who have experienced DNA damage and the effects on their performance and health. In summary, this article offers valuable perspectives on the intricate relationship between physical activity and DNA damage as well as the importance of addressing this issue in the context of athletic performance and health.

Keywords: athlete, DNA damage, exercise, RONS

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1. Introduction

Exercise has been widely known for its beneficial effects for human health and wellness [1]. However, evidences show some opposite effects of exercise associated with DNA damage. This occurs mainly due to the production of reactive oxygen and nitrogen species (RONS) in exercises. Overproduction of RONS exceeds antioxidant production in neutralizing the effects of RONS that can result in DNA damage. As vulnerable target of RONS, DNA damage may cause genetic instability if happens continuously [2,3].

RONS are generated in skeletal muscles mainly through mitochondrial electron transport chain during cellular respiration [4]. As reactive oxygen and nitrogen species (RONS) gather within the cell, whether generated through metabolic signaling pathways such as NADPH or originating from external sources, they are counteracted by scavenging

antioxidant mechanisms. Reactive oxygen and nitrogen species (RONS) play significant roles in controlling fundamental processes like cellular growth, differentiation, and apoptosis [2,5,6].

However, accumulation of RONS also has negative impact in human body, especially in athletes. Studies reported the incidence of sudden cardiac death (SCD) in young athlete are most commonly caused by left ventricular hypertrophy (LVH) which are clinically indistinguishable from physiological LVH in response to exercise and probably related to DNA damage [7]. Other study showed some cases of DNA repair deficiency and senescence in contact sports athletes with history of mild traumatic brain injury (mTBI) [8]. Study conducted with comet assay also suggested that endurance exercise results in DNA damage [9]. Based on the previous background, the objective of this study was to examine and evaluate the collective impact of research studies which discusses DNA damage resulting from various forms of exercise.

2. Methods

The method we use in this study is a study literature from reliable scientific references in search engines such as Web of Science, PubMed, MEDLINE, EMBASE, and Scopus in last ten years up to 2022.

3. Results and Discussion

Some studies find that physical activities could lead to DNA damage, but some also suggest otherwise. Study by Laomax suggest that chlorine exposure and chronic endurance training reduce Club cell protein 16 (CC16) levels and the ratio of CC16 to surfactant-associated protein D. that leads to lung growth restriction and this study was carried out to swimmer athletes. They also suggest that retirement will help to partially reverse this condition in children and adolescence athletes [10]. Study by Arent, 2010 and Carrera-Quintanar, 2022 show the occurrence of oxidative stress through increase of MDA level and creatine kinase in football player [11][12].

In contrast, several studies show protective effect of exercise such as regular physical activity protective effect against DNA damage in lymphocytes by increase in antioxidant capacity [13]. Neubauer also proposes that oxidative DNA damage may have been offset by the antioxidant responses triggered by training and exercise [14]. Liang, 2022 also noted that treadmill exercise decreases Bcl-2 and Bax values in the body, enhances

mitochondrial DNA integrity and increases telomerase activity in myocardial cells [15]. Study by Tryfidou, 2019 described that in acute aerobic exercise, there is a notable rise in DNA damage right after engaging in short-term aerobic exercise, and this elevation remains notable between 2 hours and 24 hours afterward, but not within the period of 5 to 28 days following the exercise session [2]. The opposite effect of various exercise related to DNA damage describes in detailed in Table 1.

The most important thing is that Short-term exercise leads to a temporary rise in RONS production as a result of heightened activity in mitochondria and other metabolic pathways. This may subsequently induce oxidative stress and DNA damage. The extent of ROS increase is influenced by exercise factors such as intensity, duration, and frequency. Numerous studies also propose that the connection between exercise and DNA damage might be elucidated by the hormesis theory, which is somewhat simplistic and therefore constrained [2][16]. Top of Form

The hormesis theory explains how exercise regulates both beneficial and detrimental effects mediated by RONS, by amplifying DNA oxidation between two poles: physical inactivity and overtraining. It is noteworthy that consistent exercise has been demonstrated to boost the body's antioxidant defense mechanisms and promote DNA repair mechanisms. Conversely, excessively intense activity levels may result in DNA damage, leading to the suggestion that the interplay among exercise, ROS, and DNA damage could adhere to the curve depicted in Figure 1 [2][16].

Nevertheless, the precise threshold between the advantageous outcomes of consistent exercise and the onset of overtraining, which is linked to heightened oxidative damage and inadequate DNA repair, remains uncertain. Taking this into account, engaging in regular exercise, especially aerobic exercise, is likely to mitigate DNA damage owing to elevated antioxidant capacity and enhanced DNA repair capabilities [2][16].

4. Conclusion

In summary, the relationship between exercise and DNA damage could potentially be elucidated by the hormesis theory where the dose of exercise, training intensity and frequency, plays significant roles to the outcomes. The hormesis theory outlines how exercise influences both beneficial and detrimental effects of RONS activity, by enhancing DNA oxidation which results in DNA damage.

TABLE 1: Opposite effects of various exercise related to DNA damage.

Types of DNA Damage	Exercise / Sport	Long-term Effect	Prevention	References
Reduction in levels of Club cell protein 16 (CC16) and the ratio of CC16 to surfactant-associated protein D. Top of Form Bottom of Form	Swimming: chlorine exposure and chronic endurance training	lung growth restriction	partially reversible in early retirement	Lomax, 2016 [10]
Oxidative stress	Football: increase MDA level	-	polyphenolic antioxidant and anti-inflammatory supplements	Carrera-Quintanar, 2022 [12]
Oxidative stress	Football: increase creatine kinase and oxidative stress biomarker	-	antioxidant and nutraceutical blend supplements	Arent, 2010 [11]
Oxidative stress by increasing ROS and creatine kinase (CK) level	Athletic sprint	-	strength training should be combined with proper recovery in between each session	Affonso, 2019 [17]
Protective effect against DNA damage in lymphocytes by increase in antioxidant capacity	Regular physical activity	-	-	Soares, 2015 [13]
The oxidative damage to DNA may have been offset by the antioxidant responses triggered by training and exercise.	Regular physical activity	-	-	Neubauer, 2019 [14]
Decrease the levels of Bcl-2 and Bax in the body, enhance mitochondrial DNA integrity, and increase telomerase activity in myocardial cells.	Treadmill exercise	slow down the aging process	-	Liang, 2022 [15]
A notable elevation in DNA damage after a short-term aerobic exercise that persists significantly between 2 hours and 1 day afterward.	Aerobic exercise	-	-	Tryfidou, 2020 [2]
Reduction of DNA repair proteins levels in mild traumatic brain injury (mTBI) or concussion brains resulting in DNA damage and cellular senescence that lead to neurobehavioral and/or psychiatric symptoms or cognitive dysfunction and/or dementia.	Contact sports: football, hockey, rugby, and boxing.			Schwab, 2019 [8]

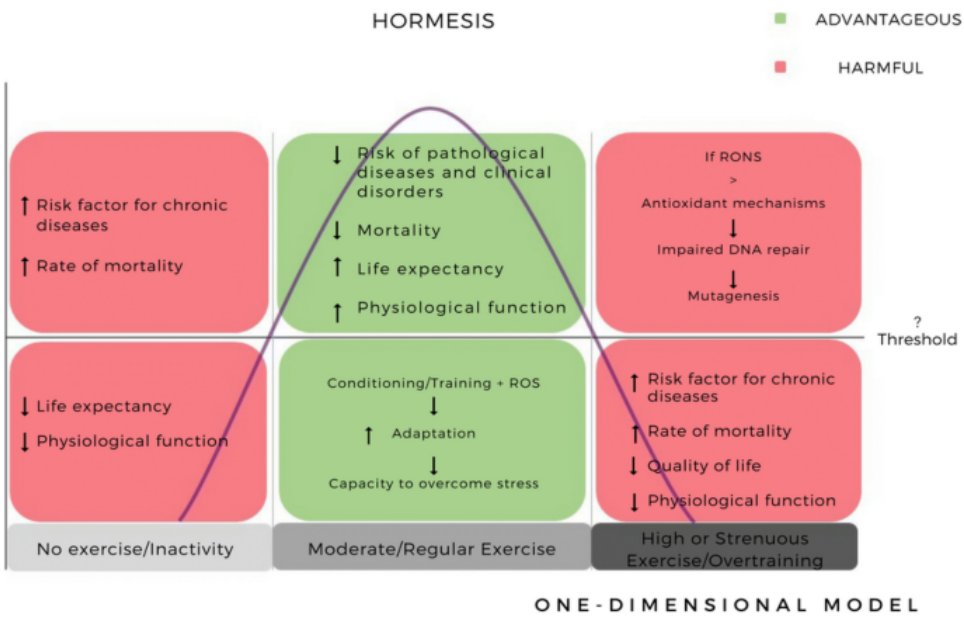

Figure 1: Hormesis theory in different intensity of exercises [2].

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Research Article

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Electrostatic Mapping of Rabies Anti-Idiotype Antibody Compared to Rabies Virus Glycoprotein

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Abstract.
Rabies is a lethal viral animal disease that assaults the central nervous system. Its glycoprotein is a viral protein that is essential for viral pathogenicity. Initially, the rabies vaccine was produced from nerve tissue, but it is no longer recommended since it causes adverse effects and is less effective. The anti-idiotype antibody vaccination is one option that functions as homologous artificial antigens to the glycoprotein of the rabies virus. The CDR is the structure of anti-idiotypic antibodies that play a role in mimicking epitopes. It may resemble or be identical to epitopes seen in rabies virus glycoproteins. The objective of this study is to determine the affinity of the CDR anti-idiotype antibody for the rabies virus glycoprotein epitope by studying the CDR electrostatic value. This electrostatic value was analyzed by bioinformatics approaches using a webPIPSA server. The findings revealed an electrostatic resemblance between the structure of anti-idiotype antibodies and the rabies virus glycoprotein. Further study will be aimed at collecting electrostatic values from each structure to create an anti-rabies vaccine.

Keywords: anti-idiotype antibodies, CDR, electrostatic, rabies, glycoprotein, webPIPSA

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1. Introduction

Rabies is a contagious viral disease that, when clinical symptoms appear, is almost invariably fatal. This disease is a zoonotic disease, which means that it is spread from animals to people via bites or scratches, most commonly through saliva [1]. Rabies is expected to cause 55,000 deaths worldwide each year, with Asia accounting for 56% of them. In Indonesia, there are nine rabies-free provinces and 24 rabies-endemic provinces [2,3].

Rabies treatment options include post-exposure prophylaxis (PEP) and pre-exposure prophylaxis (PrEP) [4,5]. Vaccines can protect against rabies either before or after

exposure. Louis Pasteur and Emile Roux created an attenuated rabies vaccine in 1885 [6]. There are four types of vaccines: mouse nerve tissue, human diploid cell cultures, monkey vero cells, and chicken and duck embryos (CCEEVs).[6] Because of their lower immunogenicity, the World Health Organization (WHO) has advised that nerve tissue vaccines be discontinued and replaced with CCEEVs since 1984 [5,6]. Virus attenuation can potentially raise the risk of virulence. Another option is to create an anti-idiotype antibody vaccination [7,8].

Anti-idiotype antibodies act as vaccines by mimicking antigens and stimulating an immune response. TCRs and immunoglobulins have a complementarity-determining region (CDR) with a distinct amino acid structure known as an idiotope. The term idiotype is frequently used to describe a group of numerous idiotopes [9]. Idiotype is frequently connected to an antibody's Complementarity Determining Region (CDR), which specifies specific binding locations. The diversity of antigen-binding sites, notably the CDR hypervariable domain, is connected to idiotypic variability. CDRs are found in both the light and heavy chains, with CDR H3 playing an important role in antigen recognition by changing form upon binding [8,10].

A rabies vaccine based on anti-idiotype antibodies from chicken immunoglobulin (IgY) has been demonstrated to be highly immunogenic and capable of inducing a protective immunological response in animals [11,12]. Animal trials, on the other hand, are time- consuming, costly, and produce superfluous proteins. Recombinant DNA (rDNA) vaccine technology is safer, more effective, and less expensive than traditional approaches [13,14]. The purpose of this work is to compare the epitopes on anti-idiotype antibodies to the rabies virus glycoprotein bioinformatically in order to find an immunogenic epitope for a rabies vaccine based on anti-idiotype antibodies. Protein structure modeling is a technique for determining a protein's three-dimensional structure based on its amino acid sequence. The ultimate goal is to develop a vaccine for the rabies virus that includes anti-idiotype antibody peptides as an immunogenic component to promote protective immunity. Vaccination with anti-idiotype antibodies, which contain the antigen's internal image, has significant advantages over traditional vaccines. Anti-idiotypes are not infectious, but they can evoke a stronger immune response against the pathogen than inactivated viruses and are beneficial for obtaining huge amounts of antigen [15,16].

2. Material and Methods

2.1. Material

The three-dimensional structure of rabies glycoprotein G (PDB ID: 4D6W) and the amino acid sequence of anti-idiotype antibodies (Patent No.: P00201900780) were used in this study [17]. The tools were classified into two categories: hardware and software. A set of personal computers was considered hardware. The software utilized to explore for rabies glycoprotein templates included NCBI BLAST and RCSB PDB. Biovia Discovery Studio 2019 is used to visualize the structure of glycoproteins and anti-idiotype antibodies in three dimensions form. PROCHECK was used to evaluate the quality of the structure that has been formed. The ROSETTA are utilized to build anti-idiotype antibody structures. Then, for electrostatic mapping analysis, use webPIPSA.

2.2. Methods

The study aims to design an anti-idiotype antibody model and conduct a structural comparison between the anti-idiotype antibody and the rabies glycoprotein. The CDR section was then analyzed to determine the structure of the anti-idiotype antibody. The NCBI server was used to search for templates, and a model was created using MODELLER software. The 3D structure was evaluated by PROCHECK.[18,19] The Biovia Discovery Studio 2019 program was used to create a three-dimensional view of the anti-idiotype antibody and rabies G glycoprotein.

The anti-idiotype antibody was marked as a ball on the CDR section and arranged with the glycoprotein's 3D structure in various positions. Compilation results were obtained, and the files were analyzed using webPIPSA to display an epogram diagram illustrating the degree of proximity of structures based on electrostatic values [20,21].

3. Results

3.1. Rabies virus glycoprotein sequences

The protein sequence of the rabies virus is the glycoprotein part. Glycoprotein proteins are antigenic specific components that induce immune system responses. Through the

NCBI GenBank server (http://www.ncbi.nlm.nih.gov/), protein sequences were shown in Figure 1.

```
MVPQALLFVPLLVFPLCFGKFPIYTIPDKLGPWSPIDIHHLRCPNNLVVEDEGCTNLSGFSYMELKVGYI
LAIKMNGFTCTGVVTEAETYTNFVGYVTTTFKRKHFRPTPDACRAAYNWKMAGDPRYEESLHNPYPDYRW
LRTVKTTKESLVIISPSVADLDPYDRSLHSRVFPSGKCSGVAVSSTYCSTNHDYTIWMPENPRLGMSCDI
FTNSRGKRASKGSETCGFVDERGLYKSLKGACKLKLCGVLGLRLMDGTWVAMQTSNETKWCPPDQLVNLH
DFRSDEIEHLVVEELVRKREECLDVLESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEADAHYKS
VRTWNEILPSKGCLRVGGRCHPHVNGVFFNGIILGPDGNVLIPEMQSSLLQQHMELLESSVIPLVHPLAD
PSTVFKDGDEAEDFVEVHLPDVHNQVSGVDLGLPNWGKYVLLSAGALTALMLIIFLMTCCRRVNRSEPTQ
HNLRGTGREVSVTPQSGKIISS WESHKSGGETRL
```

Figure 1: The amino acid sequence of rabies glycoprotein.

3.2. Rabies virus glycoprotein model

The NCBI BLAST server was used to identify templates that are comparable to the structure of the rabies virus glycoprotein (Table 1). BLAST is a search tool that searches the GenBank DNA database for sequences that are similar to ours using sequence comparisons.

TABLE 1: BLAST analysis of rabies virus glycoprotein based on the amino acid sequence.

Description	Max Score	Total Score	Query Cover	E-value	Per. Ident	Accession
Chain A, GLYCOPROTEIN G [Chan virus]	66.2	66.2	53%	3e-11	24.24%	4D6W_A
Chain B, C-terminal motif from Glycoprotein [synthetic construct]	30.4	30.4	2%	0.31	100.00%	2KQF_B
Chain A, Spike surface glycoprotein [Human coronavirus OC43]	32.7	32.7	20%	1.7	27.83%	6NZK_A

Table 1 illustrates the results of the BLAST analysis, including the Score, Query Coverage, E-value, and Percent Identity. The score represents the alignment of all database sequence segments that match the protein sequence, with higher scores indicating higher homology levels. The percentage of protein length that is aligned with the database is shown by query coverage. Higher E-values indicate lower homology, while lower E-values indicate higher homology. Percent Identity represents the best match between the query and database sequences being compared.

With a score of 66.2, the chandipura virus glycoprotein has the highest resemblance to the rabies virus glycoprotein, suggesting that 66.2 database sequences match the rabies virus glycoprotein sequences. The Chandipura virus glycoprotein has the highest coverage question, with a 53% E-value. The chandipura virus glycoprotein with PDB ID 4D6W serves as the template for homology modeling, with the structure displayed in Figure 2 corresponding to that in PDB.

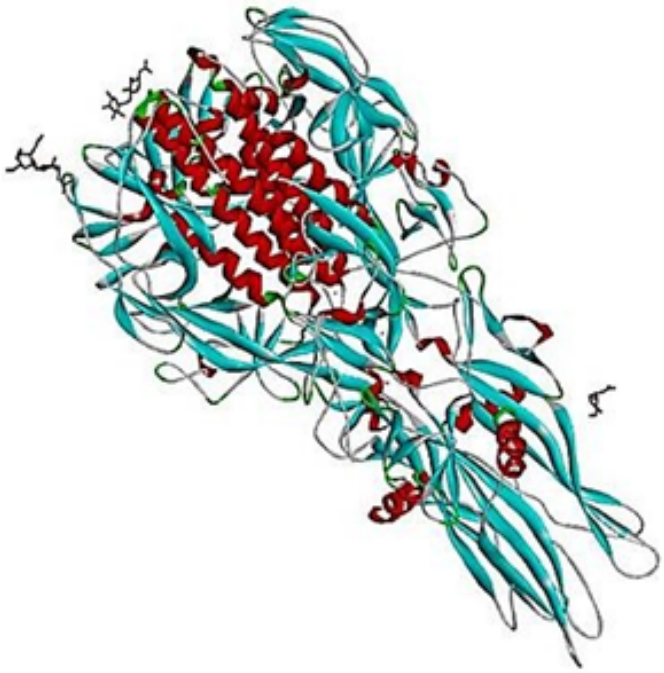

Figure 2: The Chandipura virus glycoprotein structure according to the PDB database.

Figure 2 is the result of validating the structure of the Chandipura virus glycoprotein in the PDB database. The protein structural resolution attained in this experiment was rather low, 3.6Å. resolution is considered to be ideal if it is less than 2Å. The low resolution does not hinder an accurate final model in this case.

3.3. Modeling and evaluation of the rabies virus glycoprotein model

The SWISS MODEL server is used for modeling, and the results are displayed in Figure 3A. The model structure is evaluated by examining overlapping residues and analyzing the Ramachandran plot (Figure 3B). The allowed and generously allowed regions are indicated by light green, while the most favored region is indicated by dark green. The

disallowed region is marked in white. The model is accepted based on data on non-glycine residues in the disallowed region, with a 93.91% percentage of the most favored region.

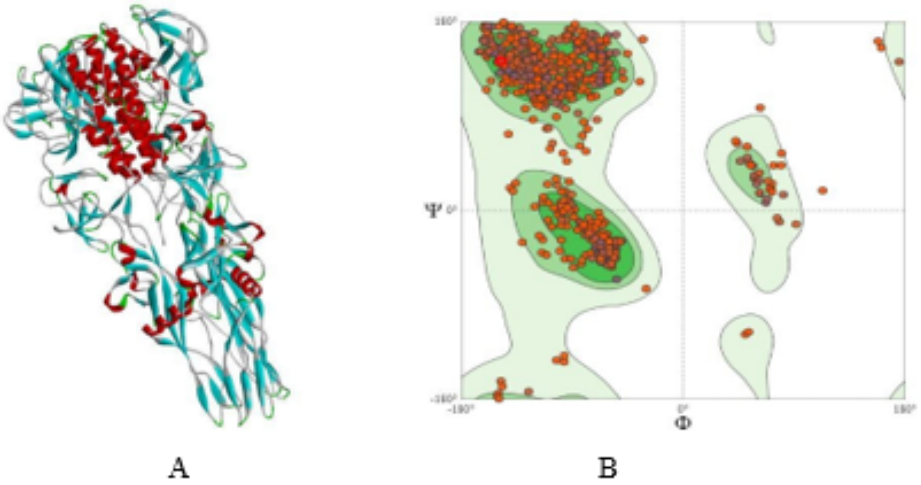

Figure 3: (A) Structure model and (B) Ramachandran plot of Rabies virus glycoprotein modelled by SWISS-MODEL.

3.4. Identification of anti-idiotype antibody templates

The NCBI BLAST server was utilized to explore anti-idiotype antibody templates by evaluating sequences from previous report. 18 As demonstrated in Tables 2 and 3, the results were classified as heavy chains or light chains.

TABLE 2: Heavy chain analysis of IgY anti-idiotype.

Description	Max Score	Total Score	Query Cover	E-value	Per. Ident	Accession
Chain A, IgY Fcu3-4 [Gallus gallus]	406	449	55%	5e-142	91.23%	2W59_A
Chain A, single chain variable fragment [Gallus gallus]	159	159	24%	3e-45	70.73%	5VF6_A
Chain A, Antibody Scfv 180 [Gallus gallus]	147	147	25%	2e-40	68.94%	4P48_A

Table 2 shows that IgY from Gallus gallus has the highest similarity score with anti- idiotype antibodies, with an E-value of 5e-142. IgY Gallus gallus has the highest coverage and Percent Identity queries, with values of 55% and 91.23%, respectively. The template for homology modeling of the anti-idiotype antibody heavy chain is IgY from Gallus gallus, with PDB ID 2W59_A.

TABLE 3: Light chain analysis of IgY anti-idiotype.

Description	Max Score	Total Score	Query Cover	E-value	Per. Ident	Accession
Chain I, Anti-ptau Light Chain [Gallus gallus]	185	185	90%	6e-59	58.49%	4GLR_I
Chain L, Fab 218 anti-SIRP-alpha antibody Variable Light Chain [Homo sapiens]	201	201	89%	2e-65	58.17%	6NMV_L
Chain A, H5.3 FAB Light Chain [Homo sapiens]	179	179	87%	1e-56	54.81%	4XNM_A

According to Table 3, IgY from Gallus gallus shares 185 similarity scores with anti-idiotype antibodies. It has an E-value of 6e-59, 90% coverage, and 58.49% identity queries. IgY from Gallus gallus, PDB ID 4GLR_I, was used as the template for homology modeling of the anti-idiotype antibody light chain. This is due to the fact that a 3D structure template must be of the same species.

3.5. Modeling and evaluation of the anti-idiotype antibody model

The next stage is to generate the model after determining the template that will be used to create a 3D structural model of the target sequence. The SWISS MODEL server (https://swissmodel.expasy.org) is used for modeling, and the results are shown in Figure 4.

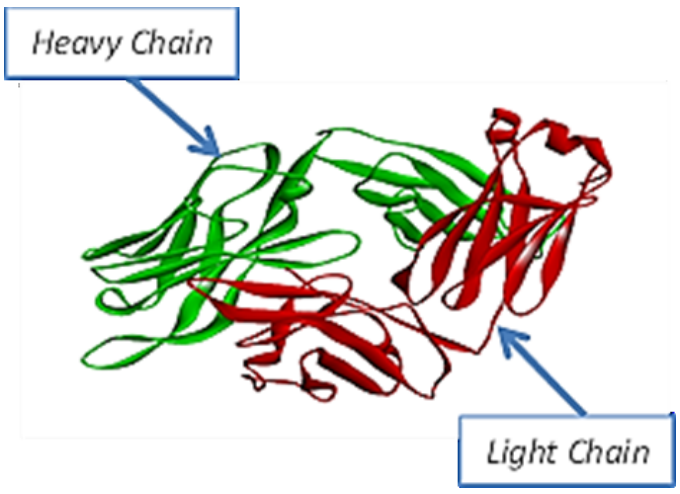

Figure 4: The structure model of the Rabies anti-idiotype antibody.

Figure 4 depicts the 3D modeling of the structure of anti-idiotype antibodies. The structure was divided into two colors: green and red denotes a heavy- and a light-chains, respectively. The model structure is evaluated by examining the GMQE (Global Model Quality Estimation) and Ramachandran plot of the produced model. GMQE is an estimate of structure quality based on target-template alignment. The obtained GMQE value is 0.75, indicating that the model is good. According to Ramachandran's plot, the model is acceptable based on non- glycine residual data. The disallowed region percentage is just 3.3%, while the most favored region percentage is 91.88%.

3.6. The electrostatic mapping value of anti-idiotype antibodies in relation to glycoprotein rabies

Prior to analysis, the CDR part of the anti-idiotype antibody was first determined by changing the appearance of the surface structure of anti-idiotype antibodies with the aromatic, and then given a ball-shaped marker, so that the electrostatic value of the part to be analyzed is only on the structure present within the ball. Aromatic residues, particularly Tyr and Trp residues, are abundant in antigen identification sites and have a significant impact on the process of antibody-antigen interaction. As illustrated in Figure 5, after converting the structure visual to an aromatic appearance, a blue and orange surface is formed, indicating the presence of aromatic residues and most likely the CDR of anti-idiotype antibodies against rabies.

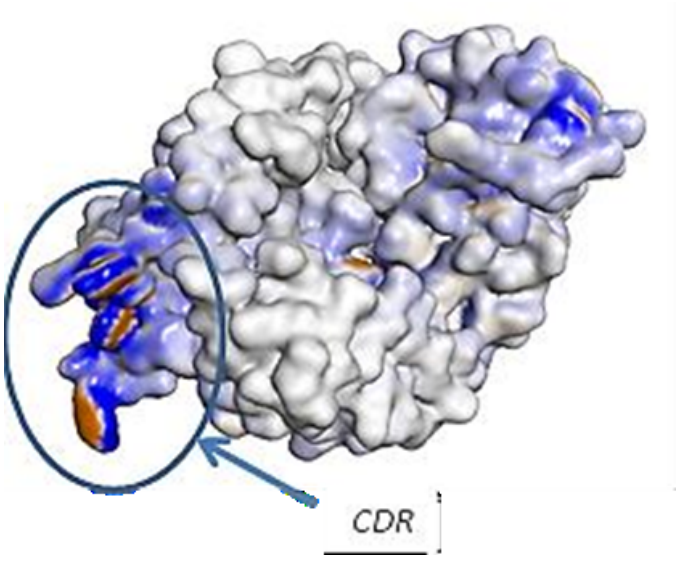

Figure 5: Aromatic residues and potential CDR positions within the structure of antibody anti-idiotype from rabies virus glycoprotein.

The 3D rabies virus glycoprotein structure was docked to a previously generated CDR to analyze the region of interaction (Figure 6A). To detect the entire glycoprotein structure, it was moved to the CDR and compared with anti-idiotype antibodies (assigned as antiabnew). The PDB file format preserves all components imprinted into the CDR, resulting in 27 PDB files obtained from the CDR-glycoprotein structure.

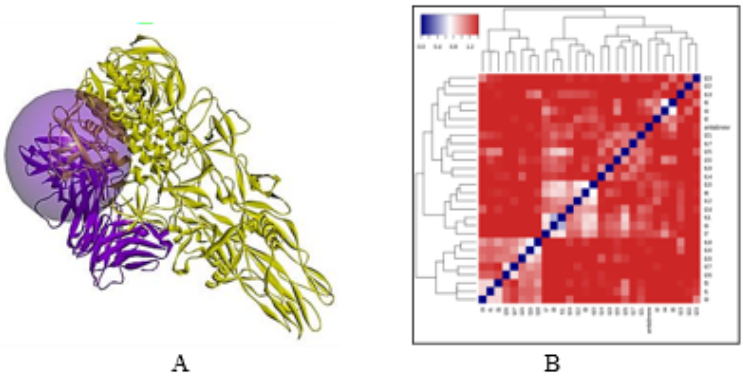

Figure 6: A. Rabies virus glycoprotein structure was docked to a previously generated CDR of anti-idiotype antibodies and B. Electrostatic value heatmap of anti-idiotype antibodies with glycoprotein virus rabies.

The web server PIPSA (https://pipsa.h-its.org/pipsi/) was used to map the electrostatic values of anti-idiotype antibodies with rabies virus glycoprotein. A heatmap of electrostatic values between anti-idiotype antibody structures and rabies virus glycoprotein was demonstrated at Figure 6B. Figure 6B depicts the electrostatic values of rabies virus glycoprotein structures in relation to anti-idiotype rabies antibodies, as indicated by color and values 0-1,4. The blue indicates that the structure's electrostatic values are becoming increasingly comparable to those of other structures, whereas the red indicates that the structure has no electrostatic similarities.

4. Discussion

The relevance of this work is that changes in the electrostatic characteristics of Rabies anti-idiotype antibody compared to rabies virus glycoprotein with their binding properties were identified. Both proteins exhibited a significant number of short-range electrostatic interactions, extremely strong salt bridges, and a very strong salt-bridge at the binding site, as well as the highest electrostatic contributions to binding in, which limit flexibility and making the binding site shape relatively rigid.

Electrostatics have a global and local impact on protein-protein interactions, impacting specificity and affinity by restricting conformational flexibility and modifying structural

and thermodynamic interactions. Because of the lack of substantial electrostatic interactions, the binding site conformation is variable, allowing for greater cross-reactivity with mutant antigens. The overall electrostatic contributions to binding and the scope of the electrostatic environment in protein are determined by the amount and distribution of charged residues [10,16]. The electrostatic characteristics of antibody anti-idiotype and rabies virus glycoprotein differ in this investigation. The enormous number of electrostatic interactions and binding contributions limit flexibility, making the binding site shape stiff. These variances in specificity and affinity can be attributed to these variations [8-10].

Electrostatic interactions affect specificity and affinity directly through complementary polar and charged intermolecular contacts at the binding interface and indirectly through stabilizing partners and their complexes. The quantity and distribution of charges determine the overall electrostatic contributions, whereas the local electrostatic contributions via binding-site salt bridges control the intensities of local interactions [8,9].

Charge mutations affect complexes' association processes, implying methods of association in both complexes. Three main implications may be derived from the findings: changes in electrostatic versus other interactions correlate with differences in antibody specificity and affinity [15]. Electrostatic interactions alter specificity and affinity directly at the binding interface through complementary polar and charged-based intermolecular contacts, and indirectly by stabilizing partners and their complexes, regulating antibody flexibility properties. Electrostatic effects, also known as diffusional electrostatic steering effects, are interactions that occur after a collision that change encounter complexes and transition-state energies [8-10].

5. Conclusion

The study revealed that the 3D rabies virus glycoprotein structure has the most favored region on Ramachandran Plot at 96.6%, with a disallowed region of 1.08%. The 3D structure of anti-idiotype rabies antibodies has the most favored region at 91.88%, with a disallowed region of 3.3%. Electrostatic similarities were found, but electrostatic values were not obtained for each structure.

Acknowledgment

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Research Article

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Relationship Between ODF and PHBS to the Incidence of Diarrhea in South Cimahi Public Health Center

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Abstract.

Diarrhea is a defecation disorder with a frequency of more than three times in 24 hr with watery stools. Based on data from the World Health Organization (WHO), there are 1.7 billion cases of diarrhea that occur globally each year, and in Indonesia, 200 to 400 per 1000 residents each year with 60–80% of cases being children under five years old. Based on data collected by South Cimahi Public Health Center, there were 922 cases of diarrhea in 2022. Diarrhea can be prevented by implementing ODF and Clean and Healthy Life Behavior (PHBS). The Open Defecation Free (ODF) program aims to help people in the area to be free from open defecation. This study aims to understand the relationship between ODF and PHBS behavior with diarrhea incidence of residents in the South Cimahi Health Center area using analytical observation with a cross-sectional study design. A total of 112 residents were selected using a simple random sampling technique. The results showed as many as 56.4% of families who have implemented ODF and have no incidence of diarrhea, and 86.2% of families who have not implemented PHBS with diarrhea. There is a relationship between ODF and PHBS with an incidence of diarrhea (P = 0,00) among residents in the South Cimahi Public Health Center area.

Keywords: diarrhea, ODF, PHBS, public health center

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1. Introduction

Diarrhea is one of the environment-based diseases which can cause death. Diarrhea could be prevented by health and clean hygiene and sanitation. Though there are other etiology of the disease, about 88% of diarrhea-associated deaths are attributable to unsafe water, inadequate sanitation, and insufficient hygiene [1]. Based on data from World Health Organization (WHO), there are 1.7 billion cases of diarrhea occur globally each year. The incidence of diarrhea in Indonesia is estimated at around 200 to 400 per 1000 residents each year with 60 to 80% of cases being children under five years old. Based on data collected by South Cimahi Public Health Center in 2019, among 10.998 diarrhea cases, 3.883 cases were children under five years old. Another data

recorded 512 cases of acute diarrhea and 20 cases of children under five years old in 2021. Meanwhile, in 2022 as many as 922 cases of acute diarrhea with 390 cases were children under five years old [2-5].

Open Defecation is a behavior of defecating other than on a latrine, which can contaminate the environment, soil, air, and water. Open Defecation Free (ODF) are villages in which the community in its area has carried out defecating in healthy latrines/toilets. The evaluation criteria in verification of ODF behavior in the Community-Based Total Sanitation Verification Guidebook include toilet holes with lids, feces disposal distance from water sources more than 10 meters, toilets made of strong material, baby/elderly feces (if there is any) is flushed down the toilet, every resident in the household uses the toilet, availability of anal cleansing, and no human excrement is seen around the house, the yard, outside/nature, and the rivers [4,6]. The objective of the ODF program planned by the government in 2019 is that 100% of residents in the area are defecating on latrines. Cimahi City achieved the 79.9% target of the ODF program while in the area of South Cimahi Public Health Center is 66.9% [4-6].

Clean and Healthy Living Behavior (PHBS) is a form of embodiment of a healthy life orientation in individuals, families, and in the community whose purpose is to improve, maintain, and protect their health physically, mentally, spiritually, and socially. There are 10 indicators of PHBS including childbirth assisted by health workers, exclusive breastfeeding, weighing infants and children up to six years of age, using healthy latrines, eradicating mosquito larvae, eating nutritious and healthy food, doing physical activity every day, and not smoke [3,4]. In 2020, Cimahi City was listed as one of 11 areas in Indonesia with coverage of households already applied PHBS less than 60%. Data in 2022 shows only 2598 (58.55%) from 6339 households have carried out PHBS in the area of South Cimahi Health Center [3,7,8].

Based on Tafere's study in the North Central Ethiopia, the prevalence of diarrhea among children residing in open-defecation-free areas was lower than that among children those who resided in non-open-defecation-free areas. In the area of South Cimahi Health Center, that have not reached the target and fact of high incidence of diarrhea in children under five years old, we are interested to know the relationship between ODF and PHBS programs with an incidence of diarrhea in South Cimahi Public Health Center area. The implementation of PHBS and ODF behavior is related to individuals, families, communities, and their environment's health which could be correlated with the incidence of diarrhea. The incidence of diarrhea in Cimahi City was still high and the achievement of ODF and PHBS programs didn't meet the target, the

health workers and government of the South Cimahi area still have to work hard to reduce the incidence of acute diarrhea [9,10].

2. Methods

The research method used was analytic observation with a cross-sectional design. Measurement for ODF and PHBS done using the questionnaire of Cimahi City Health Office's ODF Verification, Indonesian Ministry of Health's PHBS, and incidence of diarrhea for the last year. The population was residents who owned a place to live in the area of South Cimahi Public Health Center, which registered as much as 6.191 households. The number of samples calculated using the Slovin formula produced a minimum sample of 100 houses of residents who live in the area of South Cimahi Public Health Center and are willing to fill out the questionnaires. Exclusion was made for non-residents and residents who were not willing to fill out the questionnaire.

Evaluation ODF program was carried out by observing the house latrines and the environmental condition of the house according to the questions in the questionnaire. Evaluation of the PHBS program is carried out by interviewing and observing residents and their house environment. According to the criteria from WHO is the incidence of defecation disorder with a frequency of defecation more than three times in 24 hours with the consistency of stools softer or more watery, asked directly to know how many incidences of diarrhea in the last year. See Tables 1–3 below.

3. Results and Discussion

As many as 112 residents who met the inclusion criteria were involved in this study in February 2023. See Table 4 below.

Education is an important factor of education, shaping attitudes, perceptions, beliefs, and one's assessment of health. It could be assumed that a higher level of education is related to higher health awareness as they maintain their hygiene and environmental cleanliness [9,11]. There was 51,8% of respondents completed Senior High School, 31.3% completed Junior High School, and 11.6% are undergraduate/bachelors. This result shows that most of the respondents received sufficient formal education to know, shaping attitudes and healthy living behavior by implementing ODF and PHBS [9,11,12]. See Table 5 below.

Table 1: Open Defecation Free (ODF) verification criteria.

No.	Criteria	Yes or no	Explanation
1.	The toilet/latrine has a lid/cover so no insects around the toilet/latrine		If the latrine/toilet is in a goose-neck shape, then a lid is no longer needed.
2.	The distance between the septic tank and dug wells/water source is >10 meters		If < 10 meters, the septic tank must be watertight (eg concrete septic tank, bio septic tank, etc.)
3.	The squat part of the toilet is made of strong material		Not made of bamboo or weathered wood.
4.	Baby/elderly feces (if there is any) is flushed down the toilet		If there are sanitary pads/diapers then it is treated like waste.
5.	Every resident in the household uses the toilet		Observing the residents and environment
6.	Availability of anal cleansing		Depending on user habits such as the use of water and soap
7.	No human excrement is seen around the house, the yard, outside/nature, and the rivers		Observe

Table 2: Clean and Healthy Life Behavior (PHBS) indicator.

No.	Clean and healthy living behavior (PHBS) indicator	Yes	No
1.	Childbirth assisted by health workers		
2.	Exclusive breastfeeding (6 months)		
3.	Routine body weighing (Ideal weight)		
4.	Using clean water (odorless, colorless, tasteless)		
5.	Washing hands with clean water and soap		
6.	Using healthy latrines (clean toilet, no rats, septic tanks > 10 meters)		
7.	Eradication of mosquito larvae in the house		
8.	Consumes fruit and vegetables every day		
9.	Doing physical activity every day (15 – 30 minutes/day)		
10.	No smoking inside the house		

Table 3: Incidence of diarrhea.

In the last year experienced diarrhea?	
Yes	No

ODF can reduce or prevent the incidence of diarrhea. One of the influential environmental factors is poor environmental sanitation, including fecal disposal facilities. Indiscriminate disposal of feces can cause soil contamination and affect the supply of

Table 4: Respondent education characteristic.

Level of Education	Total	Percentage
Elementary School	6	5,4
Junior High School	35	31,3
Senior High School	58	51,8
Undergraduate/ Bachelor	13	11,6
Total	112	100

Table 5: Distribution of ODF behavior.

ODF	Total	Percentage
ODF	55	49,1
Not ODF	57	50,9
Total	112	100

clean water [9,11,12]. The result showed there are not quite some respondents who have not implemented ODF. See Table 6 below.

Table 6: Distribution of Clean and Healthy Living Behavior (PHBS).

PHBS	Total	Percentage
PHBS	18	16,1
Not PHBS	94	83,9
Total	112	100

The number of families that have implemented PHBS is shown in Table 3. Most of the respondents did not practice PHBS namely 83.9% and the rest 16.1% had PHBS. The assessment is based on a questionnaire with ten indicators with conditions that all points must be met to complete the criteria called implementing PHBS.

A person's clean and healthy living behavior (PHBS) is closely related to improving the health status of individuals, families, communities, and their environment. Healthy behavior in daily life will protect us from various diseases, especially infectious common diseases such as diarrhea [9,11,12]. The result shows there are still very few respondents aware of living a clean and healthy life in the South Cimahi Public Center area. See Table 7 below.

Table 7 describes there were many respondents have experienced diarrhea in the last year. As many as 81 respondents or 72.3% answered that they ever had diarrhea and only 27.1% had not. We asked directly to respondents about the signs and symptoms of diarrhea based on established criteria by WHO.

Table 7: Incidence of diarrhea within the last year in the respondent family.

Diarrhea	Total	Percentage
Diarrhea	81	72,3
No Diarrhea	31	27,7
Total	112	100

Diarrhea is characterized by 3 times or more frequency of defecation in 24 hours with the consistency of stool being more liquid than usual. This disease can be caused by bacterial, viral, and parasitic infections. Infection transmission through contaminated food or drinking water. Diarrhea could be caused by many other factors besides infection [2,13-16]. The etiology of multifactorial diarrhea will not discussed in this study.

3.1. Analysis of the relationship between Open Defecation Free (ODF) and diarrhea

Table 8 below shows the result of the Chi-Square test to determine the relationship between ODF behavior and the incidence of diarrhea within the last year.

Table 8: Relationship between ODF behavior and diarrhea incidence.

Variable	**Diarrhea**				**Total**	**p-value**
	Yes		**No**			
	n	**%**	**n**	**%**	**n**	
ODF						
Yes	24	43,6	31	56,4	55	0,000
No	57	100	0	0	57	
Total	81	72,3	31	27,7	112	

The result of analyzing the relationship between the implementation of ODF and the incidence of diarrhea shows a significant p-value (P= 0,000). It can be concluded that there is a significant difference proportion amount of diarrhea cases between respondents who implemented and respondents who did not implement ODF behavior.

It is my theory that ODF behavior can reduce or prevent diarrhea. One of the influential environmental factors is poor environmental sanitation, including fecal disposal facilities. Indiscriminate disposal of feces would contaminate soil and affect the supply of clean water. Those factors could cause people to get waterborne diseases such as diarrhea [12,17-21].

Febryani in her study also had the same conclusion which proves the relationship between latrine ownership, the condition of latrine sanitary, the distance from the water source to a septic tank, and the use of latrine by all family members with the incidence of diarrhea [22].

3.2. Analysis of the relationship between Clean and Healthy Living Behavior (PHBS) and Diarrhea

The Table 9 below was the result of the Chi-Square test to determine the relationship between PHBS behavior and the incidence of diarrhea within the last year.

TABLE 9: Relationship between PHBS and diarrhea incidence.

Variable	Diarrhea				Total	p-value
	Yes		No			
	n	%	n	%	n	
PHBS						
Yes	0	0	18	100	18	0,000
No	81	86,2	13	13,8	94	
Total	81	72,3	31	27,7	112	

The Table 9 showed that the relationship between the implementation of PHBS and the incidence of diarrhea is significant with a p-value of 0,000 after Chi-square analysis. Therefore it can be concluded that there is a significant difference proportion amount of diarrhea cases between respondents who implemented and respondents who did not implement PHBS behavior.

The result is the theory that an individual's clean and healthy lifestyle is closely related to an increased health status of individuals, families, communities, and their environment. It is clearly explained that implementing clean and healthy living behavior will prevent us from various common diseases, especially infectious diseases such as diarrhea [8-10,23].

Anggraini proves in her study that, the population who has not implemented PHBS has ten times the risk of suffering from diarrhea compared to the population that implementing PHBS. Therefore, it can be concluded that there is a significant relationship between PHBS and the incidence of diarrhea in children under five years old [8,10,17,23].

3.3. Researchers limitation

The limitation of this study is the presence of some confounding cause factors for diarrhea outside of this study such as people who have low immunity, comorbidities, or others. We only put information on the history of diarrhea without asking about other causative factors. In this study, intervention cannot be carried out before filling out the questionnaire.

4. Conclusion

There 50.9% of families have not implemented ODF behavior while 49.1% of families have implemented it.

There 83.9% of families have not implemented PHBS while 16.1% of families have implemented PHBS.

The incidence of diarrhea within the family in the last year is 72.3% and the other 27.1% family has no experience.

There was a relationship between ODF behavior and the incidence of diarrhea in the last year (p=0,000). Families who have not implemented ODF had 100% of diarrhea. Families who have implemented ODF and have had no experience of diarrhea within one last year are 56.4%.

There is a relationship between PHBS and the incidence of diarrhea in the last year (P = 0,000). As much as 86.2% of families who have not implemented PHBS had 100% diarrhea. The rest families who have implemented PHBS and have had no experience of diarrhea within the last year are 100%.

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Research Article

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Effect of Mediterranean Diet Composition on Metabolic Syndrome Marker Parameters Based on NCEP-ATP III Criteria

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Abstract.
Metabolic syndrome refers to a cluster of metabolic abnormalities in the body, including central obesity, elevated blood pressure, high glucose, and triglyceride levels, as well as low HDL levels. The Mediterranean diet, known for its specific composition, is believed to mitigate metabolic syndrome. This study aims to assess the impact of adhering to the Mediterranean diet on individuals with metabolic syndrome. It employs an observational analytic approach, utilizing a prospective cohort of 30 participants who followed the Mediterranean diet regimen as part of their treatment at a clinic in Bandung City. Systematic Random Sampling was used to select participants, and blood pressure was measured at the beginning (T1) and end (T2) of the study. Data on dietary intake were collected using the Relative Mediterranean Diet (rMED) scale via a Food Frequency Questionnaire (FFQ). Statistical analyses, including ANOVA and Tukey's post-hoc test, were conducted to compare dietary compositions across adherence groups and to assess the impact of the Mediterranean diet on metabolic syndrome components. The findings revealed that participants, with an average age of 57.94 years and 69.44% female, experienced significant improvements in certain metabolic syndrome components, such as waist circumference (fruit and nut intake), triglycerides (vegetable intake), HDL (meat intake), and blood glucose (dairy product intake). However, no significant effects were observed for legumes, seeds, fish, and olive oil components on MetS.

Keywords: components, Mediterranean diet, MetS, NCEP-ATP III

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1. Introduction

Metabolic syndrome (MetS) is a collection of interconnected symptoms and risk factors that increase the risk of developing cardiovascular disease and type 2 diabetes mellitus (T2DM) [1]. A person experiences MetS if ≥ 3 symptoms are found which include obesity, high triglycerides, low levels of HDL-cholesterol, increased blood pressure, and increased blood sugar levels [2]. Data from the Framingham Offspring Study, in 2005 SM affected 20-25% of cases in the world with the prevalence of respondents aged 26-83 years being 29.4% in men and 23.1% in women [3]. According to the National Basic Health Research (RISKESDAS), the prevalence of MetS in Indonesia has risen from 14.8% in 2013 to 21.8% in 2018 [2,4]. As outlined by the National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP) III, metabolic syndrome symptoms align with three of the five criteria. These criteria include: 1) waist circumference > 90 cm for men and > 80 cm for women, 2) HDL value < 40 mg/dL, 3) triglyceride value ≥ 150 mg/dL, 4) blood pressure ≥ 130/85 mmHg, and 5) fasting blood glucose ≥ 110 mg/dL [5,6].

According to the American Heart Association (AHA), the Mediterranean Diet is one of the recommended dietary therapies for SM therapy [7-9] The Mediterranean Diet, originating from the Mediterranean region, emphasizes the consumption of plant-based foods like cereals, fruits, vegetables, legumes, nuts, and whole grains, along with olive oil as the primary source of additional fat. It includes moderate intake of fish, seafood, and dairy, while limiting the consumption of red meat and other meat products [10-12].

The Mediterranean diet is well-known, especially in Europe, but its benefits are still not widely experienced by the Indonesian population. This study seeks to elucidate the impact of Mediterranean diet composition on the parameter markers of metabolic syndrome as defined by the NCEP-ATP III criteria.

2. Methods

The research method used is observational analytic with the principle of the prospective cohort in 30 research sample, with man 26,67% and woman 73,33% an average age of 58 years and exhibiting symptoms of metabolic syndrome meeting three out of the five criteria outlined by the NCEP-ATP III. The population in this study were MetS patients who had undergone the Mediterranean diet and followed prolanis at the Bandung city clinic. The sample selection used the Systematic Random Sampling method for

research subjects, blood pressure was checked in the first month (T1) and the last month of the study (T2). Information regarding the consumption of individual components was gathered using the relative Mediterranean Diet (rMED) assessed through the Food Frequency Questionnaire (FFQ). ANOVA test and Tukey's post-hoc test were employed to assess disparities in Mediterranean and MetS diet composition among adherence groups, as well as to evaluate proportion comparisons among rMED adherence groups. Linear regression analysis was utilized to examine the mean and standard deviation ($M \pm SD$) of each Mediterranean diet component consumption, aiming to ascertain the influence of Mediterranean diet composition on MetS.

3. Results

The objective of this study was to investigate the impact of the Mediterranean diet composition on the parameters of metabolic syndrome, as defined by the NCEP-ATP III criteria. From the research results obtained as many as 30 metabolic syndromes that have met the criteria.

MetS individuals adhering to the Mediterranean diet had an average age of 58.12 years, with a median of 59 years and a standard deviation of 8.48 years. The youngest participant in this study was 40 years old, while the oldest was 73 years old. Age can increase the risk of Mets. Increasing age causes changes in body composition including an increase in fat mass to central obesity in MetS patients. Aging can also occur from several mechanisms resulting from the symptoms of MetS with inflammation, oxidative stress, and endothelial dysfunction [13-18].

The majority of metabolic syndrome patients who underwent the Mediterranean diet were female, namely 22 people (73.33%), while there were 8 male patients (26.67%). The rise in MetS incidents is believed to be linked to lifestyle changes influenced by globalization. There's a shift towards modern living, where traditional dietary habits are being replaced by the consumption of instant and Westernized foods. Based on research by Srilaning et al. in 2018 showed women were four times more at risk than men with the results of a sample of women who experienced MetS, namely 26.6%, while men were 18.3%, or women almost 1.5 times compared to men [3].

The statistical analysis indicates a significant correlation between adherence to the Mediterranean diet and the consumption of fruit, nuts, vegetables, legumes, whole

grains, fish, olive oil, and dairy products. However, there is no significant association between adherence to the Mediterranean diet and the consumption of meat.

The multiple comparison test in this study was tested using Tukey's Post Hoc test if the components were significantly significant, whereas if the food components were not significant, then the data was not subject to a Post Hoc test on these components.

Diagram illustrating the mechanisms underlying the impact of bioactive compounds found in the components of the Mediterranean diet. Dietary fiber plays a role in reducing LDL cholesterol levels, synthesizing cholesterol, enhancing insulin sensitivity, and lowering hyperglycemia. Additionally, polyphenols present in fruits and vegetables can enhance antioxidative capabilities, enhance endothelial function, and exhibit anti-inflammatory effects [19-21]. The insignificant content of biologically active compounds in Mediterranean diet components such as legumes and nuts which contain dietary fiber, vitamins, minerals, antioxidants, lignans, and phytosterols, is lost during processing into daily food. This study illustrates that the association between legume consumption and health outcomes is multifaceted and can vary across different populations or circumstances [22-25].

The effect of the Mediterranean diet food components on waist circumference, HDL, triglycerides, blood pressure, and fasting blood glucose is presented in Table 1.

TABLE 1: The effect of the total rMED score and Mediterranean food components on waist circumference, HDL, triglycerides, blood pressure, and fasting blood glucose.

	p-value					
	Waist circumference	**HDL**	**Triglycerides**	**Systolic Blood Pressure**	**Diastolic Blood Pressure**	**Fasting Blood Glucose**
Fruits and Nuts	0,042	0,333	0,156	0,729	0,147	0,886
Vegetables	0,690	0,650	0,019	0,428	0,415	0,396
Legumes	0,466	0,052	0,134	0,886	0,905	0,339
Whole grains	0,316	0,754	0,449	0,960	0,689	0,386
Fish	0,404	0,063	0,537	0,704	0,552	0,089
Olive oil	0,203	0,781	0,762	0,790	0,513	0,702
Meat	0,217	0,008	0,193	0,868	0,616	0,204
Dairy products	0,496	0,337	0,780	0,906	0,071	0,027

According to the study findings, MetS patients following the Mediterranean diet had an average age of 57.94 years, with 69.44% being female. Significant effects were

observed, with fruit and nut components impacting waist circumference, vegetable components affecting triglyceride levels, and meat components influencing HDL levels. dairy products on blood glucose. There is no significant effect between the components of legumes, seeds, fish, and olive oil on MetS.

4. Discussion

Research on the Effect of the Composition of the Mediterranean Diet on the Metabolic Syndrome Marking Parameters based on the NCEP-ATP III criteria shows several influences on the components of the metabolic syndrome. From these data the researchers suggest that the composition of the Mediterranean diet can be applied as a food requirement in everyday life. In addition, further research is needed with a longer period of monitoring the Mediterranean diet to determine the long-term benefits of the Mediterranean diet. Further research is needed regarding the effect of the Mediterranean diet in people who have genetic risk factors for metabolic syndrome to see the benefits of the Mediterranean diet as an eating pattern that can prevent metabolic syndrome. Further research is also needed to examine other factors that influence the control of the 5 components of metabolic syndrome with the Mediterranean diet, such as differences in each group's level of physical activity so that they can find out the therapeutic options given to patients with metabolic syndrome.

5. Conclusion

MetS patients following the Mediterranean diet had a mean age of 58.12 years. The majority of these patients were female, comprising 73.33% of the sample, and most had low dietary adherence, accounting for 36.67%. Significant effects were observed, with fruit and nut components impacting waist circumference, vegetable components affecting triglyceride levels, meat components influencing HDL levels, and dairy product components affecting blood glucose. However, there were no significant effects observed between the components of legumes, seeds, fish, and olive oil on MetS.

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Subchronic Toxicity of Ethanol Extract of Mangosteen Rind (Garcinia Mangostana L.) on Kidney Function in Wistar Rats

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Abstract.

Mangosteen (Garcinia mangostana L.) is a fruit that possesses antioxidant, antibacterial, antihistamine, anti-inflammatory, and antidiabetic properties. Before this, an acute toxicity test was conducted on the ethanol extract of mangosteen rind. However, further testing is required, specifically the subchronic toxicity test, to identify any hazardous effects that may not have been found in the previous study. The objective of this study is to evaluate the amount of toxicity based on the mortality rate, alterations in the relative weight of the kidneys, and changes in renal function as shown by levels of urea and creatinine. This study is an experimental investigation that employs the Post-test Only Control Group Design methodology, with a sample of 40 rats. The rats were categorized into two groups: the control group and the treatment group. The treatment group was divided into three subgroups, each receiving a dosage of 250 mg/kg body weight. The doses were 500 milligrams per kilogram and 1000 milligrams per kilogram of body weight, respectively. The control group received simply water and feed. The duration of the treatment was 28 days. Observations were conducted over 28 days, which involved monitoring animal mortality. On the 29^{th} day, a surgical procedure was carried out to assess the comparative weight of the kidneys and collect blood samples for analyzing the levels of urea and creatinine. The findings demonstrated no mortality in the test subjects, although there was an elevation in the relative mass of the kidneys and an increase in urea concentrations. The increase in urea levels was observed in female rats using the Kruskal–Wallis test (P = 0.019). The study was further conducted using the post-hoc Mann–Whitney test. Control female rats exhibited notable disparities when administered dosages of 250 mg/kgBW and 500 mg/kgBW. The repeated administration of an ethanolic extract derived from the peel of the mangosteen fruit had a detrimental impact on the functioning of the kidneys, as evidenced by a rise in urea concentrations.

Keywords: mangosteen, toxicity test, subchronic, kidney

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1. Introduction

The discovery of new drugs were expected to create effective results and reduce side effects [1,2]. The existence of synthetic drugs as a solution has a high price, and the side effects underlie people's mindset to return to traditional medicine because it is easier to treat [3,4]. Disadvantages of traditional medicine include the lack of standardization of raw materials and the lack of research to test their efficacy and safety, so a toxicity test is needed [5].

Indonesia was known as a fertile and prosperous country. There were 40,000 species of medicinal plants identified in the universe, and approximately 30,000 species are estimated to grow in Indonesia. From these data, only 9,000 species were thought to have medicinal properties. About 5% of that amount was used as phytopharmaka, one of which was the mangosteen fruit (Garcinia mangostana L.) [6,7]. Mangosteen has many benefits because it contains α-mangosteen, which was helpful as an antioxidant, antibacterial, antihistamine, anti-inflammatory, and antidiabetic effects [8,9]. The acute toxicity has tested by Hana [10] and the results showed that the ethanol extract of the mangosteen rind was included in the "Practically Non-Toxic" category. Subchronic toxicity test has been carried out by Chayaburakul et al [11]. The subchronic toxicity test of mangosteen extract at doses of 0, 10, 50, 100 mg/kgBW did not find any damage to kidney function [11]. Hence, it is necessary to carry out a subchronic toxicity test to determine the effects that will be caused in the long term [12,13].

One of the organs that are important for drugs and is the main route of elimination of potentially toxic metabolites and foreign substances for the body is the kidney [14,15]. The basic process of elimination occurs in three processes, glomerular filtration, tubular reabsorption, and secretion. This study will examine the effect of subchronic toxicity of mangosteen rind ethanol extract at doses of 250 mg/kgBW, 500 mg/kgBW, and 1000 mg/kgBW on urea and creatinine levels as parameters to assess the presence of toxic effects that appear on the kidneys. National Agency for Drug and Food Control of Indonesia (BPOM) recommended that the experimental animals were white rat rodents (Sprague Dawley or Wistar strains) male and female [12]. The use of male and female rats as experimental animals is due to differences in anatomy, physiology, biochemistry, and behavior, so the bodies of male and female animals will respond to chemicals in different ways [16,17].

2. Material and Methods

2.1. Plant material

Mangosteen was obtained from Tasikmalaya, Indonesia. Mangosteen was identified and characterized at the School of Life Sciences and Technology (SITH), Bandung Institute of Technology-Indonesia. Mangosteen rind criteria for this study were fresh, dry, and purplish-red to dark purple.

2.2. Mangosteen extract preparation

The ethanol extract of mangosteen rind was prepared in the School of Life Sciences and Technology, Bandung Institute of Technology-Indonesia, using the maceration process. First of all, the mangosteen rind is detached and then cleaned and drained. The mangosteen rinds were sliced, then dried in an oven before being processed by a machine to acquire the powder. Mangosteen rind powder was macerated three times using 96% ethanol till submerged for 24 hours. Next, the filtering process is carried out, and the extract is concentrated using a rotary evaporator so that the extract gets concentrated.

2.3. Experimental animals

Subchronic toxicity test investigation employed standard male and female Wistar rats with criteria of 6-8 weeks of age, weighing 150-200 g and acclimatization for seven days before treatment. Acclimatization was carried out at room temperature with 12 hours light and 12 hours of dark cycle. animals were given water and food available at any time (ad libitum), and animals were randomized into control and experimental groups. All experimental protocols were in line with the Guide for the Care and Use of Laboratory Animals and approved by the local animal care committee, The Health Research Ethic Committee of Faculty of Medicine Universitas Padjajaran Bandung-Indonesia, with ethical approval # 686/UN6.KEP/EC/2021.

2.4. Subchronic toxicity test

Determination of dosage following the guidelines of the National Agency for Drug and Food Control of Indonesia (BPOM) and the Organization for Economic Co-operation and Development (OECD). The procedure of animal care begins with acclimation. The experimental animals are separated into 4 groups, each consisting of 10 rats consisting of 5 rats with female sex and 5 rats with the male sex. The control group was just given water and feed, while the treatment group was the group that was given water, feed, and a dose of mangosteen rind ethanol extract in a varied amount of 250 mg/kgBW, 500 mg/kgBW, and 1000 mg/kgBW, respectively. The test preparation was given orally with multiple dosages per day. Observations were taken for 28 days, for the number of rat deaths. On the 29th day, Animals were sacrificed using the cervical luxation procedure, which was previously anesthetized using ketamine, urea and creatinine levels were studied, the relative organ weight of the kidneys was estimated.

2.5. Biochemical analysis

The principle utilized in testing urea is the Enzymatic ultraviolet (UV) test, the Urease–GLDH method. Urea nitrogen was oxidized using urease and Glutamate dehydrogenase (GLDH) enzymes, and absorbance changes were recorded using a spectrophotometer at a wavelength of 340 nm at 37°C. The test process involves 10 μL of test serum to be reacted with 1000 μL of test reagent for urea nitrogen testing in a 5 mL test tube, homogenized with the help of a vortex. Absorbance was measured with a spectrophotometer at 37°C at precisely 30 seconds at a wavelength of 340 nm, and then absorbance was measured again at precisely 60 seconds.Creatinine examination uses the kinetic test without deproteinization Jaffe technique. Creatinine interacted with alkaline picrate to generate a red hue and was measured using a spectrophotometer at a wavelength of 492 nm, temperature 37°C. The examination process requires 50 μL of test serum to be reacted with 1000 μL of test reagent for creatinine examination in a 5 mL test tube, homogenized with the help of a vortex. Absorbance was measured by spectrophotometer at 37°C after 60 seconds at a wavelength of 492 nm; absorbance was measured again after 120 seconds.

2.6. Relative organ weight

The relative organ weight (ROW) of each animal was calculated as follows:

$$ROW = \frac{Organ\ weight\ (g)\ x\ 100}{body\ weight\ of\ the\ animal\ on\ sacrifice\ day\ (g)}$$

3. Results and Discussion

3.1. Effect of ethanol extract of mangosteen rind on death of experimental animals

The data showed no fatalities of test animals during the observation duration. No deaths suggested that the ethanol extract of mangosteen rind with doses of 250 mg/kgBW, 500 mg/kgBW, and 1000 mg/kgBW was not hazardous. Research conducted by Hana verified that the acute toxicity test with a maximum dose of 5000 mg/kg BW had an LD50 value in the non-toxic category [10,12].

Effect of Ethanol Extract of Mangosteen Rind on Relative Organ WeightRelative organ weight is a means to detect if an exposed organ is injured or not. In toxicity testing, relative organ weight assessment shows good sensitivity in predicting toxicity and correlates with histological abnormalities [18,19].

TABLE 1: Relative organ weight test in male and female rats.

Groups	Average relative organ weight ± SD (mg)				p
	Control	250mg/kgBW	500mg/kgBW	1000mg/kgBW	
Male	0.442±0.099	0.447±0.045	0.542±0.038	0.533±0.050	0.072*
Female	0.505±0.050	0.593±0.047	0.546±0.064	0.542±0.065	0.114*

*tested using one way Anova

There were no significant difference (p>0.05) on relative organ weights in experimental animals which fed ethanol extract of mangosteen rind at doses of 250 mg/kg BW, 500 mg/kg BW, and 1000 mg/kg BW. Kidneys are the principal route of the metabolism of potentially hazardous metabolites and foreign substances. Many medicines and phytochemicals can have a harmful effect on the kidneys by damaging the activity of mitochondria, blocking tubular transit, raising oxidative stress, or the generation of free radicals. Renal reaction to hazardous chemicals can be observed in glomerular shrinkage, cell necrosis, and loss of brush border [14]. The results of this investigation

demonstrated no significant difference between the relative organ weight of the kidneys in the control group and the group that was given a dose of mangosteen rind extract. It revealed that the mangosteen rind ethanol extract was not harmful because one of the parameters for a toxic effect was a weight change of the Relative organs. Previous research conducted by Chayaburakul et al [11] indicated that the subchronic toxicity test of mangosteen extract at dosages of 0, 10, 50, 100 mg/kg BW did not create a significant variation in the relative organ weight of the kidney.

3.2. Ureum levels

TABLE 2: Average levels of urea after administration of ethanol extract of mangosteen rind.

Groups	**Ureum average ± SD (mg/dL)**				**p**
	Control	250mg/kgBW	500mg/kgBW	1000mg/kgBW	
Male	39.84±4.93	38.48±6.51	40.16±4.43	44.98±4.81	0.260*
Female	31.38±1.05	37.12±2.98	33.52±2.84	36.54±3.56	0.019**

*Analyzed with one-way Anova
**Analyzed with Kruskall Wallis

The findings of the One Way Anova test analysis indicate that there is no statistically significant difference ($p > 0.05$) in the impact of administering ethanol extract of mangosteen rind at doses of 250 mg/kgBW, 500 mg/kgBW, and 1000 mg/kgBW on urea and creatinine levels in male rats. The Kruskal-Wallis test revealed a significant difference in the effect of ethanol extract of mangosteen rind at doses of 250 mg/kg BW, 500 mg/kg BW, and 1000 mg/kg BW on the urea levels of the female group ($p \leq 0.05$). Consequently, the analysis proceeded with the Mann-Whitney test.

3.3. Creatinine levels

The One Way ANOVA test conducted on the creatinine levels of male rats and the Kruskal Wallis test performed on females revealed no statistically significant difference ($p > 0.05$). The information is presented in Table 1.

The urea levels of female rats exhibited statistically significant results, warranting the use of a Post Hoc Mann-Whitney test to compare the control group with the three different doses. The study results presented in Table 1 indicate a notable disparity between the control group and the groups administered doses of 250 mg/kgBW and 1000mg/kgBW, respectively. The substantial elevation in urea levels seen at a dosage of

Table 3: The average creatinine levels of male and female rats after administration of ethanol extract of mangosteen rind.

Groups	Creatinine average ± SD (mg/dL)				p
	Control	250mg/kgBW	500mg/kgBW	1000mg/kgBW	
Male	0.426±0.076	0.426±0.038	0.360±0.047	0.440±0.032	0.066*
Female	0.378±0.046	0.364±0.040	0.364±0.038	0.422±0.023	0.086**

*Analyzed with one way Anova
**Analyzed with Kruskall Wallis

1000 mg/kg BW may not be attributable to impaired renal function. As per the Laboratory of Physiology at the Faculty of Biology, Gadjah Mada University in Indonesia, the typical urea levels in female rats were found to be within the normal range of 23.19-44.61 [20].

The kidney is a vital organ responsible for the elimination of potentially harmful metabolites and foreign chemicals from the body, making it crucial for medication processing [14,15]. Factors that can elevate urea levels include eating of high protein diets, gastrointestinal bleeding, corticosteroid use, dehydration, hypovolemic conditions, or kidney injury [21]. Urea and creatinine levels serve as indicators of renal function. However, their sensitivity decreases if the kidney problem is still anticipated. Measuring renal clearance can be significantly influenced by a rise in urea and creatinine levels, particularly when the glomerular filtration rate reduces by around 50-70% [22]. The elevation in urea levels may be attributed to the rat meal, which had a protein composition ranging from 18.5% to 20.5%.

Protein undergoes a process of conversion into peptides and amino acids. The acids present in the colon are absorbed and transported to the liver, with over 90% of them being successfully absorbed. The hepatocytes undergo deamination and transaminases of these amino acids. The surplus nitrogen will be incorporated into the urea cycle. Protein that is not absorbed by the small intestine, along with urea, will undergo conversion into ammonia by intestinal microbes, particularly in the large intestine. Ammonia will permeate through the portal circulation and reach the liver in order to participate in the urea cycle [18,21]. Mangosteen is also rich in xanthone chemicals, which serve as antioxidants. Antioxidants can mitigate oxidative stress caused by elevated reactive oxygen species (ROS) generation, so safeguarding the kidneys from harm marked by heightened levels of urea and creatinine [23,24].

In a study conducted by Chayaburakul et al [11], it was found that administering doses of 0, 10, 50, and 100 mg/kg BW of mangosteen extract did not result in elevated levels of urea and creatinine, indicating no subchronic toxicity. This study employed doses of

250 mg/kg BW, 500 mg/kg BW, and 1000 mg/kg BW to observe the effects of escalating doses on female rats. The results showed significant increases in urea levels [11].

4. Conclusion

The administration of ethanol extract derived from the peel of the mangosteen fruit, at dosages of 250 mg/kg BW, 500 mg/kg BW, and 1000 mg/kg BW, did not result in mortality or alterations in the relative weight of the kidneys. However, it did have a toxic impact on the kidneys, as seen by an elevation in urea levels in female rats. Additional investigation is required through the implementation of renal histological examination and the continuation of chronic toxicity studies.

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Hearing Screening of Infant and Children Suspected of Congenital Hearing Loss at Dustira Cimahi Hospital

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Abstract.
Hearing problems in children impact development, especially speech and language. Some risk factors are known to influence the occurrence of congenital deafness. For the last five years, screening with Otoacoustic Emissions (OAE) and Brainstem Evoked Response Audiometry (BERA) examinations has been used at Dustira Hospital to make a diagnosis and intervene as early as possible. This study aims to obtain the prevalence, risk factors, and OAE and BERA examination results at Dustira Cimahi Hospital. A retrospective descriptive study was conducted on children who underwent the OAE and BERA examination by collecting subject data for the period of January 2020–December 2022. The OAE examination uses distortion product otoacoustic emissions, while the BERA examination is based on the International Standard Organization (ISO). Of the 42 patients who were screened for hearing loss, the 3–5-year-old group was the most dominant (40.4%). Most of the patients were male (66%). While the prenatal risk factor was a history of TORCH infection (12%), the perinatal risk factor was low birth weight (14.2%) and the postnatal risk factor was hyperbilirubinemia (19%). The majority of the OAE examination results were refers found at 32 people (76.1%). The results of the BERA click examination were the presence of wave V found in 38 people (90.4%)). Patients with suspected congenital deafness who underwent hearing screening were in the age range of 3–5 years and were all male. The risk factors found were a history of TORCH infection, low birth weight, and hyperbilirubinemia. The majority of the results of the OAE examination was refer and that of the BERA was wave V.

Keywords: BERA, children, congenital deafness, OAE

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Selection and Peer-review under the responsibility of the 4th ICONISS Conference Committee.

1. Introduction

Hearing plays a very important role for children in learning speech and language, as well as socialization and cognitive development. Children speak based on what they hear so that hearing loss experienced by children from birth will result in speech and language delays. With consequent longer-term risk to educational attainment, mental health, and quality of life. Therefore, hearing loss in neonates must be found immediately to avoid this [1, 2]. Hearing loss in neonates or congenital deafness is the inability to hear that existed since the baby was born. The causes divided based on the prenatal,

perinatal, and postnatal period. Joint Committee on Infant Hearing (JCIH) pronounced that babies who have one or one risk factor will have an increased likelihood of hearing loss. However, it turns out that there are many cases of hearing loss in babies without risk factors. Based on the considerations of JCIH guidelines for the detection and intervention of infants, audiological and clinical evaluation is carried out until the age of 3 months and intervention is carried out before 6 months. A program called Infant Hearing Screening (IHS) has been implemented and developed in order to identify hearing loss in children as early as possible. If hearing loss is detected early, early intervention services can be performed, and prevention of child development delays can be achieved. The IHS program has developed early behavioral observation techniques to screening techniques based on physiological measurements such as otoacoustic emission (OAE) and brainstem response evoke audiometry (BERA) [3-5].

2. Method

This research method is a prospective study by taking data from medical records and interviews with parents of patients undergoing hearing screening at the ENT clinic, Dustira Cimahi Hospital. Data taken in the form of age, sex, risk factors. Then the results of the OAE and BERA examinations were recorded.

3. Results

The study was conducted on patients undergoing OAE and BERA click examinations at Dustira Cimahi Hospital from period January 2020 - December 2022 with a total of 42 patients. Patients who came were referrals from the growth and development pediatric division with complaints of late speech in 36 patients and 6 patient to perform newborn hearing screening. The results of data recording are described below.

3.1. Age characteristics of suspected congenital hearing loss patients at Dustira Cimahi Hospital

Overview of the age characteristics of suspected congenital hearing loss patients at Dustira Cimahi Hospital is shown in Table 1.

TABLE 1: Age frequency distribution.

Age	Frequency (n)	Percent (%)
<1 year	6	14.3
1-3 year	11	26.1
>3- 5 year	17	40.4
>5 year	8	19
Total	42	100%

The majority of children who carried out hearing screening examinations were aged 3-5 years (40.4%) 1-3 years (26.1%) and less than 1 year with 6 people (14.3%) and aged> 5 years with 8 people (19%).

3.2. Gender characteristics of suspected congenital hearing loss patients at Dustira Cimahi Hospital

The sex of the patients who underwent the hearing screening examination is shown in Table 2.

TABLE 2: Sex frequency distribution.

Gender	Frequency (n)	Percent (%)
Male	14	33.3
Female	28	66.7
Total	42	100

The study showed that majority of children who underwent hearing screening were female, with 28 people (66.7%) and 14 male (33.3%).

3.3. Frequency of risk factors of suspected congenital hearing loss patients at Dustira Cimahi Hospital

The risk factors for congenital deafness consist of risk factors during the prenatal, perinatal and postnatal periods. Prenatal risk factors include genetic history, TORCH infection and history of the use of ototoxic drugs. Perinatal risk factors or those during the delivery process, including asphyxia, prematurity and low birth weight (LBW). Postnatal risk factors include hyperbilirubinemia, history of exchange transfusion and history of treatment in the NICU.

The distribution of risk factor frequencies is shown in Table 3.

TABLE 3: Frequency distribution of risk factors.

Prenatal	Frequency (n)	Percent (%)
Genetic	0	0
Ototoxic medicine	1	2.4
TORCH infection	5	11.9
Total	6	14.2
Perinatal	Frequency (n)	Percent (%)
Asphyxia	3	7.1
LBW	6	14.3
Prematuritas	2	4.8
Total	11	26
Postnatal	Frequency (n)	Percent (%)
Hyperbilirubinemia	8	19
NICU Carehistory	6	14.3
Exchange Transfusion	0	0
Total	14	33.3

From the table above, it can be seen that infants who underwent screening had prenatal risk factors (14.2%) in the form of a history of use of ototoxic drugs (2.4%) and TORCH infection (11.9%). Perinatal risk factors were found in 11 people (26%) in the form of low birth weight (14.3%) asphyxia (7.1%) and prematurity (4.8%). Postnatal risk factors (33.3%) included hyperbilirubinemia (19%) and NICU care (14.3%).

3.4. Results of Otoacusticc Emmision (OAE) examination of suspected congenital hearing loss patients at Dustira Cimahi Hospital

Otoacoustic Emmision (OAE) examination is performed as an initial screening to check the condition of hair cells in the cochlea. The results of the OAE examination are shown in Table 4.

TABLE 4: OAE test results.

OAE result	Frequency (n)	Percentt(%)
Pass	10	23.9
Refer	32	76.1
Total	42	100

Based on Table 4, it can be seen that the majority of OAE examination results are refer was found at 32 people (76.1%) and pass found at 10 people (23.9%). The pass results describe hair cells responding to sound stimuli, while the reference results cannot confirm hair cells do not respond to sound and should be followed by BERA examination.

3.5. Results of Brain Evoked Response Audiometry (BERA) examination of suspected congenital hearing loss patients at Dustira Cimahi Hospital

OAE examination is followed by BERA examination to assess auditory function in the auditory system. The results of the BERA examination are shown in Table 5.

TABLE 5: BERA examination frequency distribution.

BERA Examination	Frequency (n)	Percent (%)
Wave V (+)	38	90.4
Wave V (-)	4	9.5
Total	42	

Based on the Table 5 it can be seen that the majority of BERA test results were the presence of wave V, found at 38 people (90.4.7%) and there is no wave V found at 4 people (9.5%). The absence of V waves describes the absence of a response in the brainstem to a sound stimulus, meaning the child cannot hear at all. The presence of V waves can mean normal or mild to severe hearing loss, and can be corrected with hearing aids or cochlear implants.

4. Discussion

Deafness and hearing loss are widespread and found in every region and country. Today more than 1.5 billion people (nearly 20% of the global population) live with hearing loss. 430 million of them have crippling hearing loss. World Health Organization (WHO) estimate that by 2050, there will be more than 700 million people with disabling hearing loss. Five out of 1000 children are born or have hearing loss during early childhood. Hearing loss is estimated to be the leading cause of disability worldwide. Globally, 34 million children have deafness or hearing loss, of which 60% of cases are caused by preventable causes [6-9].

This study found that the majority of children who carried out hearing screening examinations were in the age range 3-5 years (40%), meanwhile the age <1 year was 6 person (14.2%). The ideal time a baby should undergo hearing screening is before the age of 1 month. Infants who do not pass the neonatal screening should have an audiological examination before 3 months for confirmation. This is because there is an increased risk of hearing loss in infants with risk factors, and experts recommend these children should undergo a 3-year monitoring period [10].

The results of this study are in accordance with research conducted by Thirunavukarasu et al, 2015 on BERA examination in high-risk children and infants under 10 years, 68% of patients belong to the age group of 1 - 5 years. Similarly, in Thakkar et al's 2018 study on BERA examination in the pediatric age group, 80% of patients belonged to the age group 0-5 years. OAE examination by Gunawan et al, 2016 also found the same thing where almost 91% of children who underwent hearing screening examination were aged < 5 years [11-13].

Language development begins generally during the age of 1 - 3 years. This makes parents most often detect hearing loss or speech delays during this age. Patients most often come to an ENT specialist or audiologist during this age period. In conducting anamnesis or examination of previous patient medical records, most patients have risk factors for hearing loss [12].

Based on gender, the majority of children who carried out hearing screening examinations were females with 28 people (66.7%) and 14 male (33.3%). This result is different with research by Sari et al, 2015 which states that the majority of children who undergo hearing screening examinations are male (65.9%). The same thing was also said by Wiryadi et al, 2019 that more male children come for hearing screening (62%) [14, 15].

Although baby boys more often experience brain maturation disorders, impaired brain white matter development and nerve dysfunction, there is no mechanism that states which gender is prone to hearing loss [16, 17].

Infants who underwent screening had prenatal risk factors (14.2%) in the form of a history of use of ototoxic drugs (2.4%) and TORCH infection (11.9%). Perinatal risk factors were found in 11 people (26%) in the form of low birth weight (14.3%) asphyxia (7.1%) and prematurity (4.8%). Postnatal risk factors (33.3%) included hyperbilirubinemia (19%) and NICU care (14.3%).

Research by Gupta et al, 2019 also found that the majority of risk factors at baby who perform hearing screening is low birth weight (25.2%) and very low birth weight (49.4%).

In a study by Rianto et al, 2017 the incidence of SNHL in low weight was also higher than in normal body weight (p = 0.01; OR = 3.82; CI 95% = 1.18 - 12.67) thus associated with a higher need for hearing screening. According to Cristobal & Oghalai, impaired hearing function in newborns with low or very low body weight can be caused by resorption of temporal bone mesenchyma and osteoclatic erosion causes premature pneumatization of temporal bone, resulting in impaired density and air cellsm the mastoid so that this condition causes impaired transmission of acoustic emissions [18-20].

Several other risk factors associated with permanent bilateral congenital hearing loss, including low birth weight < 2500 grams, so hearing screening is needed [12]. Research conducted by Frezza et al, 2019 states that the group of babies at risk of hearing deficit are premature newborns born < 33 weeks of gestational age, especially those admitted to the NICU. Prematurity can cause impaired function of cochlear outer hair cells due to the immaturity of the cochlear organ both anatomically and functionally, so there is a significant difference between the results of OAE examination in neonates aged < 32 weeks compared to full-term. Premature infants are at high risk of hearing loss and their evaluation should be prompt and accurate to ensure activation of early habilitation (within the accepted limits of 4 - 6 months of correction age) that can, in addition, promote maturation of the auditory pathway. However, this category of children requires special caution because common changes in hearing dysfunction are observed. This aspect is very important in the choice and timing of treatment, but also in communication and counseling to the patient's parents [21].

Research conducted by Sari et al, [14] on pediatric patients who underwent hearing examination that the most birth method for these patients was spontaneous (81.9%). Labor with complications such as premature rupture of membranes, large baby size, prolonged labor can increase the risk of perinatal asphyxia leading to impaired oxygenation / hypoxia in the baby. Perinatal asphyxia is a risk factor with a strong association with the occurrence of disorders of hair cells outside the cochlea so it needs special attention immediately [22-23].

Otoacoustic emission (OAE) is a meaningful screening tool in neonatals. The procedure involves inserting a sound probe into the outer ear canal. In the probe there is a microphone and loudspeaker (loudspeaker) that functions to provide sound stimulus. The microphone functions to pick up the sound produced by the cochlea after giving a stimulus. The ear plug is connected to a computer to record the response arising from the cochlea. The inspection should be done in a quiet or soundproof room, this is to reduce environmental noise [4, 24]. The majority of OAE examination results are refer

was found at 32 people (76.1%) and pass found at 10 people (23.9%). This is similar with the research of Purnami et al, 2018 where the majority of research subjects were included in the refer category (67.75%). Likewise, the research of Sari et al, that in OAE examination, the majority of the results of the right ear OAE examination were refer (60%) and left ear OAE was also refer (55.1%) [14, 25].

OAE examination is the test that is most often done as a screening because the procedure is easy, safe, and fast. This examination is also easy to interpret, and has a sensitive indication of hearing loss. OAE also does not require special human skills [26].

Brainstem Evoked Response Auditory (BERA) is an electrophysiological recording of responses derived from auditory tissue activation to sound stimuli, starting from the cochlea to along the brainstem, aiming to assess the integrity of nerve synchronization. This examination is non-invasive and has a fairly high objective value [5, 26]. The principle of the BERA examination is to assess changes in electrical potential in the brain after the administration of sound stimuli. Sound stimuli given through the head phone or insert probe will travel through the cochlea (wave I), cochlear nucleus (wave II), superior olivarious nucleus (wave III), lateral lemnicus (IV wave), inferior colliculus (V wave) then go to the auditory cortex in the temporal lobe of the brain [27].

The majority of BERA test results V waves found at 38 people (90.4%) and there were no V waves found at 4 people (9.52%). According to research by Wiryadi et al, [28] the majority of children in hearing screening with the most BERA examination results are the presence of V waves (57.6 - 60.8%). The presence of V waves illustrates no response in the brainstem to a sound stimulus. The presence of V waves illustrates that the brainstem response is still present, even though the patient is clinically normal, or has mild to severe hearing loss. This is a consideration in carrying out hearing rehabilitation with hearing aids or cochlear implants.

V wave is the most stable wave, easy to assess even to the point of a low-intensity and clinically meaningful sound stimulus, therefore the assessment of the predominant BERA potential is based on the latent period (time interval between stimulus onset and wave peak) absolute peak [29].

In this study,10 people with OAE pass results and 4 people who did not find wave V on the BERA examination. OAE describes the response of hair cells in the cochlea, while V waves in BERA describe the brainstem response to sound stimuli. This study is similar to the study of Mirajkar et al, [30] where in 15 children obtained 11 children with bilateral

OAE refer results who had normal BERA results, while 4 other children had abnormal BERA results. Johnson et al, [31] also obtained similar results where in 21 children with OAE refer results also had normal BERA results. The results of unilateral OAE refer and BERA refer are not always associated with nerve deafness but can also be caused by disorders of the middle ear. In a study conducted by Chang et al, [32] found 12 children with unilateral OAE refer results with abnormal BERA results. One child with conductive deafness, 10 children with sensorineural deafness, and 1 child with mixed deafness.

The difference in results between OAE and BERA can be because OAE reflects problems in the cochlea, while BERA reflects problems in the auditory nerve. That is, it could be a case of referring to OAE when there is only a problem in the cochlea but there is no problem with the auditory nerve so that the test results still detect a V wave in BERA [25]. What happens in this study is that the results of OAE refer and the results of BERA pass can be due to middle ear disorders that are not eliminated during the examination so that the OAE results are not accurate, hence the need for tympanometric examination before the examination is carried out. Another possibility is the presence of auditory neuropathy. Auditory neuropathy is a condition in which the function of hair cells is abnormal, while the function of the auditory nerve is impaired. Therefore, on examination, normal OAE and abnormal BERA results will be found [33].

5. Conclusion

Patients with suspected congenital deafness who perform hearing screening are in the age range of 3-5 years, male sex, the risk factors found are history of TORCH infection, low birth weight and hyperbilirubinemia. The majority of the results of the OAE examination was refer and BERA examination was found wave . Hearing screening in children needs to be done as early as possible considering the number of hearing loss is still common at the age of speech development.

Conflict of Interest

There is no conflict of interest in the writing of this paper

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Research Article

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Description of Characteristics, Main Complaints, Duration of Treatment, Complications and Comorbidities in Covid-19 Patients at Tk.II Dustira

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Abstract.
The 2019 coronavirus disease was declared a pandemic by the World Health Organization in March 2020. By 2021, Indonesia had a total of 4,262,720 cases and 142,560 deaths. By 2021, the total number of confirmed COVID-19 cases grew to 4,262,720. Since the deaths due to the coronavirus amounted to 144,094, clinical manifestations in patients with COVID-19 have a wide spectrum, ranging from no to severe symptoms. Most patients have symptoms such as fever, cough, sneezing, and shortness of breath. Age, gender, accompanying diseases, clinical symptoms, and laboratory parameters are all related to the duration of treatment for COVID-19 patients; comorbidities can cause complications such as hypertension, heart disease, chronic kidney disease, and chronic lung disease. The research draft used in this study is descriptive. Previous research showed that the age of most participants (55%) was 50 years and 59% were male, 91% had respiratory complaints, 90% had a respiratory cough, 94% had a non-respiratory fever, the treatment length was 6–11 days in 68%, bronchopulmonary pneumonia as a complication was seen in 43%, and comorbid hypertension was reported in 35%. The results of the study showed that elderly patients are at greater risk of getting infected with COVID-19, and respiratory complaints are the most common because COVID-19 attacks the reproductive organs, and fever is a manifestation of the infection of the body with the virus. Hypertension is the most common comorbidity that causes the activation of COVID-19 caused by ACE 2.

Keywords: Covid-19, primary complaint, treatment length, complication, comorbid

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1. Introduction

The World Health Organization (WHO) has designated the coronavirus disease 2019 (Covid-19) pandemic since March 2020, and has resulted in 1,521,252 cases and 92,798 deaths across the globe. In Indonesia, cases of Covid-19 were recorded until May 18, 2020, with 18,010 confirmed positive cases and 1,191 deaths [1].

In 2021, Indonesia achieved a total of 4,262,720 confirmed cases and 142,560 deaths. Graph data always shows an increase in the number of confirmed cases every month. This is due to the high mobility of the Indonesian people who celebrate the New Year in January 2021 and Eid al-Fitr in May 2021 and the emergence of a new variant, the Delta variant, is considered to contribute to an increase in the number of confirmations [2].

In 2021 in West Java, confirmed cases of Covid-19 have reached 4,262,720 infected people. Meanwhile, 144,094 people died due to the coronavirus, and 4,292 were still sick, and 4,114,334 people were declared cured. Dustira is one of the Covid-19 referral hospitals for the cities of Bandung and Cimahi, Dustira Hospital is a reference for handling West Java Covid-19 cases because Dustira Hospital has 349 Covid-19 patient care beds. In 2021 Dustira Hospital handled 527 Covid-19 cases [3].

Most patients infected by coronavirus have symptoms such as fever, coughing, sneezing, and shortness of breath. Some other symptoms can be found productive cough, sore throat, headache, myalgia and arthralgia, chills, nausea and vomiting, nasal congestion, diarrhea, abdominal pain, hemoptysis, and conjunctival congestion [4]. Patients with severe primary complaints usually develop heart and lung complications. This is because both of these organs have ACE-2 receptors. Both organs from Covid-19 can cause Acute Kidney Injury, this is because the kidneys have ACE-2 receptors [5].

Age, sex, comorbidities, clinical symptoms, and laboratory parameters are all factors that can affect the length of stay of Covid-19 patients [6].

Comorbidities that can lead to complications such as hypertension, heart disease, chronic kidney disease, and chronic lung disease [7]. Deaths from Covid-19 infection with the highest comorbid order were diabetes mellitus 32.5%, kidney 26.5%, cardiovascular 11.3%, cancer 10.6%, and chronic obstructive pulmonary disease (COPD) 6.6%. This suggests that diabetes mellitus is a comorbid that can cause the highest percentage of deaths in patients with the highest percentage of comorbidity [8].

Coronavirus is a single-chain and positive-chain RNA virus belonging to the coronaviridae family which is divided into subfamilies according to serotype and genotype characteristics which include a, β, γ and δ. Four main structural proteins are found in coronavirus particles: spike protein (S), membran (M), envelope (E), and nucleocapsid (N). All of these proteins are encoded on the viral genome's 3' end [9].

This virus is spread by patients via droplets or aerosol particles that enter the airway. The virus binds to the ACE-2 receptor on the plasma cell membrane of humans, which is

the main factor in infecting its host species. In cells, these viruses copy genetic material and proteins to form new virions on the cell surface. Furthermore, this virus will release RNA genomes into the cytoplasm and golgi of cells, which will then be translated into two lipoproteins and structural proteins so that it can replicate. The virus is then released from the infected cells through exocytosis. It can infect kidney cells, liver cells, heart, testines, T lymphocytes, and the lower respiratory tract. When the virus enters a cell, it presents the viral antigen to the Antigen Presentation Cell (APC). T cells and B cells mediate the humoral and cellular immune systems [10].

After transmission, the virus enters the upper airway and replicates in the epithelial cells of the upper airway, then spreads to the lower airway. In acute infections, viral decay occurs in the airway, and the virus may remain decayed in gastrointestinal cells after healing. The virus incubation period is about 3 to 7 days [11].

Respiratory exposure that carries the infectious virus is the main way people get infected with SARS-CoV-2. Other ways of transmission include contact and air transmission. Viruses released in respiratory secretions (like coughing, sneezing, and talking) can infect others by touching their mucous membranes. WHO provides update pada July 9, 2020, states that airborne transmission can play a role in the spread of Covid-19, especially in closed spaces. WHO emphasized the importance of social distancing and wearing masks in prevention. WHO continues to support airborne transmission as a method of spreading disease [12].

There are other precautions that need to be followed, such as hand washing, wearing masks at all times, keeping a safe distance, avoiding crowds, and limiting mobility. When experiencing Covid-19 symptoms, individuals should self-isolate right away and perform a nasopharyngeal swab examination [13].

2. Method

2.1. Research design

In this study, the research design was descriptive. The sampling technique was accidental, and the data used was secondary data from 96.04 medical records of Covid-19 patients in 2021, rounded up to 100.

In this study, patients who have been confirmed to have Covid-19 and are currently undergoing treatment at TK.II Dustira Hospital and have moderate severe symptoms

were excluded. Exclusion criteria for this study included medical records from Covid-19 patients that were incomplete, damaged, or illegible in terms of main complaints, treatment duration, complications, and comorbidities.

2.2. Place and time of research

This research was carried out at Dustira Cimahi Hospital from October 2022 to December 2022.

2.3. Research procedure

The medical records of Covid-19 patients receiving care at Dustira Hospital were examined first by the researchers. Samples were then gathered in accordance with the inclusion and exclusion criteria. Subsequently, the information is entered and examined using SPSS statistical software.

2.4. Research analysis

Univariate analysis was performed to calculate the number, percentage (proportion) for the characteristics of each study subject and research variables (characteristics, main complaints, duration of treatment, complications and comorbidities).

2.5. Research aspects

The ethics committee of Dustira Hospital has granted permission for this research, and has obtained permission for the collection of medical record data at Dustira Hospital with the letter number Etik.RSD/006/I/2023.

3. Results and discussion

The study was conducted in January 2022. The object of research is the Medical Record of Dustira TK.II Cimahi Hospital which fulfils the inclusion and exclusion requirements. The number of respondents obtained by researchers amounted to 100 medical records where the amount of data obtained was in accordance with the plan of the researcher.

3.1. Characteristics of research subjects based on age and gender

Table 1: Features of research subjects at TK.II Dustira Hospital in 2021: Distribution by age and gender in Covid-19 patients.

Characteristic	n	%
Age		
<20 years	2	2
20-35 years	13	13
36-50 years	30	30
>50 years	55	55
Gender		
Man	59	59
Woman	41	41
Total	100	100

Table 1 shows that the lowest number of patients is <20 years old 2 people (2%), and the highest number of patients in 2021 aged >50 years is 55 people (55%).

Mohitosh Biswas et al.'s 2020 study in China found that age factors, which categorise people into two age groups—less than 50 and beyond 50—were connected to death. The study's findings indicate that the age group over 50 who contract Covid-19 has a higher mortality risk than the age group under 50 [14].

In addition, elderly individuals have taken many drugs or consumed several types of drugs at the same time in comorbid therapy efforts that result in decreased organ function. Excessive expression of ACE2 may occur in patients over 50 years of age due to impaired immunity, impaired organ function. Comorbidities and many other causes that increase the risk of death [15].

Table 1 displays the majority of Covid-19 patients' genders at TK.II Dustira Hospital in 2021 with 59 men (59%) and 41 women (41%).

According to a meta-analysis study, men are 28% more likely than women to get Covid-19 infection. This finding links sex with the risk of infection [14]. It is well known that men express more of the sexual hormone ACE2, which is linked to an increased risk of SARS-CoV-2 infection in men. Smoking is a lifestyle choice that puts males at increased risk of contracting the corona virus and maybe dying [15].

TABLE 2: Overview of the main grievances raised by TK.II Dustira Hospital Covid-19 patients in 2021.

Main Complaints	n	%
Respiratorik	91	91
Non-Respirator	9	9
Total	100	100

TABLE 3: Description of the most respiratory complaints raised by TK.II Dustira Hospital Covid-19 patients in 2021.

Respiratory Complaints	n
Cough	90
Shortness of breath	89
Chest pain	2

TABLE 4: Description of the most non-respiratory complaints raised by TK.II Dustira Hospital Covid-19 patients in 2021.

Non Respiratory Complaints	n
Fever	94
Headache	32
Liquid defecation	2
Loss of consciousness	1
Nausea, vomiting	15
Slogans	28
Anosmia	3

3.2. Overview of the main complaints in Covid-19 patients

Table 2 shows the majority of patients with Covid-19 in TK.II Dustira Hospital in 2021 with 91 respiratory main complaints (91%) and 9 non-respiratory main complaints (9%).

Prior studies carried out in 2020 by Xiaoneng Mo in China showed that the lungs are the organs most affected by Covid-19. These diseases include fibrous proliferation of the alveolar septum, diffuse alveolar epithelial destruction, capillary damage/hemorrhage, hyaline membrane formation, and pulmonary consolidation, all of which can impair a person's ability to function [16].

Tables 3 and 4 show that the most respiratory complaints are cough 90 people (90%), complaints of shortness of breath 89 people (89%) and complaints of chest pain 2 people (2%). For the main non-respiratory complaint, the most complaints are fever

as many as 94 people (94%) and the least is a decrease in consciousness, which is 1 person (1%).

According to data from the Republic of Indonesia's Ministry of Health 2020, fever, coughing, lethargy, and shortness of breath are the most typical signs and symptoms of COVID-19 infection. Fever and respiratory problems were the most often reported symptoms in the Wuhan study [17].

The most common sign is fever. 43.8% of patients had a fever at admission, however the percentage of patients who arrived at the hospital partially without a fever rose to 88.7% while they were there. Enhanced cytokine activity releases TNFα, IFN-γ, IL 1, IL4, and IL 6 at the proper concentrations, triggering both nonspecific and cellular immunity [18].

3.3. Overview of treatment duration in Covid-19 patients

Table 5: Overview of the length of treatment based on the main complaints raised by TK.II Dustira Hospital Covid-19 patients in 2021.

Duration of Treatment	n	%
<6 days	9	9
6-11 days	68	68
>11 days	23	23
Total	100	100

Table 5 shows the average length of treatment for patients with Covid-19 in TK.II Dustira Hospital in 2021 was 10 days with a minimum length of treatment of 3 days and a maximum of 21 days and mostly patients with Covid-19 in TK.II Dustira Hospital in 2021 with a length of treatment of <6 days as many as 9 patient (9%), 6-11 days as numerous as 68 patient (68%) and >11 days as numerous as 23 patient (23%). The most treatment duration is 6-11 days of treatment with a total of 68 people (68%).

There are several studies conducted by Rizka Fahmia et al. in Indonesia in 2020 saying that significant risk factors for the length of treatment are age, gender and comorbidities owned by patients. Especially in male patients, aged over 45 years and have comorbid obesity, hypertension and asthma [19].

Table 6: Overview of the form of complications raised by TK.II Dustira Hospital Covid-19 patients in 2021.

Complications	n	%
Bronchopneumonia	43	43
Heart Disorders	24	24
Kidney Disorders	11	11
ARDS	11	11
Hepatic Disorders	7	7
Electrolyte Disturbance	2	2
Stroke	2	2
Total	100	100

3.4. Overview of complications of Covid-19 patients

Table 6 reveals that in 2021, there were 43 patients (43%) with problems from pneumonia at TK.II Dustira Hospital, while there were only 2 patients (2%), who had difficulties from stroke and electrolyte disorders.

Broncopneumonia covid-19 often occurs in both lung organs, considering that the covid virus can infect quickly. A comparative study in America conducted by comparing the condition of patients with covid broncopneumonia with other pneumonia also reinforces this fact. When compared using CT scans and lab tests, results show that most covid broncopneumonia will attack both lungs instead of one [20].

The SARS-CoV-2 coronavirus is the source of Covid-19, a condition that can harm heart muscle and impair cardiac function. Cells in the heart have Angiotensin Converting Enzyme-2 (ACE-2) receptors, to which the coronavirus attaches before entering the cell. Heart damage can also be caused by high levels of inflammation circulating in the body. As the immune system fights the virus, the inflammatory process can damage some healthy tissue, including the heart. Coronavirus infection also affects the inner surface of veins and arteries causing inflammation of blood vessels, damage to very small blood vessels (capillaries), and blood clots, all of which can disrupt blood flow to the heart or other parts of the body [21].

TABLE 7: Overview of comorbid factors raised by TK.II Dustira Hospital Covid-19 patients in 2021.

Comorbid	**n**	**%**
Hypertension	35	35
DM	19	19
Heart Disorders	13	13
Limfoma post chemo	1	1
Meningitis	1	1
Indigestion	8	8
Kidney Disorders	4	4
Neurological disorders	3	3
Dengue	1	1
Old Age	12	12
Pregnant	2	2
Overweight	1	1
Total	100	100

3.5. Description of comorbid factors in Covid-19 patients

Table 7 shows that most Covid-19 patients at TK.II Dustira Hospital in 2021 with comorbid factors of Hypertension were 35 people (35%) and the least with comorbid factors of Post chemo, Meningitis, Dengue and Overweight Lymphoma each 1 person (1%).

Some studies say hypertension has a relationship with COVID-19, where hypertension will aggravate Covid-19 infection and can even be a pathogenesis of Covid-19 infection. This virus will bind to Angiotensin Converting Enzyme 2 (ACE2) in the lungs. Several studies state that the severity and mortality of Covid-19 are influenced by several comorbid diseases, one of which is hypertension, where existing hypertension can aggravate 2.5 times COVID-19 [22].

The position of hypertension as the most comorbids in Covid-19 patients is supported by research conducted in 2020 and it was found that hypertension became the most comorbid, namely 21.1%. In hypertensive patients suffering from Covid-19, there is an increase in ACE-2 expression which causes high susceptibility to Covid-19 infection, especially treatment with angiotensin II receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors (ACEi). This can lead to worsening and severity of Covid-19 infection due to increased binding of the virus to target cells that utilize ACE-2 [23].

At 33.6%, diabetes mellitus is the second most common concomitant condition among Covid-19 patients in Indonesia. Covid-19 attaches itself to the surface of human body

cells via ACE-2 receptor binding, which allows Covid-19 to enter the cell. Studies by Marko Marhl et al. demonstrated that ACE-2 receptor expression is elevated in diabetes situations. The higher expression of ACE-2 in individuals with both type 1 and type 2 diabetes mellitus provided additional evidence for the study [24].

4. Conclusion

Considering the findings of the studies that have been done, the results of Covid-19 patients with the most main complaints are respiratory complaints as many as 90 people (90%). Covid-19 patients carried out 6-11 days of treatment as many as 68 people (68%). The major complications in Covid-19 patients were Broncopneumonia as many as 43 people (43%). The major comorbid in Covid-19 patients is hypertension as many as 35 people (35%).

Looking at the results obtained by researchers with the most main complaints, namely respiratory complaints, health workers must prepare qualified facilities to handle the conditions experienced by patients so that there are no ongoing complications and worsen the condition of patients. Hospitals must also prepare a sufficient number of treatment rooms to treat patients. Make sure health workers are competent to handle complications and comorbidities experienced by patients to prevent further worsening.

Conflict of Interest

The author of this paper affirms that there is no conflict of interest in any of the scholarly publications we produce.

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Research Article

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Linezolid As a Treatment for Multidrug-resistant Tuberculosis: A Literature Review

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Abstract.

Multidrug-resistant tuberculosis (MDR-TB) emerges when Mycobacterium tuberculosis develops resistance to both rifampicin and isoniazid, representing a significant threat that undermines global efforts to combat tuberculosis. The unfavorable prognosis of MDR-TB can be attributed to prolonged treatment duration, the utilization of multiple medications, and the adverse effects associated with drug therapy. This drug moved from Group C (third line) in 2016 and Group 5 (unclear efficacy) in 2011. This is a synthetic oxazolidinone antimicrobial drug and a non-selective mono oxidase inhibitor. Antimicrobials that are both vulnerable to and resistant to gram-positive bacteria can be effectively combatted by linezolid. This study investigates and appraises the utilization of linezolid as a therapeutic intervention in individuals afflicted with MDR-TB, while also scrutinizing the pharmacological attributes of the drug. We also discuss Linezolid's safety, efficacy, and tolerability for treating MDR-TB. Linezolid medication should be utilized for most patients and is a part of more recent short-course regimens since it has been known to increase the success rate of treatment of DR-TB by increasing the conversion sputum rate. However, primarily hematologic and neurologic, linezolid toxicity is typically treatment-limiting yet should be monitored. Recent studies suggest that dose modification and intermittency can reduce linezolid toxicity. Also, using linezolid in the regimen potentially reduces the treatment duration, but it needs further research.

Keywords: efficacy, linezolid, safety, MDR-TB, tolerability

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1. Introduction

Multidrug-resistant tuberculosis (MDR-TB), marked by resistance to both isoniazid and rifampicin, presents a formidable challenge to the global community, complicating tuberculosis treatment regimens and necessitating the implementation of innovative therapeutic approaches [1]. To effectively treat MDR-TB, several medications must be used in combination, including linezolid, fluoroquinolones, bedaquiline, ethambutol, clofazimine, terizidone or cycloserine ,imipenem-cilastatin, pyrazinamide, delamanid or ethionamide, meropenem, streptomycin, amikacin [2]. On the WHO linezolid include of The three medicines in Group A used for classification of second-line medicines with

bedaquiline and fluoroquinolones [3]. In vitro and animal investigations have demonstrated the promising effectiveness of linezolid, the inaugural oxazolidinone sanctioned for human use, against drug-resistant variants of Mycobacterium tuberculosis [4-6]. We tought to evaluate the function of linezolid in MDR-TB, talk about this medication and its characteristics, and present fresh data on the clinical advantages of clofazimine in MDR- TB treatment.

2. Multidrug-Resistant Tuberculosis

2.1. Epidemiology

According to the WHO, between 2015 and 2020, the predicted number of people who annually developed MDR-TB or RR-TB (MDR/RR -TB) was largely steady; however, it increased in 2021. The range of estimated incident cases was 450 000 to 501 000 (95% UI: 399 000 to 501 000), representing a 3.1% increase from 437 000 (95% UI: 390 000-483 000) in 2020. The main cause of the increase is believed to be the general increase in tuberculosis incidence between 2020 and 2021, which was exacerbated by the COVID-19 pandemic's effects on tuberculosis detection. Twenty-six percent of cases worldwide in 2021 came from three countries: Pakistan (79.9%), the Russian Federation (8.5%), and India (26%). With more than 50% of previously treated MDR/RR-TB patients, the Russian Federation and a number of other countries in Eastern and Central Asia have the highest rates [2].

The COVID-19 pandemic continues to exacerbate the burden of tuberculosis and hinder access to its diagnosis and treatment [7-9].

Global TB objectives are not on pace, and progress made in the years leading up to 2019 has slowed, stopped, or even reversed. The most obvious and immediate result was a notable and immediate drop in the reported number of new TB diagnoses worldwide. At its highest point of 7.1 million in 2019, this fell to 5.8 million in 2020 (-18%), reaching a level last observed in 2012. At 6.4 million, there was a little uptick in 2021 (compared to the 2016–2017 level). The three countries that made up the majority of the drop in 2020 were Indonesia, India, and the Philippines, which together accounted for 67% of the entire world population [9].

2.2. Treatment of MDR-TB

There are currently two regimens available for treating MDR-TB. Based on the length of the treatment, the WHO classed these regimens as either short or lengthy. A long regimen lasts 18–20 months (15 months following culture conversion, at least), while a short regimen lasts 9–11 months [10]. WHO made a public appeal (44) for IPD for the treatment of DR-TB in June 2021. Following details were requested for each patient: bacteriologically proven MDR/RR-TB patients (including MDR/RR-TB, MDR/RR-TB with added resistance to second-line TB medicines, and patients with pre-XDR-TB or XDR-TB) application of the modified, shorter (12 months or less) all-oral regimens that include at least linezolid and bedaquiline. Application of the shorter, all-oral bedaquiline-containing regimen (9–11 months), as advised by the WHO, in the following combinations: Levofloxacin (or moxifloxacin), ethambutol, pyrazinamide, clofazimine, ethionamide, and high-dose isoniazid were administered for 4 or 6 months (used for at least 6 months), and then for 5 months clofazimine, pyrazinamide, ethambutol, and levofloxacin (or moxifloxacin). WHO also recommend use of the longer, all-oral treatment plan that the WHO recommends, which must include at least linezolid and bedaquiline [2].

3. Linezolid

3.1. Introduction to linezoid

Linezolid belongs to the oxazolidinone drug class that is effective against gram-positive rods, such as corynebacteria, L monocytogenes, and Nocardia sp., and gram-positive bacteriae, including enterococci, streptococci, staphylococci, and gram-positive anaerobic cocci. Its primary activity is bacteriostatic, even if it has antibacterial properties against streptococci. It also exhibits anti-Mycobacterium TB properties [11].

Linezolid is used for disease treatments resulting from gram-positive bacterial infections, not negative gram bacterium treatment. It was first introduced in 1978 because of the effectiveness of the linezolid drug on plant diseases. Then linezolid was introduced as antimicrobial medicine; after 6 years, linezolid had antibacterial properties that felt better than the previous antibacterial compounds [12]. Linezolid is an effective drug against drug-resistant TB treatment, and linezolid is effective for treating pneumonia bacteria, skin infections, Infections, and complications from bacterial infection [13].

3.2. Chemical properties of linezolid

The chemical known as linezolid is an organofluorine made composed of 1,3-oxazolidin-2-one containing an N-3-fluoro-4- (morpholine-4-yl) phenyl group and an acetamido methyl group at position 5 (see Fig. 1). A synthetic antibacterial drug that stops the development of a functional 70S initiation complex by binding to a specific location on the 50S subunit of the 23S ribosomal RNA and inhibiting the synthesis of proteins by bacteria. As a protein synthesis inhibitor and antimicrobial, it serves both purposes. It belongs to the class of acetamides, morpholines, oxazolidinones, and organofluorine compounds [14].

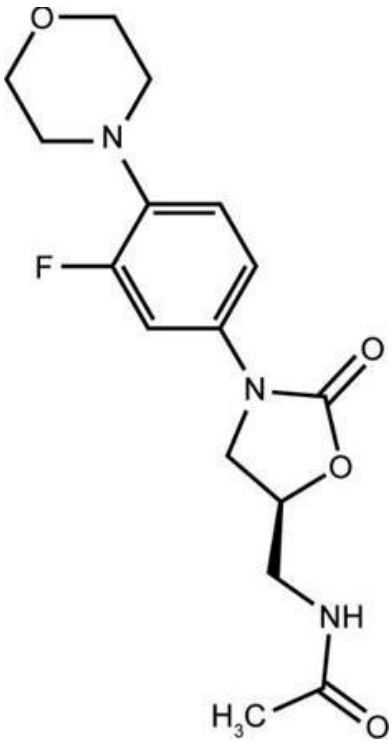

Figure 1: Chemical properties of linezolid.

3.3. Mechanism of action of linezolid

Linezolid, a synthetic antibiotic, functions by binding to the rRNA within the 30S and 50S ribosomal subunits, thereby impeding bacterial protein synthesis. This interference hampers the formation of the initiation complex, resulting in the truncation of the peptide chain and a decrease in the translation rate. While certain inhibitors of protein synthesis target elongation, they do not necessarily operate at this specific level of inhibition. Moreover, there is no indication that inhibition at this precise site leads to

cross-resistance to other protein synthesis inhibitors. Additionally, linezolid possesses the capability to suppress the synthesis of virulence factors, thereby diminishing the production of toxins associated with gram-positive bacterial infections [15]. Linezolid is bactericidal against most streptococcal strains and bacteriostatic against staphylococci and enterococci [13].

Linezolid is nonselective monoamine oxidase (MAO) inhibitor. Monoamine oxidase inhibition in the central and sympathetic nervous system can contain higher concentrations of the neurotransmitters dopamine, serotonin, norepinephrine, and epinephrine. Beta- and alpha-adrenergic and serotonin receptors may become desensitized because of inhibition. Inhibiting MAO can enable significant levels of tyramine from food to be absorbed throughout the body, which could lead to life-threatening hypertension. This can happen in the liver and digestive tract [13].

3.4. Synergism activity of linezolid

Synergism activity of linezolid with other drugs shown in some research, one showed between linezolid and clarithromycin. In MDR-TB patients, clarithromycin raises linezolid exposure by 44%. The research findings suggest that clarithromycin holds potential as a synergistic agent to enhance the therapeutic levels of linezolid, alongside low-dose ritonavir and protease inhibitors. Incorporating clarithromycin into multidrug-resistant tuberculosis (MDR-TB) treatment protocols is recommended based on the observed in vitro synergy. Consequently, with the heightened exposure to linezolid and enhanced drug susceptibility observed in both clarithromycin and linezolid, the dosage of linezolid can be further minimized, leading to cost reductions and mitigated adverse effects [16].

According to the in vitro findings from the current investigation, the majority of the combinations showed no difference or an additive impact, except LZD + CPM, which showed partial synergism (FIC = 0.75) for three of four isolates. Discussing the standards that characterize medication interaction patterns is essential because different definitions determine them. A common definition of interaction in antimicrobial drug combinations is as follows: FIC 0.5 indicates cooperative relationships; FIC > 0.5 but 4 indicates no cooperation; and FIC > 4.0 indicates aggressive behavior. All varieties examined would have 'no interaction' if this criterion evaluated the interaction patterns between LZD and second-line medications. LZD and CPM are potentially effective combinations that could be used in MDR-TB regimens. In vivo, LZD + CPM had the highest level of efficacy against H37Rv and only demonstrated limited synergism.

Compared to LZD and CPM alone, LZD + CPM had more excellent bactericidal action (p 0.05). After two months of treatment, LZD + CPM showed the highest activity against H37Rv of all the treatment groups, lowering CFU by 3.55 log10 [17].

3.5. Pharmacokinetics of linezolid

In healthy people, linezolid has a bioavailability of about 100% and is well absorbed.5 This feature is a significant advantage because it enables the agent to be used intravenously before moving to oral therapy or even starting infection treatment with oral therapy. After 600 mg oral dosages, steady- state peak serum concentrations (Cmax) are reached 0.5-2 hours later and range from 15 to 27 mg/L.3–8 Additionally, oral absorption was unaffected by co-administration with antacids such as magnesium hydroxide and aluminium hydroxide [12].

The volume of distribution is close to the 40–50 L total body water content, and the percentage of plasma protein binding is 31%. Half-life of plasma elimination is 3.4–7.4 hours. Two inactive metabolites, hydroxyethyl glycine (metabolite B) and aminoethoxy acetic acid (metabolite A), are produced during the metabolism of linezolid. The clearance rate (±SD) is 80 ± 29 mL/min and by non-renal (65%) and renal processes. Renal tubular reabsorption is possible. A percentage of the dosage is eliminated in the urine unaltered [12,18].

A portion of the dosage is eliminated in urine in its original state. In-depth research has been done on linezolid's pharmacokinetics in various patient groups receiving doses. After a dose was multiplied by five, there was discovered to be just a minimal amount of nonlinearity, with a 30% fall in clearance. The therapeutic dosage window has nothing to do with the nonlinearity. Plasma levels of linezolid exhibited comparability across various demographics, including young, healthy volunteers, elderly patients, and individuals with mild or chronic renal dysfunction. It has been suggested that there is no need to change the dosage when the concentration of females is higher than that of males. According to reports, persons with normal renal function are exposed to drug metabolites seven to eight times less frequently than patients with severe renal impairment who need hemodialysis. Patients who have significant renal impairment are advised to take Linezolid with caution. Pediatric patients necessitate a greater daily dosage per kilogram of body weight compared to adults due to the demonstrated higher clearance of linezolid in children relative to adults [12].

3.6. Effectivity of linezolid for treating MDR-TB

Irrespective of dosage regimen, linezolid is strongly advocated as the primary therapeutic agent for managing MDR-TB, contingent upon the absence of contraindications. However, this linezolid drug has side effects from this drug which is still a problem [3,19]. Several research, including the most recent ZEPHYR experiment, revealed higher linezolid effectiveness than vancomycin. However, the efficacy of linezolid, better than vancomycin, is still being debated for indications such as SSSI or nosocomial pneumonia. Linezolid's clinical usage in complex MRSA-SSSI, such as DFI without osteomyelitis, has been validated by multiple recent trials [20]. According to data from real-world studies, linezolid has been shown to be safe and effective in treating Gram-positive bacterial infections in critically ill patients. When treating SSTIs (pulmonary infections) brought on by Staphylococcus aureus, linezolid demonstrated superior clinical efficacy [21].

Linezolid use has been proven to boost effectiveness for at least 6 months, although its use may be restricted by harmful effects. According to the analysis, linezolid should be used for the entire course of therapy to have the most impact (According to the data received, almost 70% of patients used linezolid for six months or longer, and 30% for a full year). There are no patient factors for an early linezolid withdrawal shown by the IPD sub-analysis [3]. Some of the following studies demonstrate the effectiveness of linezolid (Table 1). The average result from 5 studies showed that linezolid became an effective drug for MDR TB treatment.

3.7. Linezolid's effectiveness in treating XDR-TB

Linezolid has been shown to be effective in the treatment of MDR-TB and XDR-TB in five case-control studies. Toxicity and side effects, including optic neuropathy, peripheral and bone marrow suppression, limit the use of linezolid. Contrarily with MDR-TB, reported their study for seven XDR-TB patients, all patients showed initial culture conversion and a low incidence of myelosuppressive and hematologic side effects although the use of the US Food and Drug Administration (FDA)-approved dosage of 600 mg orally twice a day and the result of this study showed that two patients had peripheral neuropathy, and none of them experienced severe myelosuppressive side effects [8].

TABLE 1: Description of safety and tolerability of linezolid.

Study	Drug Resistance	Linezolid Daily Dose	Outcome
			Treatment Success Rate
Rodvold and McConeghy [22]	MDR-TB (resistant to isoniazid and rifampicin), XDR-TB (resistant to at least one injectable drug, amikacin, capreomycin, or kanamycin), and MDR-TB (resistant to any fluoroquinolone)	1200 mg daily Duration: 26 weeks	Ninety-eight individuals (90%; 95% CI 82.7-94.9%) showed positive results at six months after the completion of treatment. Of the 38 MDR patients, 35 (92%) had good results (78.6-98.3%). A total of seven deaths occurred due to withdrawal of consent during treatment (n=1), relapse in follow-up (n=2), and loss to follow-up (n=1), as opposed to one death from an unidentified cause that was not connected to TB or drugs during follow-up (n=1).
Conradie et al. [23]	MDR-TB is distinguished by its resistance to isoniazid and rifampicin, streptomycin/ amikacin/ capreomycin, XDR-TB	600 mg once day and 300 mg twice day. Time: 56 days	This study involved eight patients. A median cumulative dose of 51 000 mg (IQR 33 850-60 450 mg) was administered throughout the course of a median 56-day course of linezolid treatment (interquartile range [IQR] 44-82 days). The median linezolid AUC over 12 hours (AUC12) values were 57.6 mgh/L with the 300 mg dosage and 145.8 mgh/L with the 600 mg dose (IQR 101.2-160.9 mgh/L). The AUC24/MIC ratios for the 300 and 600mg doses were 452 (IQR 343-513) and 1151 (IQR 656-1500). It was easy to tolerate linezolid.
Esmail et al. [24]	MDR-TB, which is susceptible to rifampicin, isoniazid, fluoroquinolones, and aminoglycosides but resistant to both of these drugs.	Dose: NA Duration: 6 months or 24 months	A favorable outcome was recorded in 22.7% (10 of 44) of the patients in the SOC arm and 51.0% (25 of 49) of the participants in the intervention arm 24 months after the start of treatment, representing a relative risk of 2.2 (95% CI, 1.2-4.1) and risk difference of 28.3% (9.6-46.7)
Singla et al. [25]	Capreomycin, moxifloxacin, levofloxacin, and amoxicillin-clavulanic acid	600 mg (N/A). Duration: >12 months	The high cost of therapy at 10 and 14 months, respectively, and one was displaced at 16 months. These three had negative ongoing smear and culture results at default. Due to chronic sputum positive and no clinical response at 15 months, two patients (6.8%) were deemed unsuccessful, and the therapy had to be stopped. Nine patients have been cured and receive follow- up care for an average of 12.8 months (6-28 months). So yet, nobody has relapsed. Two individuals with XDR-TB had surgery. The outcomes for both patients were favorable.

TABLE 1: Description of safety and tolerability of linezolid.

Study	Drug Resistance	Linezolid Daily Dose	Outcome
			Treatment Success Rate
Anger et al. [26]	KAN, CIP, PZA, LVX, ETH, CAP, AMK, OFX, RFB, PAS, AMC, CLO	600 mg twice daily and 600 or 400 mg once daily dose. Duration: 16 months	MDR TB regimens included linezolid for a median of 16 months (range: 1-29). 11 patients (69%) finished their treatment; four (25%) passed away, and one (6%) stopped without relapsing. After beginning treatment with linezolid, myelosuppress ion happened over in a median of 5 weeks (range: 1–11) in 13 patients (81%), gastrointestina l side effects in 13 (81%) patients in a median of 8 weeks (range: 1-57), and neurotoxicity in seven (44%) patients in a median of 16 weeks (range: 10-111). Combinations of brief linezolid suspension, linezolid dose decrease, and symptom management were used to treat adverse effects. Five patients (31%) needed to stop using Linezolid eventually. Compared to neurotoxicity, myelosuppress well to clinical treatment techniques. Males were likelier to experience leucopenia and neuropathy, and greater age was linked to thrombocytopenia (P 0.05).

LZD, linezolid; INH, isoniazid; INH (900), 900 mg high dose isoniazid; RIF, rifampicin; EMB, ethambutol; PZA, pyrazinamide; STR, streptomycin; CAP, capreomycin; KAN, kanamycin; AMK, amikacin; ETH, ethionamide; CYC, cycloserine; CIP, ciprofloxacin; LVX, levofloxacin; OFX, ofloxacin; RFB, rifabutin; PAS, para-aminosalicylic acid; AMC, amoxicillin/clavulanate; CLO, clofazimine; MXF, moxifloxacin; GAT, gatifloxacin; IPM, imipenem; IFN-G, recombinant human interferon-γ; COPD, chronic obstructive pulmonary disease; M, male; F, female.

3.8. Safety and tolerability of linezolid

According to recent study, dose modification and intermittent therapy may help reduce the toxicity associated with linezolid. Linezolid is secure and generally well tolerated in 600 mg twice daily doses for up to 28 days. Adverse drug reactions are often mild to moderate in severity and transitory. Common adverse reactions observed predominantly in adults include nausea, diarrhea, headaches, loose stools, while vomiting is prevalent in children [27].

It is safe to combine linezolid with aztreonam, yet not enough information is available to assess how linezolid and rifampicin interact. The co-administration of ceftazidime, ciprofloxacin, gentamicin, and meropenem with Gram-negative antibiotics did not result in any unfavorable side effects. Linezolid did not interfere with the efficacy of other antifungal medications, including aminoglycosides, antivirals, amphotericin B, fluoroquinolones, azoles, or -lactams. Therefore, linezolid can be combined with other antibiotics without causing any interactions [28,29]. When used with serotonin

reuptake medications, linezolid, a non-specific inhibitor of monoamine oxidase, can cause lethal serotonin poisoning [28,29]. Findings show that Linezolid does not present contraindications in this particular illness, even though doctors need to be aware of this potentially dangerous combination and keep careful monitoring on patients receiving linezolid in addition to serotonergic medications [30].

3.9. Mechanisms of resistance to linezolid

Linezolid binds to the 23S rRNA nucleotides around the 50S ribosomal subunit gap, according to an examination of high-resolution structures of the medication [31]. Accordingly, 23S rRNA mutation has been identified as one of the mechanisms behind linezolid resistance. Furthermore, mutations in a few of these proteins are increasingly associated with resistance to linezolid, despite the fact that the ribosomal proteins uL3 and uL4 are situated farther away from the bound drug. Additionally, new studies on the Cfr methyltransferase have shown that considerable linezolid resistance might result from the transferable alteration of 23S rRNA. The creation of a new class of oxazolidinones with improved characteristics against the identified resistance mechanisms has been made possible by the accurate localization of the linezolid-binding site [32].

Recently, optr A is resistance gene of a novel oxazolidinone has been discovered, and this gene has been expressed in E. faecium and E. faecalis [33]. OptrA is an adenosine triphosphate-binding cassette (ABC) transporter. A common mechanism showed that the antibiotic-resistant bacteria use the ABC transporters to forcefully pump the medications out of the cell. Proteins which confer resistance to a variety of therapeutically relevant ribosome-targeting antibiotics are found in the ABC-F family. It has been observed that these proteins interact with the ribosome and move the drug away from its binding site in order to use ribosome protective mechanisms [34].

4. Conclusions

The requirement for a more practical treatment is highlighted by the growing incidence of MDR-TB and the poor efficacy of existing therapies. When it comes to bacteria that are resistant to many drugs, linezolid works well. Further study is required to find the best effective treatment approach to lessen neurological adverse effects without compromising multidrug-resistant tuberculosis results and predisposing factors for linezolid toxicity.

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Research Article

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Effect of the Composition of the Mediterranean Diet on Body Mass Index, Waist Circumference, Fat Level, and Visceral Fat in Patients with Obesity

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Abstract.
Excessive and unhealthy fat storage is known as obesity. Both monounsaturated and polyunsaturated fatty acids are highly encouraged in the Mediterranean diet. Obese patients at a Bandung clinic were studied to examine how the Mediterranean diet affects their BMI, waist size, fat percentage, and visceral fat. Analytical observational methods utilizing prospective cohort observations were employed. A systematic random sample technique was used to carry out the sampling. Participants were 34 overweight individuals who had twelve monthly food pattern assessments and two monthly examinations for body mass index (BMI), waist size, fat percentage, and visceral fat. To compare proportions between the rMED adherence groups and to look for differences in the composition of the Mediterranean diet on BMI, waist circumference, fat content, and visceral fat, we used the ANOVA test and Tuckey's post-hoc test. In addition, the eight tenets of the Mediterranean diet were assessed using linear regression. The average age of the obese patients who followed the Mediterranean diet was 57.54 years, and 79.41% of them were female. It was found that the components of vegetables, fruits, nuts, seafood, and dairy products had a substantial impact on the body mass index of the participants, while the components of vegetables, fruits, nuts, legumes, seeds, meat, fish, olive oil, and dairy products did not have a significant impact on the waist circumference, fat content, or visceral fat.

Keywords: body mass index, fat content Mediterranean diet composition, obesity, visceral fat, waist circumference

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1. Introduction

Obesity is defined by the World Health Organization (WHO) as an abnormal accumulation of fat in the body that causes a variety of health concerns. According to World Health Organization report, 1.9 billion adults were overweight in 2016, with an additional 650 million classified as obese [1]. Obesity rates in Indonesia increased from 7.5% in 2007 to 14.8% in 2013 and 21.8% in 2018, as reported by RISKDESDAS in 2018. North Sulawesi province has the most obesity prevalence rate in Indonesia at 30.2%, followed by DKI Jakarta province and other regions. The prevalence of obesity in West Java Province in 2018 was ranked 14th out of 34 provinces in Indonesia with a rate of 23% and one of the cities with the greatest prevalence of obesity in Indonesia was Cimahi City with a rate of 24.87% followed by Bandung with a rate of 23.84% incidence of obesity [2].

Non-pharmacological management that can be done in obese people is lifestyle adjustments such as altering diet, and increasing physical activity. The Mediterranean diet is considered to be shown to reduce weight because the Mediterranean diet is a diet that stresses intake of monounsaturated fatty acids (MUFA) and consumption of polyunsaturated fatty acids (PUFA). This diet has been approved by The European Association for the Study of Obesity (EASO), and the Indonesian Endocrinology Association (PERKENI). In 2022, the Mediterranean diet is ranked top according to U.S News and World Report for the best plant-based diet, the best heart healthy diet, the best diabetic diet, the best diet for nutritious food, and the easiest diet [3-7]. This study intends to assess the influence of the composition of the Mediterranean diet on BMI, waist circumference, fat content, and visceral fat in obese patients. The hypothesis of this study is that there is an effect of Mediterranean diet components on body mass index, waist circumference, fat content, and visceral fat in obese people.

2. Material and Method

The study design used an observational analysis approach and prospective cohort principles to find out the influence of Mediterranean diet composition on body mass index, waist circumference and levels of fat and visceral fat in obese individuals for 3 months. This investigation was conducted at a clinic in the city of Bandung which contained prolanis volunteers. The study was done from September to December 2022. The subjects of this study were obese patients who met the inclusion criteria. The

inclusion criteria in this study comprised patients with a body mass index (BMI) ≥25 kg/m2, subjects with low physical activity, and subjects with a MEDAS score >9.

The sample size of this study is a minimum of 30 samples with data collection using systematic random sampling technique. The instruments and materials utilized for this investigation included a beam balance to measure body weight, a microtoise to measure height, a measuring tape to assess waist circumference, and Bioelectrical Impedance Analysis to test fat and visceral fat levels. This study used the Food Frequency Questionnaire (FFQ) as an extra tool to describe the frequency of food consumption in research respondents.

The analysis employed in this study was ANOVA or Kruska Wallis tests to find out comparisons in all adherence groups and then Tuckey or Mann Whitney post-hoc tests to find out which groups had differences in the degree of consumption of Mediterranean diet components. This study also employed a linear regression test to assess the effect of the total rMED score and the composition of the Mediterranean diet on BMI, waist circumference, fat content and visceral fat.

3. Result and Discussion

The study took place from September to December 2022 in a Bandung clinic. The study's findings informed the selection of a sample of 34 participants who fulfilled the study's inclusion criteria. The average age of the obese patients who followed the Mediterranean diet in this research was 57.59 years old, according to the statistics. Participants' ages ranged from 41 (the youngest) to 73 (the oldest) years old. Evidence suggests that white fat tissue accumulates with age. Triglycerides, which make up 90–95% of white fat tissue, are stored in white fat, and the larger size and quantity of fat in white fat contribute to the effect of increasing fat, which is a rise in body weight [8-10].

The results showed that out of the obese patients who followed the Mediterranean diet, 27 were female (or 79.41%), compared to 7 males (20.59%). Obesity is more common in women after menopause, suggesting that gender plays a role in its development. Reduced estrogen levels have an effect on lipoprotein production via lipoprotein lipase, which in turn causes triglyceride buildup. With a range of 23.98 to 34.25 kg/m2, the average body mass index (BMI) among obese patients following a Mediterranean diet was 27.99 kg/m. Among overweight people following a Mediterranean diet, the average

waist measurement was 87.82 centimeters. Obese people often have a waist size ranging from 71 cm at the bottom to 101 cm at the top [11].

TABLE 1: Effects of mediterranean diet food components on body mass index, waist circumference, fat content, and visceral fat.

	p-value			
	BMI (kg/m^2)	**Waist Circumference (cm)**	**Fat Level (%)**	**Visceral Fat (%)**
Vegetables	0,011	0,206	0,320	0,635
Fruit and Nuts	0,012	0,846	0,172	0,545
Legumes and Seeds	0,156	0,773	0,767	0,788
Whole Grain	0,999	0,345	0,968	0,478
Meat	0,237	0,876	0,660	0,158
Fish	0,025	0,628	0,619	0,566
Olive Oil	0,151	0,813	0,192	0,469
Dairy Milk	0,030	0,274	0,734	0,771

A statistical analysis reveals a noteworthy correlation ($p=0.000<0.05$) between vegetable components and adherence to the Mediterranean diet. Adherence to the Mediterranean diet is significantly correlated with fruit components and nuts ($p=0.000<0.05$). Adherence to the Mediterranean diet is significantly correlated with legume components ($p=0.000<0.05$). The correlation between whole grain components and following the Mediterranean diet is statistically significant ($p=0.000<0.05$). Adherence to the Mediterranean diet is significantly correlated with red meat components ($p=0.000<0.05$). The fish component is significantly related to following the Mediterranean diet ($p=0.000<0.05$). The components of olive oil and compliance with the Mediterranean diet are significantly related ($p=0.000<0.05$), while the components of dairy products are significantly related to adherence to the Mediterranean diet ($p=0.006<0.05$).A statistically significant relationship between vegetable consumption and body mass index (BMI) is seen in Table 1. This is because eating veggies can help curb hunger pangs because of the fiber they contain, which is known to speed up the process of feeling full by increasing the amount of chewing required to break down food. Eating speed and volume are influenced by the activation of hypothalamic histamine neurons, which in turn suppresses H1 receptors, as can happen when food is chewed. Cholecystokinin is involved in stomach emptying regulation and promoting fullness; eating foods high in fiber can enhance its release [12,13].

A statistically significant relationship between fruit and nuts and body mass index (BMI) is seen in Table 1. It is known that the fruit's polyphenol content works to enhance thermogenesis and calorie expenditure. Additionally, it can hinder the differentiation of adipose tissue creation, boost liposis, activate β-oxidation, and thus transform energy intake into energy more rapidly. This state can aid in avoiding the buildup of TAG [14]. Because they contain fiber, which the digestive system has a hard time digesting, nuts are believed to aid weight loss by providing sustained energy throughout hunger pangs [12,15].

Table 1 shows that for all variables, there are no significant conclusions between seeds and legumes. The subject's failure to ingest the prescribed amount increases the likelihood that this will occur. The suggested daily allowance for legumes is 3-6 cups, while the recommended weekly allowance for whole grains is 60 grams, or three servings [16,17].

There are no statistically significant relationships between red meat and any of the other variables, according to Table 1. Red meat's protein and saturated fat content make it a potential trigger for this illness. A varied method of processing meat is another factor that causes weight gain when eating it. Weight gain due to processed beef is a real possibility, according to studies conducted in 2022 by Khodayari et al. This is due to the fact that processed meat is rich in calories and fat but short in protein, and it also contains nitrosamines, a carcinogenic substance with four times the salt level of original meat. Adipose tissue fat will accumulate under these circumstances [18,19].

Table 1 shows that there is a statistically significant relationship between fish and body mass index. This is due to the fact that fish is a good source of omega-3 polyunsaturated fatty acids (PUFA), which have anti-obesity effects by regulating gene expression to boost energy expenditure and decrease body fat storage and improve fat oxidation [20,21].

There are no statistically significant relationships between olive oil and any of the other factors, according to Table 1. Because linolenic acid is naturally more susceptible to oxidation, which causes cellular instability and inflammation and ultimately makes obesity more likely. An example of this mechanism in action is the conversion of linolenic acid to arachidonic acid, which in turn triggers the creation of adipose tissue, activates macrophages, and increases inflammation, ultimately leading to obesity [22,23].

There is a statistically significant relationship between dairy products and body mass index (BMI), as seen in Table 1. Dairy products' high calcium content causes this

condition by preventing the production of fat by lowering the expression of two genes that are involved in adipogenesis: peroxisome proliferator-activated receptor gamma (PPAR) and CCAAT/enhancer-binding protein alpha (C/EBP). By influencing adipocyte intracellular Ca^{2+} levels, calcium consumption can likewise reduce lipogenesis and enhance lipolysis. According to a 2012 study by Sun et al., people whose diets are rich in calcium have lower body fat percentages and lower expression of genes involved in lipogenesis (such as fatty acid synthetase, or FAS), and higher expression of genes involved in lipolysis (such as hormone sensitive lipase, or HSL). A reduction in body fat and subsequent weight loss are symptoms of this illness [24,25].

4. Conclusion

Patients with obesity who followed the Mediterranean diet in this research had an average age of 57.59 years, according to the results. A whopping 79.41% of the obese people who followed the Mediterranean diet plan were women. Vegetables, fruits, nuts, seafood, and dairy all have a role in determining body mass index (BMI) in overweight and obese people. In individuals who were overweight, the impact of various food groups on waist size, total fat, and visceral fat was insignificant. This included fruits and nuts, legumes, seeds, meat, seafood, olive oil, and dairy products.

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Research Article

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Safety and Tolerability of Antihypertensive Agents in Long-term: A Literature Review

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Abstract.
Hypertension is known to affect more than one billion people globally and is estimated to increase to 1.5 billion by 2025. It is considered one of the leading causes of death and cardiovascular disease worldwide. The safety of long-term antihypertensive use is also a concern. Through a narrative or literature review, this study evaluated antihypertensive agents based on the results of various literature searches. Researchers reviewed the safety and tolerability of five classes of antihypertensive agents such as Diuretics, Angiotensin-Converting Enzyme Inhibitors (ACEI), Angiotensin Receptor Blockers (ARB), Beta-Blockers, and Calcium Channel Blockers (CCB). The use of ARB antihypertensive drugs like valsartan is well tolerated and safe. Moreover, the mortality rate associated with enalapril was 16% lower (95% CI 0.76-0.93; $P < 0.001$). Captopril and lisinopril of Angiotensin-converting Enzyme Inhibitors (ACEIs) have significant side effects (SEs) compared to any antihypertensive drug. Therefore, this study recommends using the ACEIs group, especially captopril and lisinopril, due to the minimal side effects produced compared to other antihypertensive drugs. In addition, the CCBs or Ca antagonists class like amlodipine is potentially well tolerated and safe as a first-line drug for hypertension treatment.

Keywords: antihypertensive drugs, hypertension, safety, side effect, tolerability

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1. Introduction

Hypertension is known to affect more than one billion people globally and is estimated to increase to 1.5 billion by 2025. This disease is also has the highest number of cases, which is 63,309,620 with 427,000 deaths. Symptoms of hypertension manifest in an increase in systolic and diastolic blood pressure that exceeds the limit of ≥140/90 mmHg. Thus, this condition is perceived as one of the significant risk factors for the occurrence of cardiovascular disease, as well as being the leading cause of death worldwide, especially in Indonesia [?]. Based on the National Coordinating Council for Medication Error Reporting and Prevention (NCCMERP), medication errors are defined as “Any preventable event that may cause or contribute to the inappropriate use of

a medication or harm to a patient when the medication is within the control of the health professional, patient, or consumer." Nevertheless, any medication error can be prevented before complications occur or do not harm the patient. In the recent study titled "Incidence of Medication Errors in the Intensive Care Unit", it was explained that the majority of medication errors were caused by prescribing anti-hypertensive drugs with a percentage of cases amounting to (11.21%) [?].

The treatment of hypertension aims to prevent complications of the disease and control blood pressure. In general, doctors routinely use various classes of antihypertensives, namely Angiotensin Converting Enzyme Inhibitors (ACEI), Angiotensin Receptor Blockers (ARB), Beta Blockers, Diuretics, and Calcium Channel Blockers (CCB). However, hypertension patients must control their blood pressure (BP) for high-risk complications, The application of pharmacologic therapy for hypertension is considered to have the potential to reduce disease severity and mortality. Therefore, the long-term safety aspects of various antihypertensive agents are a subject of serious concern [?]. This is supported by the high percentage of cases of errors in prescribing antihypertensive drugs by doctors to patients.

Therefore, researchers consider that the discussion of safety and tolerability of antihypertensive Agents in a long-term is needed with the aim of evaluating the prescribing of antihypertensive drugs to reduce cases of medication errors in the use of antihypertensive drugs by doctors.

2. Methods

The research method is in the form of a narrative or literature review, the comprehensive literature search performed in the MEDLINE database, Crossref, and Google Scholar equipped with a manual literature search from the bibliography of the articles retrieved. Search terms used were hypertension, cardiovascular disease, arterial hypertension, blood pressure, "antihypertensive drug, long-term safety, side effect, efficacy, captopril, lisinopril, valsartan, furosemide, amlodipine, and propranolol. As a result of this, the literature review involved 18 scientific journals in writing this literature review. After a search, the articles selected were limited to only papers published in English and without a time limit.

3. Antihypertensive Agent

3.1. Angiotensin-converting Enzyme Inhibitors (ACEIs)

Angiotensin-converting enzyme inhibitors (ACEIs) have become a highly desirable option in the treatment of hypertension. It is due to their function of not only regulating blood pressure by inhibiting angiotensin-converting enzyme (ACE) to convert angiotensin (AT) I into AT II, which also maintains sodium and water homeostasis in the body. In addition, ACEIs have also shown efficacy in the prevention of renal damage in patients with diabetic nephropathy and non-diabetic renal failure, by the mechanism of modification from AT I to AT II [?].

Captopril and lisinopril are two examples of drugs in the ACEI group. Of 7,103 hypertensive patients receiving captopril medication for 3 months-4 years, 627 patients decided to discontinue, with 230 patients due to failure to maintain adequate blood pressure reduction and 397 patients due to Side Effects (SEs), Historically, the condition has been associated with increased disease severity and mortality in patients suffering from aortic stenosis [?].

The main side effect of using ACE inhibitors is mainly related to the risk of hyperkalemia, with a percentage of patients around 2% to 6%, which is due to the mechanism of action of ACE inhibitors in the body. Angiotensin II blockade inhibits downstream aldosterone secretion. Aldosterone affects the reabsorption of sodium and, therefore, water, which in turn causes the secretion of protons and potassium into the urine. Because potassium secretion is inhibited by aldosterone inhibition, potassium levels may increase significantly in patients taking ACE inhibitors. Commonly, patients taking lisinopril, a type of ACE inhibitor, often develop dry cough between one week to six months after the start of treatment, with an incidence of about 10% to 20% of total patients. Angioedema, although rare, is a potentially life-threatening side effect of taking ACE inhibitors, with incidence rates ranging from 1% to 2%. Angioedema is an adverse drug response characterized by episodic swelling of the face, lips, and upper airway region. Extensive accumulation of bradykinin in a given individual causes angioedema. Hypotension is one of the side effects, with a percentage of patients around 7% to 11%, which causes intolerance to therapy and discontinuation of treatment in a small percentage of patients using lisinopril or other antihypertensive agents. Dizziness is a reaction to drug use that is considered typical in ACE inhibitor therapy, around 12% to 19%. Once treatment is started, it is expected that the glomerular filtration rate (GFR)

will drop slightly. Discontinuation of ACE-Inhibitor therapy may be required in patients with renal insufficiency of 2% to 11%, chronic kidney disease, and bilateral renal artery stenosis with poor renal perfusion [?].

Lisinopril-type drugs have low bioavailability ranging from 10-30% after being taken orally. This side effect occurs more frequently in patients with conditions such as autoimmune, kidney disease or collagen vascular disease. Based on the latest 2017 American College of Cardiology (ACC) guidelines for the Prevention, Detection, Evaluation, and Related Management of High Blood Pressure in Adults, among patients requiring pharmacologic therapy, ACE inhibitors are the first recommended choice for hypertension management globally. However, captopril has minimal side effects that can potentially reduce disease severity and mortality [7, 8].

3.2. Angiotensin Receptor Blockers (ARBs)

Angiotensin Receptor Blockers (ARBs) these antihypertensive agents have a mechanism of action similar to ACEi. ARB-class drugs will reduce the likelihood of recurrent stroke in patients who have previously experienced a stroke or transient ischemic attack. This mechanism was accomplished by acting as an antagonist at the AT I receptor, which results in blocking the Renin-Angiotensin Aldosterone System (RAAS) and resulting in vasoconstriction. One example of a drug in the ARB class, In long-term studies, Valsartan is usually well tolerated. Serious adverse event (EDS) reports did not include any deaths in patients. The incidence of serious adverse events (SAEs) tended to be higher in the use of valsartan in combination with other groups of antihypertensive agents (19.5%) compared to the use of single valsartan (6.4%). However, treatment discontinuation due to SEs was remarkably lower in valsartan than in enalapril (ACEIs) (10.7% vs. 12.3%; P = 0.03) [9–11].

Some studies consider that valsartan is well-tolerated in this long-term study. In the article on achieving maximum dose over time, valsartan was titrated to a dose of 97/103 mg twice daily in approximately 35% of patients (95% confidence interval: 23-47), and there was no significant association with age or gender. Based on the analysis of the nine studies reviewed, the proportion of subjects who discontinued valsartan was 12.8% (95% confidence interval: 7.4-18.3). One of the adverse effects of use was hyperkalemia, assessed in six investigations (a total of 1076 people), where cases occurred in approximately 12 (95% CI 5-19)/100 person-years. In the literature, 16 selected articles evaluated the renal function of the patients. Still, of the 16 articles,

only 6 reported worsening renal function, defined in two types (an increase in serum creatinine ≥0.3 mg/dL in 18 articles and a case of a decrease in glomerular filtration rate ≥30% in 24 articles). Hence, these studies reported a reduction of renal function in 5.1% (95% CI 2.8-7.4) of treated patients who were CKD patients and were not drug related [?].

Five studies reported all-cause mortality due to the use of valsartan that included approximately 684 subjects, with the incidence rate of death being 8 (95% CI 4-12)/100 person-years. As concluded by 3 studies with a total of approximately 390 patients, the hospitalization rate was 24 (95% CI 5-42)/100 person-years experiencing hospitalization. Valsartan was well-tolerated in this long-term study [10, 12].

3.3. Beta Blockers (B-Blockers)

β-Blockers are a class of hypertension drugs recommended by the Joint National Committee 7 (JNC 7) as a particular choice of medications for essential hypertension. This drug works by inhibiting beta-adrenergic receptors, which could reduce the body's response to adrenaline and noradrenaline, thus helping to lower blood pressure. However, research for β-Blockers is still considered vague and inconclusive. Propranolol, a type of antihypertensive drug in the β-Blockers class regarded as non-cardioselective, exhibits membrane stabilizing properties in the absence of intrinsic sympathomimetic activity, as presented in the study [?].

Propranolol has shown effectiveness in controlling various medical conditions, such as hypertension, pheochromocytoma, myocardial infarction, cardiac arrhythmias, angina pectoris, and hypertrophic cardiomyopathy. Propranolol also has benefits in reducing symptoms of sympathetic overactivity in the treatment of hyperthyroidism, anxiety disorders, and tremors. Additional indications include prevention of migraine and upper gastrointestinal bleeding in patients with portal hypertension. It is still a concern that in the experience of one of the researchers, there was a patient who experienced severe hypotension during exercise and experienced sinoatrial block causing syncope after using beta-blockers for >15 years [13, 14].

Along with the required effects, the drug may also cause unwanted effects. In a meta-analysis conducted in the journal, researchers identified 26 patients (2.1%) who experienced (SEs) related to the treatment process with propranolol. More than 73% of adverse events occurred within the first 30 days of propranolol use. One of the adverse events was severe sleep disturbance that led to the discontinuation of

propranolol, representing 65.4% (17/26 patients), with 3 patients (11.5%) experiencing extreme agitation. 4 patients (approximately 15.4%) experienced severe respiratory distress. 2 of them developed significant bronchial hyperreactivity within 10 days of initiation of propranolol therapy. Bronchial hyperreactivity can be life-threatening, leading to permanent discontinuation of propranolol treatment due to its severe symptoms. Meanwhile, the other two patients experienced bronchospasm associated with viral infections but experienced rapid recovery after discontinuation of propranolol [?].

3.4. Diuretic

Diuretics are a class of hypertension drugs used for decades. Several prospective studies have shown that the use of thiazide diuretics is safe and effective in reducing disease severity and mortality in patients with hypertension. However, the use of diuretics in patients with diabetes poses a 3.8 times higher risk of cardiovascular death compared to patients not receiving such treatment [11, 16].

Furosemide is a loop diuretic approved by the Food and Drug Administration (FDA), which works by inhibiting the reabsorption of sodium and chloride in the proximal tubule, distal tubule, and ascending loop of Henle. It does this by inhibiting the sodium-chloride transporter and is used to treat conditions of volume overload and edema caused by exacerbations of congestive heart failure, liver failure, or renal failure, including nephrotic syndrome. The use of furosemide reduces costs and hospital stays. Furosemide administration in heart failure patients was considered well-tolerated and relatively safe after hospital discharge. Side effects (SEs) can be classified based on the organ system affected and ranked in order of severity. In the digestive system, hepatic encephalopathy may occur in patients with cirrhosis, pancreatitis, jaundice (intrahepatic cholestatic jaundice), elevated liver enzymes, and anorexia. Extreme hypersensitive responses, such as severe anaphylactic or anaphylactoid reactions (with possible shock), as well as systemic vasculitis, can also occur in response to sensitizing stimuli, As for metabolism, hyperglycemia, hyperuricemia, and hypokalemia may occur [17, 18].

3.5. Calcium Channel Blockers (CCBs)

Calcium Channel Blockers (CCBs) have become the primary choice as antihypertensive agents. The action binds to transmembrane sites located in L-type calcium channels,

especially in heart muscle and smooth muscle cells. interfering with the influx of calcium ions. Medicine and patients appreciate this class of drugs for their effectiveness, metabolic neutrality, and lack of side effects. However, recent studies have shown that hypertensive patients taking CCBs may have a higher risk of developing myocardial infarction and have a higher mortality rate compared to patients taking other types of antihypertensive drugs [?].

Individually, amlodipine (dihydropyridine) is generally considered safe with a low incidence rate of adverse events. Combined use of amlodipine may be a particularly appropriate option for patients with diabetes or metabolic syndrome as it does not exacerbate metabolic complications related to the cause. Most studies evaluating the safety of both monotherapy and the combination of telmisartan and amlodipine were conducted over a short period, while the most common adverse effect is peripheral edema, which is a result of the vasodilatory capacity of amlodipine [?]. Description of safety and tolerability of the antihypertensive agents in long-term use can be seen in Table 1.

TABLE 1: Description of safety and tolerability of the antihypertensive agents in long-term use.

Classes	**Antihypertensive Agents**		
	Drug's Name	**Daily Dose**	**Side Effects (SEs)**
Angiotensin Converting Enzyme Inhibitors (ACEIs)	Captopril	50-75 mg	Paroxysmal cough (1% to 10%), proteinuria (1 of 100 patients), Renal insufficiency or renal failure (found in 1 to 2 percent of every 1000 patients).
	Lisinopril	Start at 10 mg and titrate upwards to 40 mg once daily.	Dry cough (found in 10% to 20), dizziness (found in 12% to 19%), renal failure or insufficiency (2% to 11%), and hyperkale mia (2% to 6%).
Angiotensin Receptor Blockers (ARBs)	Valsartan	40-80 mg	Hyperkalaemia (5.1%) and the incidence rate of death was 8/100 person-years.
Beta Blocker	Propranolol	At first, 40 mg thrice daily	Chest tightness, cough-producing mucus, and difficulty with breathing.
Diuretic	Furosemide	20-80 mg	Electrolyte disturbances, hypokalemia, and kidney disease
Calcium Channel Blockers (CCBs)	Amlodipine	5-10 mg	Peripheral edema (18%), headache, and dizziness.

4. Conclusion

This study recommends using the ACEIs group, especially captopril, and lisinopril, due to the minimal side effects produced compared to other antihypertensive drugs. In the ARB group in the form of valsartan, administering valsartan at the time of hospital discharge was explicitly associated with decreased mortality and hospitalization after hospital discharge. In addition, the CCBs class amlodipine is potentially well tolerated and safe. Furthermore, to effectively lowering blood pressure, long-term amlodipine therapy can reduce seasonal blood pressure variations in high-risk hypertensive patients. However, the antihypertensive agents should be tailored to the patient's underlying disease; hence, the antihypertensive agent could work optimally.

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