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RESEARCH ARTICLES

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The submitted manuscript must be the article that has never been published, and the author must ensure that all co-authors have agreed by signing a statement on the seal. For original research, we accept the study which is last then 7 (seven) years when the manuscript is submitted. The manuscript is an original article free from plagiarism. When the article published in another journal then in the next journal, the article will be disallowed.

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Table title is the typed center, font size 10 pt, bold, initial letter of each word written with capital letter, except conjunctions. The titles are numbered and written on top of the table. Example: Table 3 *Neisseria gonorrhoeae* Resistance to 8 Types of Antimicrobials in 20 Specimens. Table, no vertical dividing line, and there are only three horizontal borderlines. Created tables in sequence two spaces from the text. Table descriptions and abbreviations are placed in the table description, not on the table title.

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The article contains results of original research in the field of basic medical or applied, and health. The article format consists of Title & Abstract (English) and *Judul* & *Abstrak* (Indonesian), Introduction, Methods, Results, Discussion, Conclusion(s), Conflict of Interest, Acknowledgments, and References.

Articles Title

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Introduction

The introduction is written succinctly to stimulate the reader's interest include all the necessary information. At the end of the introduction was written the purpose of the study.

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Methods contains the material under study, and the way described briefly by the order of operation as well as the location and time of the study. Explain statistical methods in detail. Consideration of ethical issues is included. If the protocol has been approved then the ethical clearance/approval letter number and the health research ethics committee must be written.

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The result is the core of scientific writing. This section presents data and information that will be used as

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Discussion of the article reveals, explains, and discusses the results of the study with an analysis by the research design, interpretation, and explanation of its synthesis. Also, the results obtained are compared with the results of previous research of others.

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Acknowledgments should be provided to research contributors without writing a degree.

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King P. Haemophilus influenzae and the lung (Haemophilus and the lung). Clin Transl Med. 2012;1:10 [cited 2015 August 15]. Available from: https://clintransmed.springeropen.com/articles/10.1186/2001-1326-1-10.

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RESEARCH ARTICLE

An Overview of Knowledge Levels about Organ Topography and Structure among Grade IV Medical Students

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Abstract

One of the competencies the students of the graduate medical education is to apply the basic principles of the science of biomedicine, epidemiology, clinical, and behavior in the practice of medicine. Anatomy is one of the biomedicine sciences learned in medical education, including learning about the body's structure and organ topography. This research aims to describe the organ's structure and topography knowledge among the faculty of medicine students. This survey with 88 students was conducted in a faculty of medicine in Bandung city from October to December 2018 using a random sampling method. Statistical analysis using frequency distribution, percentage proportions, and Wald's statistics in the 95% confidence interval. The instrument for validity analysis is Pearsons's correlation, and the instrument for reliability analysis is Kappa's percent agreement. The results showed the average level of knowledge on organ structure and topography. It shows anatomical teaching and learning about the organ's structure, and the topography still needs to be optimized.

Key words: Anatomy, structure, topography

Gambaran Tingkat Pengetahuan tentang Struktur dan Topografi Organ pada Mahasiswa Tingkat IV Kedokteran

Abstrak

Salah satu kompetensi lulusan mahasiswa pendidikan kedokteran adalah mampu mengaplikasikan prinsip ilmu dasar biomedik, epidemiologi, klinis, dan perilaku dalam praktik profesi kedokteran. Anatomi merupakan salah satu ilmu biomedik dalam pendidikan kedokteran yang mempelajari struktur dan topografi organ. Penelitian ini bertujuan mengetahui tingkat pengetahuan mahasiswa tingkat IV fakultas kedokteran mengenai struktur dan topografi organ. Survei terhadap 88 mahasiswa ini dilaksanakan di sebuah fakultas kedokteran di Kota Bandung dari bulan Oktober hingga Desember 2018 menggunakan metode random sampling. Analisis statistik menggunakan distribusi frekuensi, persentase proporsi, dan *Wald's statistic* dalam 95% interval kepercayaan. Analisis validitas menggunakan *Pearson's correlation*, sedangkan analisis reliabilitas menggunakan *Kappa's percent agreement*. Hasil penelitian menunjukkan tingkat pengetahuan rerata tentang struktur organ dan topografi dalam kategori cukup. Hal ini menunjukkan pembelajaran anatomi tentang struktur organ dan topografi masih perlu dioptimalkan.

Kata kunci: Anatomi, struktur, topografi

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Introduction

The free entry of health workers, including doctors from ASEAN countries, to Indonesia, requires doctors in Indonesia to improve the quality to compete with the foreign doctor.[1] To improve Indonesia's doctor's quality, governments make laws No. 20 of 2013 of medical education, mentioning that to become a doctor has to be through academic and professional medical education. The curriculum used during the academic excellence should refer to national standards of Medical Education Core Curriculum, namely physician education Indonesia (KIPDI) III. Based on KIPDI III, basic medical science should be integrated with graduates' needs in the exercise of the profession.[2,3]

Based on the National Accreditation Agency higher education (BAN-PT), 72 medical schools have produced graduates. Of the 72 faculty, only 21 medical faculties are accredited A, 46 faculty have the B accreditation, and five are accredited C. It takes the role of many stakeholders in improving the quality of education in Indonesia.[4]

One of the graduates' competencies is to apply the basic principles of the science of biomedicine, epidemiology, and clinical behavior and practice in the medical profession. One of the biomedicine sciences is anatomy.[5] Anatomy is the cornerstone of medical education; anatomical knowledge is undoubtedly essential for doctors regardless of their specialty, particularly since they continue to perform physical examinations, make medical decisions, communicate with colleagues, and provide explanations to patients.[6] Anatomists and clinicians agree that accurate knowledge of anatomy is vital to ensure a safe and efficient clinical practice.[5,7]

We survey to observe the competence in the field of anatomy to understand the level of knowledge of the organ's structure and topography among the medical student.

Methods

This research was conducted in the faculty of medicine in Bandung from October to December 2018. This study using a survey approach is applying the random sampling method. The validation and reliability testing questionnaire is done before doing the survey. The subject in this research was medical students at level IV 2018/2019 school year. The subject was prompted to fill in a questionnaire that contains the knowledge of the structure and topography of the organ, and the result is a level of knowledge grouped into low, medium, and high levels of knowledge.

The study has been ethically approved by the Research Ethics Committee of Faculty of Medicine, Universitas Islam Bandung with the certificate number is 373/Komite Etik. FK/X/2018.

Results

The research recruited 88 students.

The highest median value of the knowledge of the organ structures is in the endocrine metabolic system (EMS), while the lowest is present in the special sense system (SS). SS system with minimal value in a cardiovascular system (CVS) and SS that is 0. The highest value of 100 was found in the reproductive system (RPS), SS, and CVS (Table 1).

Knowledge of organ topographic shows

Table 1 Frequency Distribution of Knowledge about the Structure of the Organ

	DMS	EMS	NBSS	GUS	RPS	SS	CVS	GIS	RESPI
Min	22.22	27.27	7.69	10	20	0	0	12.5	0
Median	55.56	63.64	53.85	50	60	40	50	43.75	33.33
Max	88.89	90.91	92.31	90	100	100	100	87.5	100
Mean	53.79	60.74	52.27	53.95	61.02	49.54	53.98	46.02	39.96
SD	14.48	16.63	20.21	17.76	14.31	23.09	25.01	17.83	17.96
LB	39.31	44.11	32.06	36.19	46.71	26.45	28.97	28.19	22
UB	68.27	77.37	72.48	71.71	75.33	72.63	78.99	63.85	57.92

Note: DMS=dermatomuscular system, EMS=endocrine metabolic system, NBSS=neurobehaviour system, GUS=genito urinary system, RPS=reproductive system, SS=special sense system, CVS=cardiovascular system, GIS=gastrointestinal system, RESPI=respiration system

Table 2 Frequency Distribution of Knowledge about the Topography of the Organ

	DMS	EMS	NBSS	GUS	RPS	SS	CVS	GIS	RESPI
Min	10	0	0	14.28	33.33	0	0	0	0
Median	60	50	44.44	57.14	66.67	50	37.5	57.14	42.86
Max	90	100	100	100	100	100	75	100	85.71
Mean	59.43	55.87	46.46	56.98	69.95	64.49	42.33	57.47	41.39
SD	18.89	24.5	22.82	20.14	14.51	25.06	18.71	22.29	19.26
LB	40.54	31.37	23.64	36.84	55.44	39.43	23.62	35.18	22.13
UB	78.32	80.37	69.28	77.12	84.46	89.55	61.04	79.76	60.65

Note: DMS=dermatomuscular system, EMS=endocrine metabolic system, NBSS=neurobehaviour system, GUS=genito urinary system, RPS=reproductive system, SS=special sense system, CVS=cardiovascular system, GIS=gastrointestinal system, RESPI=respiration system

interesting findings as listed in Table 2. The lowest values showed in EMS, NBSS, SS, CVS, GIS, and RS. The highest median value there is in the RPS. There was a maximum value of 100 in the EMS, NBSS, GUS, RPS, SS, and GIS. The comparison median value of the structure and topography is shown in Figure. The knowledge structure's median values are less than the topography knowledge in the DMS, GUS, RPS, SS, GIS, and RS. However, it does not occur in a system of EMS, NBSS, and CVS.

The value of the level of knowledge of organs' structure is smaller than the value of the level of knowledge of the organ's topography at DMS, GUS, RPS, SS, GIS, and RS. The value of the level of knowledge of an organ's structure is higher than the value of the level of knowledge of the organ's topography at EMS, NBSS, and CVS (Table 3).

The Largest proportion of results level of knowledge about the structure and topography in all organ systems are in the category of average.

Discussion

Anatomy is the study of the structure of the human body.[8] Anatomy is sound basic science to become the foundation for physicians in conducting physical examinations, diagnosis, and understanding the patient's disease condition.[9] By learning gross anatomy, medical students get a first "impression" about the structure of the human body, which is the basis for understanding pathologic and clinical problems.[10] Although anatomy is a basic science in medical education; many students feel difficulties in studying

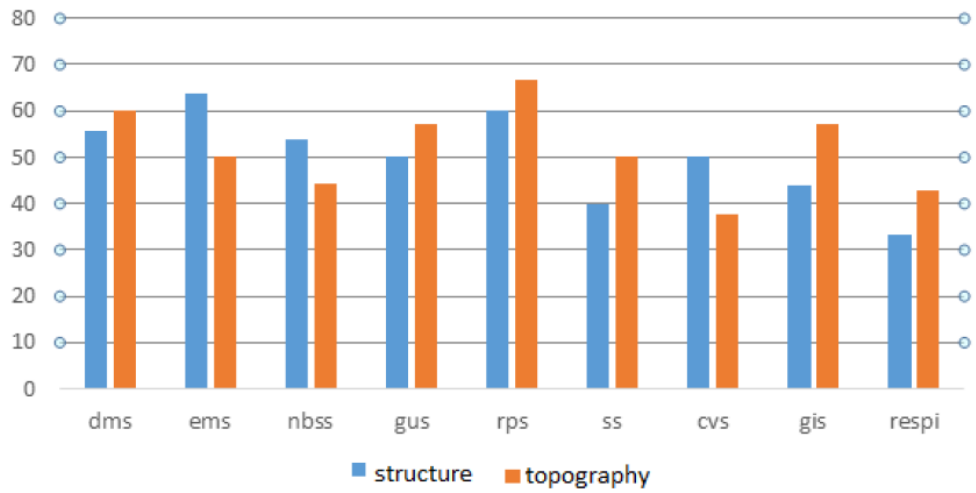

Figure Comparison Median Value of Knowledge about the Structure and Topography between Systems

Table 3 Proportion Knowledge of Structure and Topography in All Modul System

Modul System	Knowledge	Proportion of Knowledge		
		Low (%)	Average (%)	High (%)
DMS	Structure	17.04	75	7.95
	Topography	21.59	54.54	23.86
EMS	Structure	13.64	71.59	14.77
	Topography	11.36	65.91	22.73
NBSS	Structure	21.59	57.95	20.45
	Topography	23.86	60.23	15.91
GUS	Structure	11.36	80.68	7.95
	Topography	14.77	69.32	15.91
RPS	Structure	7.95	73.86	18.18
	Topography	10.23	72.73	17.04
SS	Structure	15.91	62.5	21.59
	Topography	12.5	64.77	22.73
CVS	Structure	13.64	59.19	27.27
	Topography	11.36	63.64	25
GIS	Structure	20.45	69.32	10.23
	Topography	15.91	69.32	14.77
RESPI	Structure	15.91	69.32	14.77
	Topography	17.04	71.59	11.36

Note: DMS=dermatomuscular system, EMS=endocrine metabolic system, NBSS=neurobehaviour system, GUS=genito urinary system, RPS=reproductive system, SS=special sense system, CVS=cardiovascular system, GIS=gastrointestinal system, RESPI=respiration system

anatomy.[11] This is apparent from the research results are listed in Table 1 dan Table 2, which shows the range of great value, i.e., the existence of a minimum value of 0 and a maximum value of 100.

Over the past few decades, the medical education paradigm has evolved to focus on problem-based, clinical competency-accentuated approaches, student-centered, and integrated.[12] Anatomy can be made easy by modifying the traditional anatomy education delivered by didactic lectures and cadaveric dissection. The curriculum in Anatomy should be delivered by combining different methods of teaching. Computer-assisted, problem-based learning, self-directed and directed self-learning in addition to traditional one can be used.[13] For centuries, the primary tools for teaching anatomy have been lectures, tutorials, textbooks, cadaveric dissections, and demonstrations from prosected specimens.[14] Anatomy lesson with tutorials and laboratory activities in this medical faculty using active learning principles; where students expected to be active and become the actors in the learning environment. They participate in the process of higher-order thinking, such as analysis, synthesis, and evaluation. However, in practice, most students seem to position themselves as passive learners, accepting only what is to be delivered by the lecturer.

Another thing that might cause this statement is that anatomy is a material that many use in Latin. The material for the anatomy lessons was so many, while learning time is minimal 10. Other factors some students are not ready to follow the tutorials or laboratory activities. Students sometimes do not know what material they will learn during tutorials and laboratories to not keep up with the good learning activities. Students are often not doing homework or practicing laboratory activities and not making flipcharts properly during tutorials.[15] To make the medical students interested in anatomy, various new methods have been proposed, which could improve their practical skills. In these methods, the students, while studying the theoretical subjects, are introduced to body structures and functions on the alive and healthy person and then learn their practical usage.[16]

Figure 1 shows that the value of the level of knowledge of organs' structure is smaller than the value of the level of knowledge of the organ's

topography at DMS, GUS, RPS, SS, GIS, and RS. Many organs should be studied in detail the structure of these systems, so students' chances to forget about the name of the structure in each of these organs is larger. DMS system, students study the structure of each group contains the bones of the axial and appendicular. In the system, GUS students must study the structure of the kidneys' organs, ureters, bladder, urethra, and genital organs male internal-external. In the RPS, students must learn the internal genital organs-external women following hip diversionary bones. In the special sense system (SS), students must learn the eyes and ears' organ. In GIS, students studying the organs' structure directly in the digestive system starts from the nose to anus and digestive organs accessories. Respiratory system-students learn all the organs involved in respiration starts from the nose to the lungs. The topography is the organ study based on its location and its relationship with other organs around it. Studying the layout of this organ requires students to imagine or envision an organ against the layout of other organs. Some students feel difficulties in studying topography, which is apparent from the questionnaires like EMS and NBSS. The Results showed in the CVS the level of knowledge about the organ structure that is higher than the organ's topography level. Students may have difficulty learning the subject matter of the anatomical sciences. Reasons for this may include issues with motivation, course content, and the quantity of information.[17]

Real success in education can be achieved through a combination of motivation, creativity, innovation, inspiration, and teamwork. The role of a teacher as a knowledge provider cannot be denied. However, a teacher or a facilitator as a motivator is brought out with this project. The teacher here is initiating the chain reaction with his/her motivation.[18]

Research results in Table 3 show the largest proportion of results level of knowledge about the structure and topography in all organ systems are in the category of "average." It shows anatomical teaching and learning about the organ's structure, and the topography of the organ has been running quite well but has yet to achieve maximum results. Students learn anatomy through tutorials and laboratory activities using a mannequin and saw the cadaver. Students know the science through the textbook and see in real through mannequin knowledge is the result of knowing, and this happens after people doing mainly against an object sensing. Various models (plastic, animal tissue, computer, etc.) have been useful for learning human anatomy in several different contexts. Sensing happens through sense human being; sight, hearing, smell, taste, and touch. Students should understand why specific terms are used in clinical medicine to ensure their correct use and resolve any discrepancies between the official terminologies and those specifically used in clinical medicine.[19]

Most human knowledge is obtained through the eyes and ears. The knowledge covered in the cognitive domain has six levels, knowledge, understanding (comprehension), application, analysis, synthesis, and evaluation.[20] The results of the study could not be released from the learning process. The learning process involves faculty and students.[21]

Motivation needs to be owned by a student through the process of learning, motivation can push someone, so eventually, that person becomes a specialist in a particular field of science. Someone would like to try to learn something with his best if he did not know how important and helpful the results will be from his studies.[22]

In the learning process, a student's interest is vital. For someone who has no interest in learning is not possible to do the learning activities. Interest is "symptoms that are interested in anything which further interest someone will reflect its purpose." Suppose the interested student to a particular lesson can be viewed and observed on working in this lesson. This interest plays a vital role in teaching and learning. Without any interest, then he could not master the lessons given.[23] Medical students need anatomy lessons modification of its teaching methods to makes them more interested in anatomy and can also help them improve their professional skills.[24]

In the world of education, the lecturer is the cutting edge of education. A lecturer is the direct perpetrator of the service's activities. The lecturer's quality will affect the quality of education services—the lecturer's quality is determined by educational background, personality, and ability to communicate. Interpersonal communication skills that a lecturer must master are empathy, equality, confidence, immediacy, supportiveness, positiveness, interaction management, and listening.[21]

Empathy means a lecturer should feel what is perceived by the student while teaching and

learning activities taking place. Supportiveness intent is a college lecturer should create a sense of comfort when teaching and learning take place. Positiveness intent is a college lecturer should make students feel valued and not be underestimated. Equality means a college lecturer should be able to give equal treatment to all its student in the process of teaching and learning. Confidence means a lecturer should have the self-confidence to foster students ' beliefs against the lecturer as someone who can be relied upon when they need information. Immediacy means that lecturers should show concern and interest in students' problems in the process of teaching and learning. Interaction management significance means a college lecturer should be able to manage the process of good teaching and to learn so that students can understand what is delivered by lecturers and lecturers to understand what is not understood by the student body. Listening means a lecturer should have the capability of being a good listener when the student submits an opinion in the discussion or criticism of its student gained about teaching and learning.[21]

Unlike other basic science lessons, such as biochemistry and physiology, teaching anatomy needs a particular method of teaching and appropriate tools. These tools include a cadaver, human body atlases, and educational movies and slides.[25]

Conclusion

The level of knowledge of the structure and topography of the organ among the medical student level IV is average.

Conflict of Interest

All authors stated that there no conflict of interest in this study.

Acknowledgments

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RESEARCH ARTICLE

Hepatoprotective Effect of Sun Chlorella as an Antioxidant in *Rattus norvegicus* Induced Carbon Tetrachloride

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Abstract

Carbon tetrachloride (CCl_4) is a chemical that can cause damage to liver cells. One of the natural ingredients developed to reduce liver damage due to chemicals and infections is *Chlorella* sp. The research aimed to explore the hepatoprotective effect of the antioxidant superoxide dismutase (SOD) by administering Sun Chlorella in *Rattus norvegicus* CCl_4 induced rats. The study design used a post-test with a control group design with a completely randomized design trial on 30 male rats of Wistar strains, aged 2–3 months, 200–250 grams in weight. The research was conducted in November 2016–January 2017 in Purwokerto. Rats were divided into five groups and treated for four weeks as follows: K1 group was given aquades; K2 was given CCl_4; K3, K4, K5 were given CCl_4 and Sun Chlorella 3.6 mg/200 gBW, 7.2 mg/200 gBW, and 14.4 mg/200 gBW respectively, by the gastric probe. Statistical analysis with correlation test and one way ANOVA multivariate test showed that Sun Chlorella 7.2 and 14.4 mg/200 gBW significantly increased SOD levels in rats induced CCl_4 (p=0.004, p=0.009). SOD rates were significantly strong associated with aspartate aminotransferase/AST (r=−0.685, p=0.000) and alanine aminotransferase/ALT (r=−0.659, p=0.000). The conclusion is Sun Chlorella increases SOD levels in CCl_4-induced rats. Increased SOD levels may decrease AST and ALT levels.

Key words: Antioxidant, CCl_4, *Chlorella*, SOD

Efek Hepatoprotektif *Sun Chlorella* sebagai Antioksidan pada *Rattus norvegicus* yang Diinduksi Karbon Tetraklorida

Abstrak

Karbon tetraklorida (CCl_4) merupakan bahan kimia yang dapat menyebabkan kerusakan sel hati. Berbagai bahan alami telah dikembangkan untuk mengurangi kerusakan hati baik akibat bahan kimia maupun infeksi, salah satunya adalah *Chlorella* sp. Penelitian ini bertujuan melihat efek hepatoprotektif dengan pemberian *Sun Chlorella* pada tikus *Rattus norvegicus* yang diinduksi CCl_4. Desain penelitian menggunakan *post-test with a control group* dengan rancangan percobaan rancangan acak lengkap pada 30 tikus jantan galur Wistar, usia 2–3 bulan, dan berat 200–250 gram. Penelitian ini dilakukan di Purwokerto pada periode November 2016–Januari 2017. Tikus dibagi menjadi lima kelompok dan mendapatkan perlakuan per oral dengan sonde lambung selama 4 minggu sebagai berikut: kelompok K1 sebagi kontrol negatif diberikan aquades; kelompok control positif K2 diberikan CCl_4; kelompok perlakuan K3, K4, dan K5 diberikan CCl4 dan *Sun Chlorella* 3,6 mg/200 gBB; 7,2 mg/200 gBB; 14,4 mg/200 gBB tikus berurutan. Uji statistik dengan menggunakan uji korelasi dan ANOVA satu arah menunjukkan bahwa pemberian *Sun Chlorella* 7,2 mg dan 14,4 mg meningkatkan kadar SOD pada tikus yang diinduksi CCl_4 secara bermakna (p=0,004; p=0,009). Kadar SOD berhubungan kuat dengan kadar aspartat aminotransferase/AST (r=−0,685; p=0,000) dan alanin aminotransferase/ALT (r=−0,659; p=0,000). Kesimpulan penelitian ini adalah pemberian *Sun Chlorella* meningkatkan kadar SOD pada tikus yang diinduksi CCl_4. Peningkatan kadar SOD menyebabkan penurunan kadar AST dan ALT.

Kata kunci: Antioksidan, CCl_4, *Chlorella*, SOD

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Introduction

The liver is an organ essential in the metabolism and detoxification of foreign substances in the body, such as carcinogens, chemicals, drugs, and insecticides.[1,2] These substances can cause damage to hepatocyte cells in the liver.[3] One of the chemicals that can impair the liver is carbon tetrachloride (CCl_4), which increases lipid peroxidation and hepatocyte death marked by swelling, vacuolization, and hepatocyte in rats.[4,5] It also promotes cirrhotic change.[6] Therefore, induction of CCl_4 in experimental animals is often used in research.[6,7]

Various drugs, natural ingredients, or organisms have been developed to reduce liver damage due to chemicals and infections. One of the organisms used to resolve liver damage is *Chlorella* sp.[8] *Chlorella* is a unicellular green alga that contains various antioxidants such as chlorophyll, essential amino acids, protein, minerals, vitamins, dietary fiber.[9] It also contains phytochemical composition such as alkaloids, flavonoids, triterpenes, glycosides, tannins, and phenols.[10] Carotenoid is one of the substances in *Chlorella* sp., which plays a role as an antioxidant.[11]

Many studies of *Chlorella* sp. have been extensively researched. Lee et al.[7] (2010) showed that the administration of *Chlorella* supplements to smokers has antioxidant effects. Azocar and Diaz[12] (2013) found that replenishment of *Chlorella* can reduce aspartate aminotransferase levels in adult chronic hepatitis C patients. Cai et al.[13] (2015) showed that *Chlorella vulgaris* extract led to a significant decrease in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in rats, followed by an increased antioxidant activity such as superoxide dismutase (SOD), catalase, and glutathione (GSH), which compared to the CCl_4 induced rats.[12,13]

This study aims to explore hepatoprotective effects of Sun Chlorella (CNI Sun Chlorella; PT Citra Nusa Insan Cemerlang [PT CNI], Jakarta, Indonesia) based on antioxidant status, specifically in levels of superoxide dismutase (SOD) in *Rattus norvegicus* carbon tetrachloride (CCl_4) induced rats. This study differs from Cai et al.[13] in model animals for CCl_4 and *Chlorella* dosage and treatment duration.

Methods

This research was conducted in Animal House and Research Laboratory, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, from November 2016–January 2017.

Thirty male white rat Wistar strain with 2–3 months of age and 200–250 g of weight from LPPT IV Universitas Gadjah Mada Yogyakarta was used in this study. Ethics approval for this research was issued by the Research Ethics Committee, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto No. 176/KEPK/X/2016.

The *Chlorella* preparation used in this study was the Sun Chlorella tablet produced by PT CNI (SD 081534191), which contained 170 mg of *Chlorella* powder extracted from *Chlorella pyrenoidosa*. This Sun Chlorella was chosen because it is widely available and has been commonly used as a health supplement.

This research was conducted experimentally with a completely randomized and post-test only with a control group design. The rats were divided into five groups, namely K1, K2, K3, K4, K5, and were acclimated within seven days. The negative control group (K1) were given aquades, the positive control group (K2) were given CCl_4 (Merck, Sigma-Aldrich 289116); CCl_4 + *Chlorella* 3.6 mg (K3 group); CCl_4 + *Chlorella* 7.2 mg (K4 group); CCl_4 + *Chlorella* 14.4 mg (K5 group). Aquades or CCl_4 were given orally with a gastric probe at a dose of 0.2 mL/100 g of body weight (BW) two times a week for two weeks, followed by 0.1 mL/100 gBW two times a week for two weeks. Sun Chlorella (CNI Sun Chlorella) was given orally with a gastric probe at a dose of 3.6 mg or 7.2 mg or 14.4 mg/200 gBW for four weeks.[14] *Chlorella* dose of 7.2 mg/200 gBW was obtained from converting an adult human dose to 400 mg/70 kg. Next, the dose was divided by two (3.6 mg) and multiply by two (14.4 mg).[15]

Blood collection was done through the orbitalis vein of the rat one day after the treatment. Furthermore, the blood was centrifuged at a speed of 4.000 rpm. The blood serum was then separated from erythrocytes, and then AST, ALT, and SOD were examined.

AST (DiaSys 126019910920), ALT (DiaSys 127019910026), and SOD (Randox SD126) levels were examined using a spectrophotometer (Shimadzu UV 1800). The data obtained were

analyzed using a correlation test and one way ANOVA multivariate test. One data from the K3 group was excluded because it was an outlier.

Results

The mean levels of AST, ALT, and SOD in various treatment groups are presented in Table 1. AST, ALT, and SOD levels have normal data distribution (p>0.05), but the homogeneity of AST and ALT variance showed different variants. The highest mean levels of AST (3,210.00 U/L) and ALT (2,616.66 U/L) were acquired in the K2 group, the group with CCl_4 administration, while the highest mean levels of SOD (73.00 U/mL) was in the negative control group (K1).

The results showed that administration of CCl_4 increased AST levels from 267.33 U/L to 3,210.00 U/L (p=0.027). Sun Chlorella at either dose of 3.6 mg, 7.2 mg, or 14.4 mg/200 gBW did not significantly reduce AST compared to the positive control group (respectively p=0.058, p=0.160, and p=0.073).

CCl_4 induced an increase in ALT from 233.83 U/L to 2,616.66 U/L (p= 0.016). Sun Chlorella dose of 14.4 mg/200 gBW significantly reduced ALT levels to 795.83 U/L compared to the positive control group (K2) with p=0.047.

Table 1 AST, ALT, and SOD Levels in Experimental Animals

Groups	AST (U/L) Mean (±SD)	ALT (U/L) Mean (±SD)	SOD (U/mL) Mean (±SD)
K1	267.33 (99.32)	233.83 (135.24)	73.00 (4.42)
K2	3,210.00 (154.07)	2,616.66 (1,105.72)	37.83 (4.95)
K3	821.20 (477.84)	886.00 (716.34)	40.00 (3.74)
K4	1,385.00 (643.60)	1,110.00 (503.65)	48.50 (2.94)
K5	947.50 (646.85)	795.83 (621.38)	66.00 (5.58)

Table 2 Correlation between SOD Levels with AST and ALT Levels

AST and ALT Levels	r*	Significance (p)
AST	−0.685	0.000
ALT	−0.659	0.000

Note: *Spearman's correlation

Table 3 ANOVA Test and Post Hoc Analysis of AST, ALT, and SOD Levels among Groups

Groups	AST		ALT		SOD	
	ANOVA	Post Hoc	ANOVA	Post Hoc	ANOVA	Post Hoc
	0.000*		0.000*		0.000*	
K1 - K2		0.027*		0.016*		0.000*
K1 - K3		0.235		0.392		0.000*
K1 - K4		0.039		0.037*		0.000*
K1 - K5		0.212		0.313		0.119
K2 - K3		0.058		0.074		1.000
K2 - K4		0.160		0.099		0.004*
K2 - K5		0.073		0.047*		0.009*
K3 - K4		0.498		0.973		0.043*
K3 - K5		0.995		0.999		0.000*
K4 - K5		0.765		0.866		0.000*

Note: *the mean difference is significant at the 0.05 level

SOD levels in experimental animals decreased from 73.00 U/mL to 37.83 U/mL due to CCl_4 administration (p=0.000). Sun Chlorella tablet given at a dose of 7.2 mg and 14.4 mg/200 gBW significantly increased SOD levels (p=0.004 and p=0.009) compared to the positive control group. The increase in SOD levels by Sun Chlorella administration dosed of 14.4 mg/200 gBW approached the SOD levels of the healthy control group (p=0.119). This study found a significant strong relationship between SOD levels with AST (r=−0.685, p=0.000) and ALT levels (r=−0.659, p=0.000).

Discussion

CCl_4 is proven to cause damage to liver cells, which is observed from increased AST and ALT levels. The highest AST and ALT levels were found in the positive control group that received CCl_4 only. Adipocytokine adiponectin[16] mediates liver injury by CCl_4 through bioactivation of endoplasmic reticulum and mitochondrial centrilobular hepatocytes, which contain many cytochrome P450 2E1 (CYP2E1). This mechanism cause trichloromethyl (CCl_3) and trichloromethyl peroxyl (CCl_3OO) free radicals to activate haloalkylation of cell macromolecules. Cell destruction due to CCl_3 can occur anaerobically or aerobically. The anaerobic process is in the form of dimerization of CCl_3 to form hexachloroethane. CCl_3 can also bind directly to lipids and microsomal proteins and the heme part of CYP450. The aerobic process causes CCl_3 to bind to oxygen to form trichloromethyl peroxyl (CCl_3OO). CCl_3OO can bind directly to tissue proteins or break down to form phosgene ($COCl_2$) and the electrophilic form of chlorine. CCl_3 peroxy radical is the main initiator of lipid peroxidation that is formed from carbon tetrachloride exposure. This mechanism causes lipid peroxidation, which harms membrane integrity, decreased organelle function, and cell death.[16–18]

Hepatocyte cell death causes the release of enzymes, including AST and ALT enzymes. AST (around 80%) comes from mitochondria and 20% from the cytoplasm of liver cells. ALT is an indicator of liver cell damage enzymes, mainly produced by the cytoplasm of liver cells. Increased ALT and AST are found in liver damage.[19] The results of this study indicate that liver cell damage occurs in experimental animals due to CCl_4 induction based on an increase in AST and ALT levels. The result of this study is consistent with some previous studies. AbouGabal et al.[20] found a significant increase in AST and ALT levels in the group given only CCl_4 (1 mL/kgBW mice/day for three weeks) compared to the control and olive oil groups. Likewise, the research of Fortea et al.[21] showed an increase in AST and ALT levels in the group given CCl_4 twice a week for 12 weeks compared with the control group. Ito et al.,[22] Song et al.,[23] El-Bialy et al.,[24] El-Dakhly et al.[25] also reported a significant difference in AST and ALT level between CCL_4 and control group. CCl_4 induction treatment for four weeks is likely to cause chronic liver damage, which is observed from an increase in AST levels higher than ALT.[19]

This study shows that *Chlorella* can increase SOD antioxidant and decrease AST and ALT levels in *Rattus norvegicus* rats administered by carbon tetrachloride (CCl_4). These results are similar to the previous studies. Cai et al.[13] reported that pigment-protein complex (PPC) was isolated from *Chlorella vulgaris* effectively restored SOD level. Research by Sikiru et al.[26] in the white rabbit showed the highest levels of SOD found in the group given *Chlorella vulgaris* supplements at a dose of 200 mg/gBW. Another study showed that the administration of 5 mg/100 gBW of *Chlorella vulgaris* extract could increase SOD activity to protect liver cells' damage caused by CCl_4 exposure.[27] Decreased AST and ALT levels are similar with research results from Cai et al.[13] and Ito et al.[22] Another study proved that *Chlorella* could reduce AST and ALT level in acetaminophen-induced liver damage rat model.[28]

The carotenoid content in *Chlorella* probably causes an increase in SOD levels.[11] Carotenoids are known to have antioxidant functions that significantly reduce free radicals and activate oxidative compounds. Carotenoid content in the form of ß-carotene or astaxanthin prevents the oxidation of cells by free radicals through scavenging free radicals to prevent and stop the oxidative reaction chain. The protective mechanism by ß-carotene in cells against oxidative reactions is by preventing the formation of oxygen singlets. Singlet oxygen is a non-electrophilic radical ROS that is easily bonded with organic molecules, affecting the oxidation process by directly attacking electron-rich compounds without the involvement of free radicals. ß-carotene is also efficient as an oxygen scavenger, reducing ROS release, which decreases in microsomal and cytochrome p450,

prevents chain reactions, or damages the lipid peroxidation reaction.[27,29,30]

This study found a significant relationship between SOD levels with AST and ALT. The increase in SOD levels would reduce AST and ALT levels. It proves that *Chlorella* has a hepatoprotective effect. In vivo research by Cai et al.,[13] the administration of protein pigment complexes taken from *Chlorella* showed a hepatoprotective effect. This hepatoprotective effect is expected from the carotenoid content in *Chlorella*, which can inhibit and bind ROS.[11,30]

Conclusions

CCl_4 induction cause liver damage, as shown by increased levels of AST and ALT. *Chlorella* administration has a hepatoprotective effect in CCl_4-induced *Rattus norvegicus* rats based on increased SOD levels, decreased AST and ALT levels.

Conflict of Interest

The authors state that there is no conflict of interest.

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RESEARCH ARTICLE

Hyperferritinemia Correlated with Activated Population of Natural Killer Cells in Pediatric Major β-Thalassemia Patients

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Abstract

Natural killer (NK) cells act both as cytotoxic and cytokine producers in the innate immune response. Hyperferritinemia resulting from a routine blood transfusion as a specific treatment in major β-thalassemia patients may disturb the cellular immune system's harmony. This study aims to investigate the correlation between hyperferritinemia and the NK cell subsets in major β-thalassemia settings. Pediatric major β-thalassemia patients who routinely received a blood transfusion at Dr. Hasan Sadikin General Hospital in 2016 were included in this cross-sectional study. Blood samples were treated with the monoclonal antibody of CD3, CD56, and CD16 to count the NK cells subsets as $CD56^{bright}$, $CD56^{dim}$, and $CD16^{+}$ using flowcytometry. $CD69^{+}$ used as an activation marker. The median fluorescence intensity (MFI) of CD56, CD16, and CD69 was measured. Total iron-binding capacity (TiBC), ferritin, and serum iron level examined as iron status. A Spearman correlation test was used for statistical analysis. Fifty-five blood samples were obtained for analysis. This study reveals that the percentage of $CD3^{-}$ lymphocyte population was correlated with the ferritin levels (r=−0.45, p=0.0009). Positive correlation was revealed between activated population ($CD69^{+}$) of $CD56^{bright}$ and $CD56^{dim}$ NK cell subsets and hyperferritinemia [(r=0.353, p=0.008) and (r=0.355, p=0.008)]. The activated $CD56^{bright}$ cells was associated with ferritin level (r=0.353, p=0.008) and TiBC (r=0.334, p=0.018). Hyperferritinemia in pediatric major β-thalassemia patients may influence NK cell subsets' balance population, particularly the $CD56^{bright}$ and $CD56^{dim}$ NK cell subsets, then alter their immune response to pathogens.

Key words: Hyperferritinemia, major β-thalassemia, NK cells

Korelasi antara Hiperferitinemia dan Sel *Natural Killer* Teraktivasi pada Anak dengan Talasemia Beta Mayor

Abstrak

Sel-sel *natural killer* (NK) telah diketahui memiliki peran sitotoksik dan dalam produksi sitokin pada respons imun bawaan. Hiperferitinemia merupakan hasil dari transfusi darah rutin yang dijalani sebagai terapi utama pada talasemia mayor. Penelitian ini bertujuan mempelajari hubungan hiperferitinemia dan sel NK pada talasemia beta mayor. Penelitian potong lintang ini melibatkan anak dengan talasemia beta mayor yang secara rutin menerima transfusi darah di RSUP Dr. Hasan Sadikin selama tahun 2016. Sampel darah diberi *marker* CD3, CD56 dan CD16 untuk menghitung *subset* sel NK sebagai $CD56^{bright}$, $CD56^{dim}$, dan $CD16^{+}$ menggunakan *flowcytometry*. $CD69^{+}$ digunakan sebagai penanda aktivasi. *Median fluorescence intensity* (MFI) CD56, CD16, dan CD69 diukur. Kadar TiBC, feritin, dan Fe serum diperiksa sebagai status besi. Uji korelasi Spearman digunakan pada analisis statistik. Analisis dilakukan terhadap 55 sampel darah anak dengan talasemia. Penelitian ini mendapatkan bahwa populasi limfosit CD3 berkorelasi dengan kadar feritin (r=-0,45,p=0,0009). Korelasi positif didapatkan pada populasi teraktivasi ($CD69^{+}$) dari *subset* sel $CD56^{bright}$ dan $CD56^{dim}$ NK dan hiperferitinemia [(r=0,353: p=0,008) dan (r=0,355; p=0,008)]. Sel $CD56^{bright}$ teraktivasi berkorelasi dengan kadar feritin (r=0,353; p=0,008) dan TiBC (r=0,334; p=0,018). Hiperferitinemia pada anak dengan talasemia mayor dapat memengaruhi populasi sel NK, khususnya pada *subset* $CD56^{bright}$ dan $CD56^{dim}$ sehingga berpengaruh pada respons imun terhadap patogen.

Kata kunci: Hiperferitinemia, sel NK, talasemia beta mayor

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Introduction

Major β-thalassaemia, a familial blood disorder, characterized by defects of hemoglobin (Hb) beta-chain synthesis, resulting in severe anemia and treated with a routine blood transfusion as its definite therapy.[1,2] Iron overload often comes up in major β-thalassemia due to multiple blood transfusions, increased destruction of red blood cells, and increased gastrointestinal iron.[2,3] Excessed iron stored in the reticuloendothelial system as ferritin and secreted gradually in a small amount to the blood. Therefore, iron overload presented in increased ferritin levels, also known as hyperferritinemia.[1,3,4]

Excessive iron is highly harmful to all cells, including the innate immune cells, by reducing the number of cells population or the number of cell subsets, potentially as an immune response to pathogens.[1,5–7] Therefore, major β-thalassemia patients may be more susceptible to infection. Furthermore, immune cell alteration might increase infectious disease management challenges in this population to another level, including the prevention, diagnostic effort, and treatment.[5,8] An earlier report showed a decrease in NK cell activity in major thalassemia patients, and this might occur due to iron overload.[9,10]

NK cells are prominent cells of innate immune defense, filling out approximately 10 to 20% of the lymphocytes population in normal peripheral blood.[11,12] NK cells are initially known for their ability to lyse tumor cells without prior activation.[12] NK cells are also known for their cell-killing function and cytokine production, such as interferon (IFN)-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF). Therefore, their innate immune defense role becomes more critical to counter a considerable number of viral, bacterial, and parasitic pathogens.[12–14] In addition to their critical role in innate immune response, NK cells also play a role in the adaptive immune response through direct interaction with dendritic cells, which provide negative or positive dendritic cell activity regulation.[15]

NK cells were identified by the presence of CD56 and the absence of T-cell receptor (TCR) and CD3.[16] Subsets of NK cells classified by the expression of CD56 and the presence of CD16.[17,18] CD56 expression of NK cells related to cells' ability to secrete cytokines. At the same time, CD16 is related to cell cytotoxicity function.[16,18,19] $CD56^{+}CD16^{+}$ ($CD56^{dim}$) subset is the most abundant subsets comprising approximately 90% of the NK cells population. They have the most effective cytotoxic function, with less ability to secrete cytokine.[17,20–22] $CD56^{++}CD16^{-}$ ($CD56^{bright}$) subset comprises 10% NK cells population, known as cytokine producers subsets. They can secrete many cytokines, including IFN-γ, tumor necrosis factor (TNF)-β, IL-10, IL-13, TNF-α, and GM-CSF, but have less cytotoxicity function. However, the cytotoxic ability of $CD56^{bright}CD16^{-}$ subset strengthened after activation by IL-2 or IL-12.[11,23] $CD56^{-}CD16^{+}$ ($CD56^{neg}$) subset is another mature subset, minor in number, and have impaired effector function in both cytotoxic and cytokine production. The previous study found that the $CD56^{neg}CD16^{+}$ subset increased in HIV-1, HCV, and hantavirus infection.[24–26]

Activated NK cells displayed by expression of CD69 and HLA-DR, IFN-γ secretion, and enhancement of cytotoxic function.[27] CD69 is an early membrane receptor expressed right after activation of lymphocytes, including NK cells. Not detected in resting NK cells, CD69 rapidly induced in NK cells shortly after activation.[27–31]

Iron overload in major β-thalassemia might affect the NK cells' functions and population number and might alter the immune response in time. Therefore, we examine the NK cell subsets characterization in pediatric major β-thalassemia major with iron overload and the correlation between NK cell subsets and hyperferritinemia.

Methods

A cross-sectional study was conducted from October 17th–November 15th, 2016, in Thalassemia Outpatient Clinic, Dr. Hasan Sadikin General Hospital Bandung. Pediatric major β-thalassemia patients with iron overload recruited using simple random selection.

Our study included 55 pediatric major β-thalassemia patients with multiple blood transfusions and aged less than 15 years old. All subjects had been diagnosed with major β-thalassemia through a clinical examination, confirmed by a positive result on hemoglobin electrophoresis for β-thalassemia, and had undergone a routine blood transfusion for at least two years. All patients with signs of acute infections, chronic infections such as HBV, HIV, and unhealthy conditions were excluded.

Informed consent was obtained from the parents of all subjects. Blood specimens were

acquired by venipuncture before blood transfusion and collected in three tubes (heparin-contained, EDTA-contained, and plain). Anti-coagulated blood was used to perform flow cytometry of NK cells and hematology assessment, while non-anti-coagulant blood was processed into the serum to measure iron status..

Peripheral venous blood collected in vacutainer tubes containing lithium and sodium heparin (Becton Dickinson, Franklin Lakes, NJ, USA). Immunophenotyping NK cells' subsets were done employing multicolor flow cytometry, started within 1 hour after blood collection.

Utilizing a Becton Dickinson FACS™ Calibur™ flow cytometer, according to their phenotypic marker of $CD3^-$, $CD56^+$, and $CD16^+$, NK cells were grouped into three functional subsets based on stain index guided manual gating of NK cells population of whole blood. The activation marker of $CD69^+$ was used to measure the activated cells. Afterward, the fluorescence intensity of CD56, CD16, and CD69 was measured as markers of each protein expression at every subset.

For preparation, 2,000 µL PBA 0.5% added to 200 µL heparinized blood. After vortex and centrifugation at 1,500 rpm for 5 minutes, the cell suspension was formed, and the supernatant was then discarded. Monoclonal antibodies mixture of CD3 AlexaFlour 488 (Biolegend, San Diego, CA, USA), CD16 PE (Biolegend, San Diego, CA, USA), CD56 PerCP (Biolegend, San Diego, CA, USA), and CD69 APC (Biolegend, San Diego, CA, USA) were added and vortexed to cell suspension in FACS buffer diluted solution. The antibody-cell suspension was then covered by aluminum foil and incubated for 20 minutes at 2–8°C. Ten-time diluted red cell lysing buffer (Biolegend, San Diego, CA, USA) was added to stain cells, and the cells were then re-incubated for 12 minutes. The lysed cell suspension was then vortexed and washed two times using a 2,000 µL 0.5% PBA solution, and then cells were resuspended using 200 µL of the same 0.5% PBA solution. Cells were read according to their phenotypic marker by BD Cell Quest Pro Software (Biosciences, San Jose, CA, US) for 500,000 events, and then the FCM output files were analyzed using FlowJo 10 (Tree star). The CD3 monoclonal antibody (mAb) is used to separate NK cells as $CD3^-$ cells. CD56 and CD16 mAb were used to differentiate NK cell subsets as $CD56^{++}CD16^-$ ($CD56^{bright}$), $CD56^+CD16^+$ ($CD56^{dim}$), and $CD56^-CD16^+$ ($CD56^{neg}$). CD69 mAb was used to define the activated cells as $CD69^+$.

The population of NK cells subsets delivered as a percentage, which defined the proportion of NK cells count based on $CD56^{bright}$, $CD56^{dim}$, and $CD56^{neg}$ NK cells. Activated cells of each subset also delivered as a percentage of cells expressing $CD69^+$. The expression of the surface protein (CD56, CD16, and CD69) showed by median fluorescence intensity (MFI) of each monocyte subsets' designated protein.

Hematology assessment performed using peripheral venous blood samples collected in a potassium EDTA tube (Becton Dickinson, Franklin Lakes, New Jersey, USA). An automatic hematology analyzer (Sysmex Corp., Japan) was used to measure hematology parameters.

Collected sera from plain Vacutainer centrifuged for iron status measurement, including serum iron and ferritin. Serum ferritin was measured using the Elecsys ferritin immunoassay kit (Roche, Switzerland), while the serum iron assay kit (Merck, Singapore) was used to measure serum iron.

Non-normally distributed data presented as median with interquartile range (IQR); normally distributed data presented as mean with standard deviation (SD). Correlation between parameters tested using Spearman correlation coefficient for non-normally distributed data, and the Pearson correlation coefficient for normally distributed data. All analyses were performed with GraphPad PRISM version 6.0 (Graphpad Software, Inc., La Jolla, California, USA). The result with a $p\text{-value}<0.05$ is considered statistically significant. Linear regression analysis was performed following a significant correlation result.

All procedures were conducted in conformity with the Faculty of Medicine policies, Universitas Padjadjaran and Dr. Hasan Sadikin General Hospital, Bandung, West Java, Indonesia. This study was approved by the Health Research Ethics Committee of Faculty of Medicine, Universitas Padjadjaran Bandung, with approval number 74/UN6.C1.3.2/KEPK/PN/2016 and the Ethics Committee of Dr. Hasan Sadikin General Hospital Bandung with approval number LB.02.01/C02/15691/XI/2016.

Results

Characteristics of the study participants are presented in Table 1. Their mean age is 8 (±2.9) years old with an equal proportion of

gender. Hemolytic anemia [Hb=6.3 (±1.1) g/dL, MCV=74.7 (±4.7) fL], as experienced in major thalassemia, prove the clinical setting of this study. The leucocyte profile showed, respectively, normal total leucocyte count and differential lymphocyte count. All participants have iron overload status, proved by a high iron serum level [157 (±63.9) µg/dL], and a very high ferritin level [3,183 (1,678–4,460) µg/dL].

Utilizing a negative-gating strategy to separate

Table 1 Demographic and Clinical Characteristics of Study Participants

Characteristics	Patients' Value	Normal Value
Gender {n (%)}		
Male	28 (51)	Not define
Female	27 (49	Not define
Age, year {mean(SD)}	8 (2.9)	Not define
Hematological indicators		
Hb, g/dL {mean (SD)}	6.3 (1.1)	10.9–14.9
Leucocyte, /mm^3 {median (IQR)}	6,000 (4,000–8,100)	4,500–14,500
Thrombocyte, 10^3/mm^3 {median (IQR)}	209.9 (127–328)	150–300
MCV, fL {mean (SD)}	74.7 (4.7)	79–98
MCH, pg/cell {mean (SD)}	25.7 (2.4)	25–33
MCHC, g/dL {mean (SD)}	34.4 (1.7)	32–36
Iron status indicators (µg/L)		
Ferritin {median (IQR)}	3,183 (1,678–4,460)	<1,000
Serum iron {mean (SD)}	157 (63.9)	35–150
TIBC {median (IQR)}	197 (156–219)	240–474
Cell characteristics(%)		
Lymphocyte {mean (SD)}	33.81 (10.32)	25–54
CD3$^-$ lymphocyte {median (IQR)}	46 (37.8–53.5)	Not defined
CD56^{bright} NK cells {median (IQR)}	3.1 (1.59–4.96)	Not defined
CD56^{dim} NK cells {median (IQR)}	2.49 (0.74–5.95)	Not defined
CD56^{neg} NK cells {median (IQR)}	7.04 (1.75–9.77)	Not defined
CD69$^+$ of CD56^{bright} NK cells {mean (SD)}	36.23 (9.56)	Not defined
CD69$^+$ of CD56^{dim} NK cells {mean (SD)}	35.96 (13.03)	Not defined
CD69$^+$ of CD56^{neg} NK cells {mean (SD)}	28.95 (10.33)	Not defined

Note: Hb (hemoglobin), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), and MCHC (mean corpuscular hemoglobin concentration) were presented as mean with SD (standard deviation); TiBC (total iron-binding capacity) presented as median with IQR (interquartile range); CD (cluster of differentiation) and NK (natural killer) cell are in percentage and presented as mean with SD or median with IQR

Table 2 Correlation between Activated NK Cells and Ferritin Level

Parameter (%)	TiBC		Ferritin	
	r	p	r	p
CD3$^-$ cells	−0.11	0.461	−0.45	0.0009*
CD56^{bright}	−0.246	0.089	0.029	0.837
CD56^{dim}	−0.139	0.357	−0.087	0.55
CD56^{neg}	−0.135	0.359	−0.017	0.903
CD69$^+$ on CD56^{bright}	0.334	0.018*	0.353	0.008*
CD69$^+$ on CD56^{dim}	0.136	0.375	0.355	0.008*
CD69$^+$ on CD56^{neg}	0.062	0.614	0.240	0.08

Note: *Spearman's correlation, statistically significant if $p<0.05$

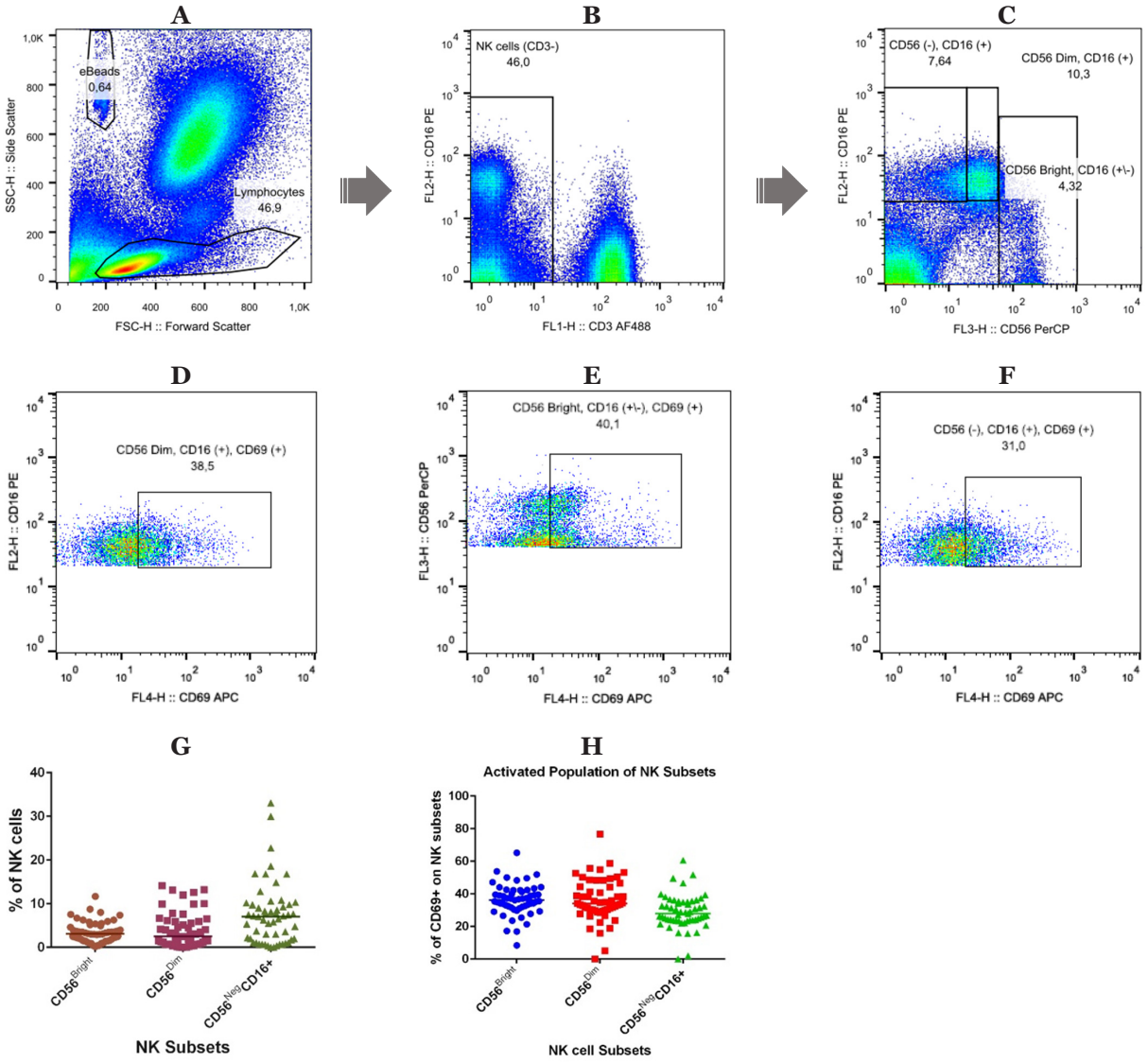

Figure 1 Identification of Blood NK Cells Subset Applying Multicolor Flow Cytometry
Identification of blood NK cells subset applying multicolor flow cytometry. Gating strategy for NK cells subsets identification presenting successive inclusion of lymphocyte population. (**A**) Identification of lymphocyte subpopulation in blood. (**B**) Selection for NK cells by gating on the CD3 negative lymphocyte population. (**C**) The selected population then differentiated on CD16 vs CD56 scatterplot to give three NK cells subsets. (**D**–**F**.) Further cell sorting on each subset against CD69 to obtain the activated NK cells. (**G**) Median and IQR of each subsets population. (**H**) Median and IQR of activated NK cells in each subset

cells with $CD3^-$ and followed by using CD56 and CD 16 as markers in cell sorting, NK cells subsets adequately identified from lysed-erythrocyte blood (Figure 1).

NK cells' selection began with gating on lymphocyte subpopulation successively continued with assort them based on the CD3 negative population. The selected cells were then classified into three distinct NK cell subsets, $CD56^{bright}$, $CD56^{dim}$, and $CD56^{neg}$ subsets, based on CD56 and CD16 surface expression (Figure 1). The median of $CD56^{bright}$, $CD56^{dim}$, and $CD56^{neg}$ subsets are respectively 3.1%, 2.49%, and 7.04% (Table 1). The mean of activated cells of $CD56^{bright}$, $CD56^{dim}$, and $CD56^{neg}$ subsets are respectively 36.23%, 35.96%, and 28.95% (Table 1).

This study showed that the percentage of $CD3^-$ cells populations in thalassemia patients negatively correlated with the ferritin levels ($r=-0.45$, $p=0.0009$). The higher ferritin level on

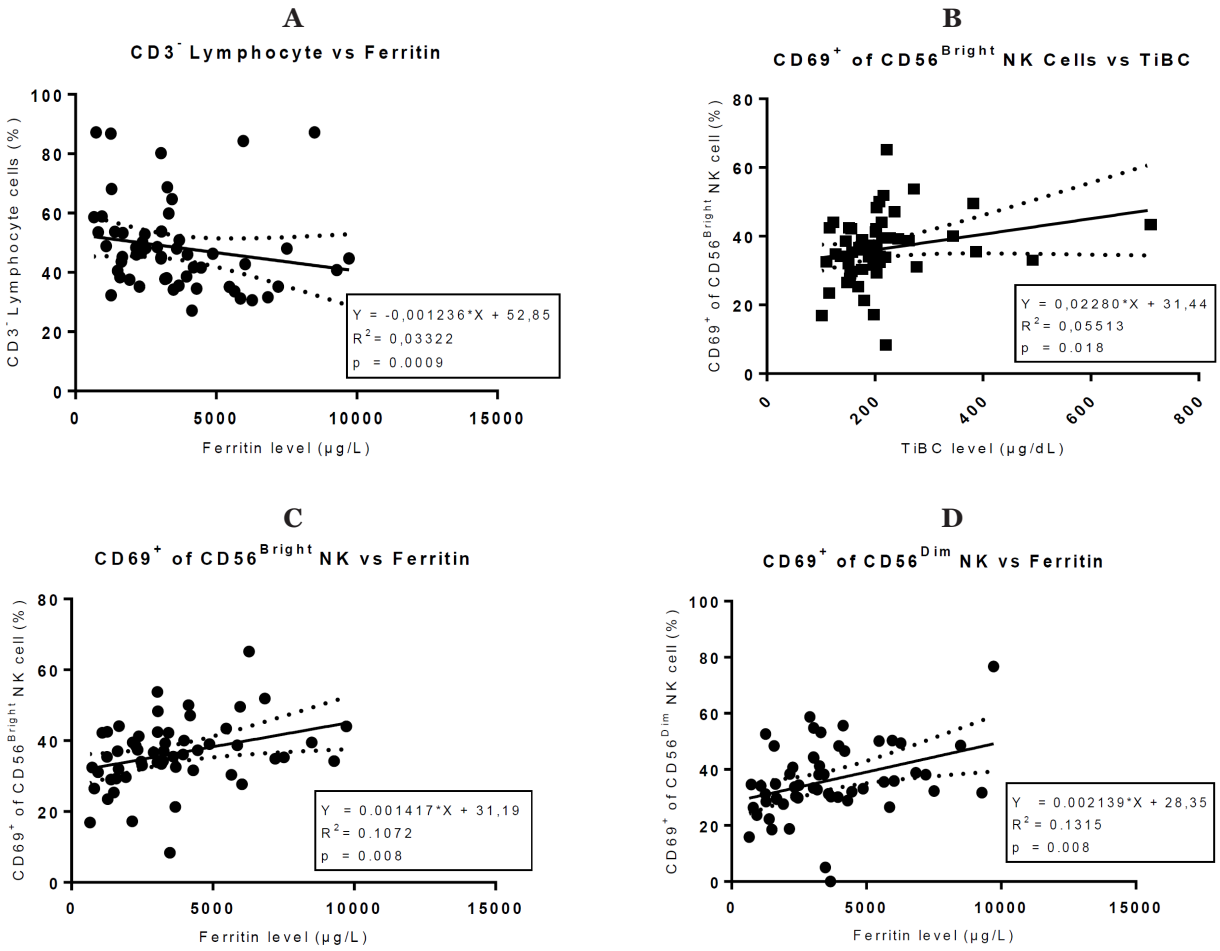

Figure 2 Linear Regression Analysis of $CD3^-$ Lymphocyte Cells

Linear regression analysis of $CD3^-$ lymphocyte cells (**A**), activated $CD56^{bright}$ NK subsets (**B**), and $CD56^{dim}$ NK subsets (**C**) against ferritin level, and also activated $CD56^{bright}$ NK subsets against TiBC. This figure shows a positive correlation between ferritin level and the number of activated $CD56^{bright}$ and $CD56^{dim}$ subsets, and also between TiBC level and the number of activated $CD56^{bright}$ subsets. Meanwhile, the ferritin level shows a negative correlation with the number of $CD3^-$ lymphocyte cells

blood, the lower $CD3^-$ cells population becomes. Other findings we have are a positive correlation between activated cells ($CD69^+$) on $CD56^{bright}$ and $CD56^{dim}$ towards ferritin level as showed in Table 2 [(r=0.353, p=0.008) and (r=0.355, p=0.008)].

This result means that the more activated cells on $CD56^{bright}$ and $CD56^{dim}$ populations, the higher the ferritin level. In terms of $CD56^{bright}$ cells, the activated cells seem associated with ferritin level and total iron-binding capacity (TiBC) (r=0.334, p=0.018). Linear regression analysis performed, as seen in Figure 2.

Discussion

Natural killer (NK) cells are unquestionably a substantial part of the innate immune defense.[14] Having been known for decades as the relatively primitive killer, their role in early host defense against a variety of pathogens (viruses, bacteria, and parasites) become more crucial coincide with increasing knowledge of NK cell biology.[23] NK cells can also produce cytokines, i.e., IFN-γ, TNF-β, IL-10, IL-13, TNF-α, and GM-CSF, as a response to stimulation from monocyte-derived cytokines (monokines), i.e., IL-2, IL-12, and IL-18. NK cells are one of the key sources of IFN-γ.[11] We sharply differentiate NK cells subsets as $CD56^{bright}$ ($CD56^{++}CD16^{+/-}$), $CD56^{dim}$ ($CD56^+CD16^+$), and $CD56^{neg}$ ($CD56^-CD16^+$) subsets as shown in Figure 1. In normal conditions, $CD56^{dim}$ subsets fill up to 90% of NK cells, and $CD56^{bright}$ comprise up

to 10% NK cells population.[23] Interestingly, our study finds that the proportion between the three subsets is barely equal, with a slightly higher of the $CD56^{neg}$ population. Unfortunately, our study does not have healthy control to be compared.

These subsets have distinct roles and function in innate immune defense. $CD56^{dim}$ NK cells have a lot higher cytotoxic ability than $CD56^{bright}$ cells, and they have more granzymes, perforin, and other cytolytic granules. Higher CD16 expression of $CD56^{dim}$ NK cells makes them efficient mediators of antibody-dependent cellular cytotoxicity (ADCC). Stimulation with cytokines such as IL-2 or IL-12 makes the cytotoxic ability of all NK cell subsets significantly augmented, but the cytokine-producing ability decreased.[16,19,23] On the other hand, $CD56^{bright}$ NK cells are the most efficient cytokine producers. The cytokines secreted by $CD56^{bright}$ NK cells are IFN-γ, TNF-β, IL-10, IL-13, TNF-α, and GM-CSF, depending on the precise conditions of stimulation. Stimulation with IL-2 and IL-18 will increase IFN-γ production.[11,12,23] $CD56^{neg}$ NK cells are functional and mature NK cells subset with less cytotoxic and cytokine-producing ability. The previous study found that the $CD56^{neg}$ NK cells population expanded in HIV-1 infection and HCV infection.[24–26] Regarding the $CD56^{bright}$ NK cells as abundant cytokine producers, particularly in IFN-γ production, alteration of this subset might lead to an inadequate innate immune response in eliminating pathogens, and contribute to diagnostic laboratory misinterpretation, such as tuberculin skin test (TST) and interferon gamma release assay (IGRA) in diagnosing tuberculosis.

We distinguished NK cells in an active state by using CD69 as an early activation marker, which rapidly expressed during activation of NK cells. As shown in Table 2 and Figure 2, we convey in our study that hyperferritinemia in pediatric major β-thalassemia patients correlated with activated $CD56^{bright}$ and $CD56^{dim}$ NK cells population. CD69 is an early expressed membrane protein that impermanent and rapidly expressed during lymphocyte activation, undetected in resting NK cells and all lymphocytes. CD69 also expressed in chronic inflammatory states.[29,30] Detection of $CD69^{+}$ on NK cells subsets clarifies the active state of NK cells. In the active state, the enhanced function of NK cells occurred, even the "cytokine producers" $CD56^{bright}$ NK subsets have an enhanced cytotoxic ability, comparable to cytotoxic $CD56^{dim}$ NK subsets. These novel findings might suggest that the higher ferritin level, the more NK cells set to face pathogens.

The limitation of our study is that we do not have blood samples from healthy children to be used as a healthy control in NK cells subset proportion to be compared. Thus, we cannot conclude the changes in subsets proportion. Further study is needed to evaluate the function of these subsets by using whole blood stimulation assay (WBSA).

Conclusions

Our study finds that hyperferritinemia correlated with the number of CD3- lymphocytes, and the number of activated cells of CD56Bright and $CD56^{dim}$ NK subsets. Considering that hyperferritinemia is the most common complication in pediatric major β-thalassaemia major, these findings explain why major β-thalassemia patients are more susceptible to infections.

Conflict of Interest

All authors declare that they have no competing interests.

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RESEARCH ARTICLE

Effectiveness of Lime Peel Extract (*Citrus aurantifolia* Swingle) against C-Reactive Protein Levels in Alloxan-Induced Wistar Rats

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Abstract

Hyperglycemia is a metabolic disease that is most often found and continuously increasing. Various complications due to hyperglycemia in the blood can cause tissue damage. It will increase free radicals that can trigger an inflammatory response characterized by an increased C-reactive protein in the blood. Prevention can be done by administering flavonoid antioxidant and lime peel containing high flavonoid. This study aims to analyze the efficacy of lime peel extract against C-reactive protein level with hyperglycemia through alloxan-induced Wistar rats (140 mg/kgBW). It is an experimental study using a post-test control group design that was carried out at the Pharmacology Laboratory of the Universitas Surabaya for the period July–August 2020. Experimental Wistar rats were divided into a negative control group, a positive control group, and three groups with different doses of lime peel extract (2.35 mg, 4.7 mg, and 9.4 mg). Treatment was carried out for 30 days before measuring the C-reactive protein levels in the blood using ELISA. The results showed a difference in C-reactive protein level between groups (Man-Whitney, p=0.004). The increase in the dose of lime peel extract (9.4 mg) showed the lowest C-reactive protein level. Therefore, it can be concluded that the administration of lime peel extract in hyperglycemia conditions can reduce the inflammatory process in the body.

Key words: Alloxan, C-reactive protein, hyperglycemia, inflammation, lime

Efek Ekstrak Kulit Jeruk Nipis (*Citrus aurantifolia* Swingle) terhadap Kadar *C-Reactive Protein* pada Tikus Wistar yang Diinduksi Aloksan

Abstrak

Hiperglikemia merupakan penyakit metabolik yang paling sering dijumpai dan terus mengalami peningkatan dari tahun ke tahun. Berbagai komplikasi akibat hiperglikemia dalam darah dapat menyebabkan kerusakan jaringan. Hal ini dikarenakan hiperglikemia akan meningkatkan radikal bebas sehingga memicu respons inflamasi yang ditandai dengan peningkatan *C-reactive protein* dalam darah. Pencegahan dapat dilakukan dengan pemberian asupan antioksidan flavonoid. Kulit jeruk nipis memiliki kandungan flavonoid yang tinggi. Penelitian ini bertujuan menganalisis efikasi ekstrak kulit jeruk nipis terhadap kadar *C-reactive protein* pada tikus Wistar dengan kondisi hiperglikemia melalui induksi aloksan (140 mg/kgBB). Metode pada penelitian ini adalah eksperimental dengan menggunakan *post-test control group* yang dilaksanakan di Laboratorium Farmakologi Universitas Surabaya periode Juli–Agustus 2020. Hewan coba tikus Wistar dibagi menjadi kelompok kontrol negatif, kelompok kontrol positif, dan tiga kelompok perlakuan dengan pemberian dosis ekstrak jeruk nipis yang berbeda (2,35 mg; 4,7 mg; dan 9,4 mg). Pemberian perlakuan dilakukan selama 30 hari, selanjutnya akan dilakukan pengukuran kadar *C-reactive protein* dalam darah dengan menggunakan ELISA. Hasil penelitian memperlihatkan perbedaan kadar *C-reactive protein* antarkelompok (Mann-Whitney, p=0,004). Peningkatan pemberian dosis ekstrak kulit jeruk nipis (9,4 mg) menunjukkan penurunan kadar *C-reactive protein* paling rendah. Oleh karena itu, dapat disimpulkan bahwa pemberian ekstrak kulit jeruk nipis pada kondisi hiperglikemia dapat menurunkan proses inflamasi dalam tubuh.

Kata kunci: Aloksan, *C-reactive protein*, hiperglikemia, inflamasi, jeruk nipis

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Introduction

Hyperglycemia is a condition where glucose levels in the blood increased due to insulin resistance, resulting in an imbalance of insulin concentration and plasma glucose levels.[1] Persisting hyperglycemia will lead to chronic diseases such as type 2 diabetes caused by decreased β cell function and insulin secretion in the blood.[2] Diabetes mellitus is classified as a non-communicable disease that requires special attention. Non-communicable diseases have increased each year significantly, estimated to have contributed 72% of all deaths due to disease in 2016.[3] The increase in people with diabetes mellitus has also increased to reach 425 million people in 2017 with around 4 million deaths. It is expected to reach 629 million people in 2045.[4] Besides, diabetes also causes disabilities and complications, mainly in the heart and kidneys.[5]

Increased blood glucose in people with diabetes will stimulate the production of excessive free radicals in the body. Various free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) will be produced and cause oxidative stress.[6,7] This happens to the increasing amount of excessive free radicals that result in an imbalance in the number of antioxidants present in the body.[8] Oxidative stress will cause direct damage to cell tissue through the lipid peroxidase reaction.[9] Cell tissue damage caused by an excessive increase of free radicals is often referred to as cell debris or damage associated molecular patterns (DAMPs).[10] Increasing the number of debris cells in the body's microenvironment will stimulate macrophages' movement as one of the body's primary defense systems for phagocytosis. Debris cells that macrophages have phagocyted will trigger inflammatory reaction through the secretion of pro-inflammatory cytokines such as interleukin-1, interleukin-6, interleukin-8, and tumor necrosis factor alpha (TNF-α).[11]

The impact of an inflammatory reaction caused by hyperglycemia is an increased risk of atherosclerotic diseases such as heart disease and stroke. Increased pro-inflammatory cytokines such as interleukin-1, interleukin-6, and TNF-α will induce the liver to release the acute phase of the protein C-reactive protein.[12] Increased levels of C-reactive protein in the blood are not only a sign of inflammation in the body, but C-reactive protein also plays an essential role in the inflammatory response, which can further accelerate cell damage.[13] Increased free radicals and inflammatory responses can be prevented by administering antioxidant intake from the outside. The number of antioxidants that the body needs to neutralize free radicals is sufficient, then the lipid peroxidase reaction that can damage the tissue can be reduced.[14]

Flavonoids are antioxidants that can be found in fruits, especially lime (*Citrus aurantifolia* Swingle). In recent years, research on the use of flavonoids as antioxidants in food sources is increasing. It has an impact on the increasing number of processed products from fruit. Each treatment process can reduce the flavonoid content by up to 50%.[15] The considerable amount of flavonoids on lime peel can be an alternative source of antioxidants.

This study used lime peel as a source of antioxidant flavonoids, so it is expected to increase antioxidant levels in the body. The intake of lime peel extract was carried out through the extraction process and initial testing of experimental Wistar rats (*Rattus norvegicus*) induced by alloxan.

Methods

This research was an experimental study using a post-test control group design. The research process was carried out on male Wistar rats (*Rattus norvegicus*) as experimental animals for 30 days and has passed the ethical test at the Institutional Ethical Committee, University of Surabaya (No.: 137/KE/VI/2020). Experimental Wistar rats will be divided into negative control groups, positive control groups, and three groups with different doses of lime peel extract.

This study used Wistar rats as an experimental animal. Some requirements are given to animals to make them homogeneous. They are 2–3 months old, weighing ±200 grams, have no macroscopic abnormalities, and have never been used for the object of research. The study was conducted at the Pharmacology Laboratory of the Faculty of Medicine, Universitas Surabaya. Each treatment group will be given a lime peel extract with different doses of 2.35 mg, 4.7 mg, and 9.4 mg.

Lime peel (about 2 kilograms) was cleaned and dried. Furthermore, powder preparations will be made using a blender and carried out

sifting to obtain fine and homogeneous powder (±40 mesh). The next step was extraction using the maceration method with 96% ethanol solvent (±10 liters). Leave for 3×24 hours, and every 24 hours, change the solvent until the resulting filtrate is clear. Concentration results will obtain dense preparation using a vacuum rotary evaporator.[16] Then, the flavonoid level in lime peel extract will be measured (51.23 mg/g), and a conversion table between organisms Laurence and Bacharach will be calculated so that doses of 2.35 mg, 4.7 mg, and 9.4 mg are obtained.[17]

After the adaptation process for 5–7 days, the experimental rats fasted for 6–8 hours. Then they will be given a single alloxan injection of 140 mg/kgBW (diluted with NaCl 0.9%) intraperitoneally. Experimental animals received blood glucose tests before the research began, so the positive control group and the treatment group reached a hyperglycemia condition. Hyperglycemia condition in experimental animals was compared with other treatment groups (mean 89.00±4.00, p=0.519), then with the negative groups (mean 75.4±3.78, p=0.001).

Measurement of C-reactive protein level in the blood serum is done by using ELISA. The result is indicated positive when agglutination is present, and C-reactive protein level reached a level of ≥6 mg/L. The result is indicated negative when there is no agglutination. The level of C-reactive protein is below 6 mg/L.[18] The C-reactive protein level can increase significantly above the normal level with the onset of substantial inflammatory stimulus.

This research was conducted for 30 days by dividing up to 5 treatment groups. The first group is a negative control group where the experimental animals were not given any treatment for 30 days. In the second to the fifth group, alloxan was administered to increase the blood glucose level to achieve hyperglycemia. The second group is a positive control group used to compare where experimental animals that have reached the condition of hyperglycemia are given glimepiride 0.36 mg (equivalent to 2 mg/day in adults). Glimepiride was used in this study because it is effective in reducing blood glucose levels.[19] For the other three treatment groups were also hyperglycemia conditioned and were given extracts of lime peel at a dose of 2.35 mg, 4.7 mg, and 9.4 mg.

The result of the study will obtain ordinal data in the form of C-reactive protein level in each group in mg/L units. C-reactive protein assessment level of >6 mg/L, 12 mg/L and 24 mg/L will be given coding (non-parametric). Data analysis was performed by using Kruskal-Wallis analysis with SPSS version 22 to see differences between groups. The difference between the two groups was significant when the p-value<0.05.

Results

The results of this study were carried out by comparing C-reactive protein levels between groups. Table 1 shows the results of measurement of C-reactive protein level in blood serum between groups. In groups, I and V, C-reactive protein levels in all experimental animals showed the lowest value <6 mg/L, while the highest C-reactive protein level was found in group II.

Table 2 shows the results of the Kruskal-Wallis test on C-reactive protein level between groups were 0.004 (p-value<0.05), so it can be concluded that there are significant differences in C-reactive protein level.

Research data on C-reactive protein levels in each group were also analyzed to show how much

Table 1 C-Reactive Protein Level in Experimental Animal

Groups	Negative Control	Positive Control	Treatment I	Treatment II	Treatment III
C-reactive protein level (mg/L)	<6	24	<6	<6	<6
	<6	24	<6	<6	<6
	<6	12	12	<6	<6
	<6	12	12	12	<6
	<6	12	12	12	<6

Table 2 Kruskal-Wallis Test Results on C-Reactive Protein Level between Groups

Groups	Kruskal-Wallis Test
Negative control	
Positive control	
Treatment I	0.004
Treatment II	
Treatment III	

Table 3 Mann-Whitney Test Result C-Reactive Protein Level between Groups

Groups	I	II	III	IV	V
I	–	–	–	–	–
II	0.008	–	–	–	–
III	0.032	0.032	–	–	–
IV	1.000	0.008	0.032	–	–
V	1.000	0.008	0.032	1.000	–

influence the lime peel extract has in reducing C-reactive protein levels than the negative and positive control groups. Based on Table 3, shows the differences in each group using the Mann-Whitney test.

The results show that groups I, IV, and V have the same C-reactive protein value (p value=1.000). In comparison, groups II and III showed significant differences with group I (p value<0.05).

Discussion

The negative control group did not show an increase in C-reactive protein levels. In contrast, the positive control group showed an increase in C-reactive protein levels, although antidiabetic drugs were administered. It can be concluded that in hyperglycemia conditions that have been given antidiabetic drugs, there is still an inflammatory reaction.

Hyperglycemia causes damage to endothelial cells, which can stimulate free radical formation.[20] Normally, free radicals can be neutralized by antioxidants in the body or often called enzymatic antioxidants.[21] Various types of enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) are types of antioxidants that can not be given through intake from outside the body yet play a significant role in neutralizing free radicals.[22] Antioxidant superoxide dismutase (SOD) can neutralize superoxide radicals ($O_2^{\bullet -}$) into hydrogen peroxidase (H_2O_2), while antioxidant glutathione peroxidase (GSH-Px) and catalase (CAT) functioning to change hydrogen peroxidase into non-radical formations that are water (H_2O) and oxygen (O_2).[23] Therefore giving antioxidant intake can be done by consuming non-enzymatic antioxidants such as flavonoids.[24]

Giving antioxidants using lime peel extract can reduce levels of C-reactive protein in the blood. The treatment group showed that the greater the dose of lime peel extract, the lower the blood's C-reactive protein level. It shows that the flavonoid content in lime peel extract can reduce free radicals and prevent an increase in the inflammatory response.

Most of the flavonoids can act as antioxidants. Flavones and catechins are the strongest flavonoids to protect the body from free radicals. Flavonoids will be oxidized by free radicals (such as superoxides and peroxynitrite) and produce more stable and less reactive radicals. Free radicals will decrease the ability to cause cell damage.[25]

This study is expected to be the first step in considering hyperglycemia conditions because anti-diabetes therapy has not fully reduced the negative impact of free radicals and will trigger an inflammatory response. The development of lime peel extract can be an alternative to reduce inflammation in hyperglycemic conditions.

Conclusions

Giving blood glucose-lowering drugs in the form of glimepiride in hyperglycemia conditions does not respond to decreased inflammation. However, the administration of lime peel extract (9.4 mg) can reduce the inflammatory reaction in the body.

Conflict of Interest

The authors declare none.

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RESEARCH ARTICLE

Effect of Gooseberry (*Physalis angulata*) Ethanol Extract in Wistar Rats Carrageenan-Induced Paw Oedema

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Abstract

Gooseberry is an herbaceous plant that contains flavonoids. Flavonoid is one of the secondary metabolites that have an anti-inflammatory effect. This study aims to determine the effect of using ethanol extract of gooseberry as an anti-inflammatory in carrageenan-induced paw edema. This study was in vivo experimental laboratory using a completely randomized design of 25 Wistar rats and divided into five groups. The negative control group was given carboxymethylcellulose. The positive control group has given diclofenac sodium 27 mg/200 gBW. The sample test group has given ethanol extract of gooseberry with 3.6 mg/200 gBW, 5.4 mg/200 gBW, and 7.2 mg/200 gBW dosage. Paw rat's inflammation induced by injecting carrageenan and measured from 1st to 6th hour using a pletismometer. This study has conducted at Pharmacology Laboratory, Universitas Islam Bandung, and the Laboratory of Therapy and Pharmacology, Universitas Padjajaran, from June to September 2019. The result of average edema volume paw rats using the Kruskal-Wallis test on the 6th hour was p=0.02 (p<0.05). The Mann-Whitney test was p<0.05, showing differences between negative control and positive control and sample test groups. One-way ANOVA test on the percentage of edema inhibition between positive control and sample test group had p=0.107. It shows no significant difference. An effect of ethanol of extract of gooseberries as an anti-inflammatory with the highest percentage of edema inhibition is 5.4 mg/200 gBW dosage. The flavonoid content in gooseberries is thought to inhibit the formation of prostaglandins by inhibiting the cyclooxygenase enzyme. In conclusion, the ethanol extract of gooseberry can be anti-inflammatory.

Key words: Anti-inflammatory, diclofenac sodium, gooseberry ethanol extract, Wistar rat

Efek Ekstrak Etanol *Ciplukan* (*Physalis angulata*) terhadap Edema Telapak Kaki Tikus Galur Wistar yang Diinduksi Karagenan

Abstrak

Ciplukan adalah tanaman herbal yang mengandung flavonoid. Flavonoid merupakan salah satu metabolit sekunder yang dapat memberikan efek antiinflamasi. Penelitian ini bertujuan mengetahui pengaruh penggunaan ekstrak etanol *ciplukan* sebagai antiinflamasi pada tikus yang diinduksi karagenan. Penelitian ini merupakan penelitian laboratorium eksperimental *in vivo* menggunakan desain rancangan acak lengkap pada 25 ekor tikus galur Wistar yang terbagi ke dalam lima kelompok. Kelompok kontrol negatif diberi *carboxymethilcellulose*. Kontrol positif diberi sodium diklofenak 27 mg/200 gBB. Kelompok uji diberi ekstrak etanol *ciplukan* dengan dosis 3,6 mg/200 gBB; 5,4 mg/200 gBB; dan 7,2 mg/200 gBB. Induksi inflamasi dilakukan dengan menginjeksikan karagenan pada telapak kaki tikus, lalu diukur menggunakan pletismometer dari jam ke-1 hingga jam ke-6. Penelitian ini dilakukan di Laboratorium Farmasi, Universitas Islam Bandung dan Laboratorium Farmasi dan Terapi, Universitas Padjajaran dari bulan Juni hingga September 2019. Volume rerata telapak kaki tikus pada jam ke-6 menggunakan Uji Kruskal-Wallis adalah p=0,02 (p<0,05). Hasil Uji Mann-Whitney diperoleh p<0,05 yang menunjukkan terdapat perbedaan bermakna antara kontrol negatif dan kontrol positif serta kelompok uji. Uji *one-way* ANOVA pada persentase penghambatan edema antara kontrol positif dan kelompok uji diperoleh p=0,107 yang menunjukkan tidak terdapat perbedaan yang bermakna. Terdapat pengaruh ekstrak etanol *ciplukan* sebagai antiinflamasi dengan persentase penghambatan edema tertinggi pada dosis 5,4 mg/200 gBB. Kandungan flavonoid pada *ciplukan* diduga mampu menghambat pembentukan prostaglandin dengan menginhibisi enzim siklooksigenase. Simpulan penelitian ini adalah ekstrak etanol *ciplukan* dapat digunakan sebagai antiinflamasi.

Kata kunci: Antiinflamasi, ekstrak etanol *ciplukan*, sodium diklofenak, tikus galur Wistar

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Introduction

The human body has a protective response to the dangerous agent that works when there is an infection, physical pressure, and injury. The protection response is called inflammation. Inflammation is signed by rubor, calor, dolor, tumor, and functio laesa. Several studies have provided evidence that inflammation is involved in the pathogenesis of various diseases, including aging, cancer, and cardiovascular dysfunction. Inflammation can be divided into acute and chronic phases. Acute inflammation cannot be sustainable because it can cause a chronic condition that triggers the chronic inflammatory disease. Chronic inflammation that arises in certain areas can result in a dangerous disease, such as if it occurs in a blood vessel, it will increase the risk of atherosclerosis. If it occurs in the joint area, it can cause rheumatic disease.[1–11]

It is necessary to use anti-inflammatory to reduce the sign of inflammation and tissue damage. Anti-inflammation commonly used in modern medicine is divided into two different types: glucocorticoid and nonsteroidal anti-inflammatory drugs (NSAIDs). Diclofenac sodium is one of the NSAIDs types commonly used, but this one can cause ulceration in the digestive tract and inhibit blood coagulation if consumed for long periods.[12–15]

Many people choose to use bioactive molecular medicinal plants in medicine throughout the country, especially in rural areas in developing countries with low socio-economic factors that make it difficult to access the health care system and modern medicines. One of the medicinal plants which have anti-inflammatory properties is gooseberry (*Physalis angulata*).[16,17]

Gooseberry is an herbaceous plant that is easily obtained and can be purchased at low prices in Indonesia. All of the gooseberries, such as the roots, stems, fruits, and leaves, are rich in chemical compounds beneficial for therapy. The content of gooseberry, which provides an anti-inflammatory effect, comes from flavonoids. Flavonoids are secondary metabolites formed through fatty acid metabolism that can inhibit the cyclo-oxygenase (COX) enzyme. Flavonoids also inhibit xanthine oxidase (XO), lipoxygenase, and phosphoinositide-3-kinase.[16–20]

One study mentioned that ethanol had the highest percentage in attracting active flavonoid substances from gooseberry.[10] For this reason, this research use ethanol as a solvent from gooseberry extract. Based on this background, the purpose of this study was to determine the effect of ethanol extract of gooseberry as an anti-inflammation in carrageenan-induced rats, to know the concentration of ethanol extract of gooseberry, which faster to eliminate inflammation, and to analyze the difference of anti-inflammatory effects between ethanol extract of gooseberry and diclofenac sodium on carrageenan-induced paw edema in Wistar rats.

Methods

The research was conducted from June to September 2019 after received ethical clearance from the Health Research Ethics Committee of Faculty Medicine, Universitas Islam Bandung, Number: 039/Komite Etik FK/IV/2019. This research method was purely in vivo experiments conducted in a laboratory using a complete randomized design. It began with collecting gooseberry plant from Yogyakarta and then determining it in Institut Teknologi Bandung by a process called determination plants to ensure the plants were *Physalis angulata* species. The gooseberry ethanol extract was made using one kilogram of dried gooseberry leaves processed into powder using an electric blender and dissolved with 95% ethanol in 3 days. The extract filtered then evaporated using a vacuum rotary evaporator to separate the solvent from the extract. This process is repeated three times, with the result was a brown paste. The phytochemical screening was conducted in Pharmacology Laboratory Universitas Islam Bandung to ensure that the extract contained flavonoids. In this study, both extract ethanol of gooseberry and diclofenac sodium diluted with carboxymethyl cellulose 0.5% to make sure it had a similar liquid type with the negative control group. The suspension of λ carrageenan was from the Laboratory of Therapy and Pharmacology Universitas Padjajaran. It is made from 5-gram carrageenan powder and diluted with 500 mL saline 0.9%. Randomization of 25 rats used paper shuffle for five numbers of groups and five numbers of rats. The adaptation rats' period was carried out for seven days on the cage in the Laboratory of Therapy and Pharmacology Universitas Padjajaran with research standards, 12 hours in a dark room and 12 hours in a bright room. It was given standard feed and water. A

day after the rats' adaptation period completed, the study was carried out by conducting initial measurements on the volume of rats paw, followed by 0.5% carboxymethylcellulose administration with 2 mL/200 gBW to the negative control group, sodium diclofenac 27 mg/200 gBW to the positive control group, and gooseberry ethanol extract with the dosages of 3.6 mg/200 gBW, 5.4 mg/200 gBW, and 7.2 mg/200 gBW to the first, second and third groups. Thirty minutes after oral treatment, all of the rats were induced to inflammation by injecting 0.1 mL subplantar carrageenan (Figure 1).

The measurements were retaken after carrageenan induction and continued every hour, started from the first hour until 6 hours after carrageenan induction.[21–24] After all the research step was done, to fulfill a good animal ethical procedure, the rats have been euthanasia with injected 1 mL of ketamine then buried the rats.

The results were displayed by the average edema volume of rats paw in the 1st, 2nd, 3rd, 4th, 5th, and 6th hours. The study's normality used the Shapiro-Wilk test, and the homogeneity used the Levene test on IBM SPSS statistics 25 application with a confidence interval of 95%. The normal and homogenous data used a one-way ANOVA test, while the abnormal nor homogenous data used the Kruskal-Wallis test. The Mann-Whitney test tested the significant result of Kruskal-Wallis..

Results

Institut Teknologi Bandung gave the result of the determination, and it showed the gooseberry's type we brought was *Physalis angulata*. The production of ethanol extract of gooseberry was carried out in the Laboratory of Therapy and Pharmacology Universitas Padjajaran, followed by a phytochemical screening in Pharmacology Laboratory Universitas Islam Bandung, which showed that the extract contained flavonoid. Research on the anti-inflammatory activity of ethanol extract of gooseberry was conducted on 25 carrageenan-induced male Wistar rats. All of the anti-inflammatory samples tests were given 30 minutes before carrageenan induction to see the percentage of edema inhibition and the measurement as in Figure 2.

The measurements were taken every hour for 6 hours by dipping the paw rats into the tube of the pletismometer. The measurement result of the paw rat volume was in Table 1.

Figure 1 Injection of Carrageenan

Figure 2 Measurement of Paw Rats Volume

In the 4th hours, the rat's paw volume from all groups was at the edema peak. The volume of paw rats in the positive control group, the first sample test, the second sample test, and the third sample test decreased than the negative control group at the 6th hours. Comparing the average paw rat volume at the initial measurements and the 6th hours showed the highest increase in the negative control group and the lowest in the second sample test group. There was a difference in rat paw edema volume of about two µL between the second sample test group and the positive control group.

Table 1 Result of Paw Rat Volume Measurement

Rat Groups	Volume of Rat Paw (µL)							
	IM*	BCI**	Hour					
			1	2	3	4	5	6
Negative control	56.0±13.4	80.0±12.3	108.0±16.4	114.0±8.2	122.0±13.0	134.0±8.9	128.0±13.0	132.0±16.4
Positive control	82.0±8.4	96.0±8.9	124.0±11.4	122.0±13.0	116.0±13.4	132.0±8.4	114.0±11.4	104.0±5.5
1st sample test	70.0±10.0	96.0±13.4	124.0±16.7	124.0±15.2	122.0±14.8	128.0±19.2	120.0±12.3	104.0±8.9
2nd sample test	76.0±5.5	90.0±7.1	112.0±14.8	114.0±15.2	118.0±16.4	124.0±16.7	110.0±12.3	100.0±12.3
3rd sample test	64.0±11.4	82.0±8.4	100.0±7.1	106.0±5.5	114.0±11.4	124.0±8.94	110.0±7.2	102.0±4.5

Note: *initial measurement, **before carrageenan induction

Based on Table 1, it can be calculated the percentage of the average edema inhibition on the rat paw through the formula:[25]

$$\% \text{ edema inhibition average} = \left\{1-\left(\frac{a-x}{b-y}\right)\right\}\times 100\%$$

Description: a=the rat's paw average volume at a certain hour in the positive control group and the test group, x=the rat's paw average volume at the positive control group's initial measurements and the test group, b=the rat's paw's average volume at a certain hour in the negative control group, y=the average volume of the rat's paw at the negative control group's initial measurements

The percentage of rat's paw edema inhibition is shown in Figure 3.

In Figure 3, in the 1st hours of measurement, the highest percentage of inhibition came from the second sample test group (30.77%) and the third sample test (30.77%), followed by the positive control group (19.23%) and the first sample test group (−3.85%). While the edema peaked in the 4th hours, the percentage edema inhibition in the positive control group (35.9%) and the third sample test (23.08%) decreased.

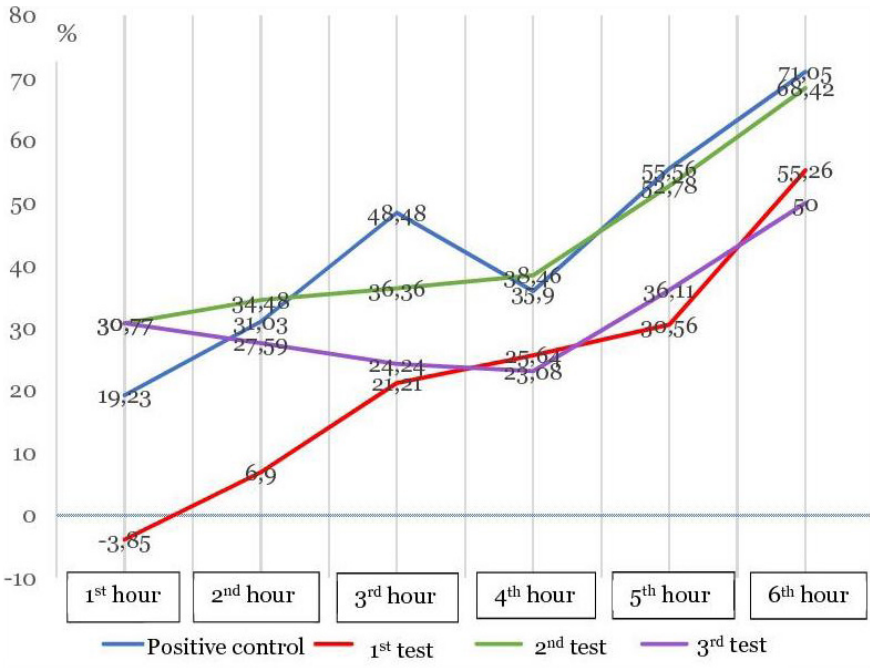

Figure 3 Percentage of Paw Rat Edema Inhibition

Table 2 Volume Difference on Paw Rats

Measurement Time	p*	p**
1st		0.068
2nd		0.260
3rd	0.855	
4th		0.651
5th		0.100
6th		0.022

Note: *one-way ANNOVA test, p<0.05 significant, **Kruskal-Wallis test, p<0.05 significant

However, it turned back to the extreme increase in edema inhibition until the last hour, followed by the second sample test group (68.47%). In the 5th hours, the first sample test group's percentage edema inhibition increased (30.56%). In the 6th hours, the positive control group (71.05%) showed the highest percentage of edema inhibition, followed by the second sample test group (68.42%), the first sample test (55.26%), and the third sample test (50%). When the sample test compared to the positive control, the first sample test group's edema inhibition rate was 16% lower than the positive control. The second sample test was 3% lower than the positive control, and the third sample test was 21% lower than the positive control.

The normality and homogeneity tests were performed on the edema volume. It showed normal and homogeneous distributed data only in the third hour. Then one-way ANOVA test was performed with a value of p=0.855 (p<0.05), which meant there was no significance. In the 6th hour measurement, data were not distributed normally nor homogenous, and it continued with the Kruskal-Wallis test with p value=0.022 (p<0.05). The difference between the one-way ANOVA test and the Kruskal-Wallis test in Table 2. Data in the 6th hour had a significant result—the Mann-Whitney test showing a significant difference between negative control and positive control and sample test groups. Also, there was no significant difference between the positive control and sample test group.

The normality and homogeneity tests were also performed on the edema inhibition percentage data, and the results showed normal and homogeneous distribution. A one-way ANOVA test was then performed with a value of p=0.107 (p<0.05), which meant no significant difference from the percentage of inhibition edema in the positive control group with the first, second and third sample tests group. It showed that ethanol extract of gooseberry provides anti-inflammatory effects such as diclofenac sodium.

Discussion

Induction of inflammation using carrageenan on the paw to make edema. In Table 1, the volume of the paw increased immediately after carrageenan injection. The peak of edema occurs at the 4th hour when carrageenan triggers maximal prostaglandin release. The process consists of the first phase mediated by histamine and 5-hydroxytryptamine, followed by a second phase mediated by kinin triggering the activation of the enzyme cyclooxygenase and the third phase of local prostaglandin production. The prostaglandin precursor is a derivative of arachidonic acid, which is activated by the enzyme cyclooxygenase.[2,3]

Gooseberry is an herbaceous plant that has various substances, one of that is a flavonoid.[15,17,18] The role of flavonoids in inhibiting the cyclooxygenase enzyme can be used as an anti-inflammatory. Flavonoid will break the chain of inflammation by inhibiting the cyclooxygenase enzyme so that prostaglandins will not be formed. Flavonoid is COX-2 selective, so it can be used as an anti-inflammatory that reduces the effects of ulceration in the digestive tract and bleeding.[3] In bio-molecular studies, the marker used in anti-inflammatory testing is nuclear factor-kappaB (NF-κB). Research shows the content of physalin E (a type of secosteroid that can form flavonoids through fatty acid metabolism in physalis plants (gooseberry) can inhibit the transcription factor NF-κB which plays an essential role in the inflammatory process. When NF-κB bind to tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), it can induce the transcription of pro-inflammatory genes, so that inhibition of NF-κB can prevent inflammation. These studies support that the reduced edema volume in the sample test group comes from flavonoid in ethanol extract of gooseberry.[26]

Gooseberry (*Physalis angulata*) has active substances such as physalin 9 and 10 that act as antiproliferative, and physalin 1, 3, 4, 9, 10, 13, 14, 16 act as an anti-inflammatory which works by inhibiting the production of nitrite oxide. The damage of deoxyribonucleic acid (DNA) and cell membranes is mediated by nitric oxide to trigger

nuclear pro-inflammatory cytokines, which can activate and recruit inflammatory cells. If nitric oxide production is inhibited, the inflammatory process can be stopped.[27]

Dosage of 400 mg/kgBW of methanol extract of gooseberries by Ukwubile and Oise[18] showed the same anti-inflammatory effect as ethanol extract of gooseberries in this research. The percentage of edema inhibition produced in Ukwubile and Oise's[18] study was 62.7%. This is different from the research results that gooseberry's ethanol extract gave the highest percentage of edema inhibition, with 68.4% from the second sample test group given a dosage of 5.4 mg/200 gBW (300 mg/kgBW). It can be caused by the differences in the solvent so that the binding of flavonoids non-optimal.

Conclusions

We conclude an anti-inflammatory effect of ethanol extract of gooseberry on carrageenan-induced paw edema in Wistar rats. There was no significant difference between the diclofenac sodium group and the gooseberry ethanol extract, which meant both had anti-inflammatory effects on edema.

Conflict of Interest

There is not any conflict of interest in this research.

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RESEARCH ARTICLE

Clinical Characteristics and Number of Valve Lesion in Rheumatic Heart Disease Severity

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Abstract

Rheumatic heart disease (RHD) occurs due to sequelae in the form of damage to the heart valves from the failure of acute rheumatic fever (ARF) therapy. Heart valve damage can cause various complications such as congestive heart failure, arrhythmias, pulmonary hypertension, atrial fibrillation, endocarditis, which can cause death. The study aimed to assess the association between clinical characteristics and valve lesion and rheumatic heart disease severity. The study was an analytic observational with a cross-sectional design of 73 patients with definite RHD from September 2019 to March 2020 in Dr. Soetomo Regional General Hospital. The majority of patients were female (80%, p=0.235) 30–39 years old (34%, p=0.157). The mean age was 42.08±12.16 years. The majority of patients have low socioeconomic status (78%, p=0.025) and rural dwelling location (70%, p=0.138) over three-quarters of patients living with more than four people in the same house (75%). Multivalvular lesions (90%, p=0.003) and severe RHD (77%) were present predominantly. In conclusion, low socioeconomic status and multivalvular lesions are associated with rheumatic heart disease severity.

Key words: Clinical characteristic, rheumatic heart disease, severity, valve lesion

Karakteristik Klinis dan Jumlah Lesi Katup pada Derajat Keparahan Penyakit Jantung Reumatik

Abstrak

Penyakit jantung reumatik (PJR) adalah penyakit yang terjadi akibat gejala sisa berupa kerusakan katup jantung dari kegagalan terapi demam reumatik akut (DRA). Kerusakan katup jantung pada PJR dapat menimbulkan berbagai komplikasi seperti gagal jantung kongesti, aritmia, hipertensi pulmonal, atrial fibrilasi, dan endokarditis yang dapat menyebabkan kematian. Penelitian ini bertujuan mencari hubungan karakteristik klinis dan jumlah lesi katup dengan derajat keparahan PJR. Penelitian ini merupakan analitik observasional menggunakan pendekatan *cross-sectional*. Sampel penelitian ini adalah pasien yang terdiagnosis definitif PJR berdasar atas ekokardiografi pada bulan Sepetember 2019–Maret 2020 di RSUD Dr. Soetomo Surabaya. Didapatkan 73 pasien sesuai dengan kriteria inklusi. Mayoritas pasien berjenis kelamin perempuan (80%; p=0,235), dengan kelompok usia 30–39 (34%; p=0,157). Usia rerata 42,08±12,16 tahun. Sebagian besar pasien berstatus sosial ekonomi rendah (78%, p=0,025) dan lokasi tinggal pedesaan (70%; p=0,138). Lebih dari tiga perempat pasien tinggal dengan ≤4 orang di satu atap (75%). Lesi multivalvular (90%; p=0,003) dan PJR berat (77%) ditemukan secara dominan. Simpulan, status sosial ekonomi rendah dan lesi katup multivalvular berhubungan dengan derajat keparahan penyakit jantung reumatik.

Kata kunci: Derajat keparahan, karakteristik klinis, lesi katup, penyakit jantung reumatik

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Introduction

Rheumatic heart disease (RHD) is heart valve damage due to the failure of acute rheumatic fever (ARF) therapy.[1] ARF is an autoimmune disease caused by Group A Streptococcus (GAS) bacterial infection, characterized by clinical symptoms such as carditis, arthritis, syndrome's chorea, erythema marginatum, and subcutaneous nodule. Throat infections due to GAS in children and adolescents with a genetic predisposition that involve the nerves, skin, joints, and heart can cause ARF.[2] Rheumatic heart disease was commonly affected in young-adult living with low socioeconomic status.[3] and it latent in children and manifested clinically in adulthood. Individuals in countries affected by RHD are endemic late in the disease process and usually leave one or more ARF sequelae. Heart valve damage can cause various complications such as congestive heart failure, arrhythmias, pulmonary hypertension, atrial fibrillation, and endocarditis, which can cause death.[4,5] According to WHO Global Health Estimates 2016, disability-adjusted life-years (DALYs) of RHD worldwide were 10,397,970; while it was 384,600 in Indonesia.[6]

RHD has several risk factors such as age, sex, socioeconomic status, occupancy density, nutritional status, prophylactic adherence, ignorance, genetic factors, and access to health facilities.[7] Poverty was related to the severity of RHD in inpatients in sub-Saharan Africa.[8] Age, sex, and area of origin were not associated with RHD severity.[9] There are still few studies in Indonesia that assess the association between clinical characteristics and RHD severity. The author intends to prove the association between age, sex, area of origin, economic status with RHD severity. Moreover, to add the association between occupancy density and the number of valve lesions with RHD severity, so the medical personnel can know the management of RHD, determine appropriate prophylaxis, and anticipate the occurrence of more severe RHD.

Methods

The study was an analytic observational study with a cross-sectional design using questionnaires and medical records from September 2019 to March 2020 in Dr. Soetomo Regional General Hospital Surabaya. The sample was 73 patients with definite RHD based on World Heart Federation (WHF) criteria in Dr. Soetomo Regional General Hospital Surabaya. Patients with standard echocardiography with clinical manifestation of RHD and non-RHD valvular heart disease were excluded.

The variables used in this study were dependent and independent. Dependent variables include age, sex, area of origin, economic status, occupancy density, and the number of valve lesions. The independent variable was RHD severity. Statistical analysis was to assess the association between dependent variables and independent variables. The data were analyzed using SPSS version 25 with the Fisher-Freeman-Halton test.

Ethical clearance was approved by Health Research Ethics Committee Dr. Soetomo Regional General Hospital Surabaya with letter number 1512/KEPK/IX/2019.

Results

The patients are dominated by women (80%), 30–39 years (34%), and living in a rural location (70%). The economic status was defined by patients' monthly income and was divided into four categories based on the Statistics Indonesia which are Low (<IDR 1,500,000), middle (IDR 1,500,000–2,500,000), high (IDR 2,500,000–3,500,000), and very high (more than IDR 3,500,000). The majority of patients have low economic status (78%). Occupancy density is divided into two categories according to the number of people in one house, namely less than four people and more than four people. The results showed that 75% had an occupancy density of more than four people.

The number of patient valve lesions based on echocardiography was divided into two categories, univalvular and multivalvular lesions. The results showed that seven patients (10%) had univalvular valve lesions, and 66 patients (90%) had multivalvular valve lesions. RHD severity was divided into mild, moderate, and severe according to WHF 2012 echocardiography criteria. Data distribution obtained 3% had mild RHD, 20% moderate RHD, and 77% severe RHD (Table 1).

Table 2 showed that the economic status and the number of valve lesion were associated with RHD severity (p=0.025, p=0.003) whereas age, sex, area of origin, and occupancy density has no statistical difference.

Table 1 Clinical Characteristics of Patients

Characteristics	n=73	Percentage
Age (years)		
<20	3	4
20–29	7	10
30–39	25	34
40–49	19	26
50–59	9	12
≥60	10	14
Sex		
Male	15	20
Female	58	80
Area of origin		
Urban	22	30
Rural	51	70
Economic status		
Low (<1,500,000 IDR)	57	78
Middle (1,500,000–2,500,000 IDR)	5	7
High (2,500,000–3,500,000 IDR)	6	8
Very high (>3,500,000 IDR)	5	7
Occupancy density		
>4 people	18	25
≤4 people	55	75
The number of valve lesions		
Univalvular	7	10
Multivalvular	66	90
RHD severity		
Mild	2	3
Moderate	15	20
Severe	56	77

Discussion

The majority of patients were aged 30–39 years (34%). A study in Papua's labor community who suffered RHD showed that nearly half of the sample was 35–44 years old.[10] According to Zhang et al.,[11] more than half of patients with RHD were in the aged group 20–39. 61% of patients with ARF developed into RHD after ten years.[12] RHD peaked in the third and fourth decades.[13,14]

The results showed that age has no statistical difference with RHD severity (p=0.157). The development of ARF into RHD for each individual varies from several years to more than 20 years.[15] RHD is initially asymptomatic, as well as ignorance and difficulty accessing health facilities that cause patients to experience delays in initial diagnosis.

Female was present predominantly in this study (80%). Females are more susceptible to developing autoimmune reactions after being infected with GAS. Social factors such as involvement in raising children and access to health services also lead to increased vulnerability and the possibility of GAS infection.[14] Females tend to have more severe aortic valve lesions compared to males.[16] Statistical analysis showed that sex was not associated with RHD severity (p=0.235). According to Lubega et al.[17] there is no association between sex and tricuspid regurgitation, mitral stenosis severity. There still many factors to considered, such as prophylactic compliance, access to health care, and adherence for RHD.[7]

RHD mostly takes place in rural areas.[18,19] This study showed that 70% of patients were living in rural areas. Rural areas tend to have insufficient clean water facilities and environmental

Table 2 Clinical Characteristics and Valve Lesion in Rheumatic Heart Disease Severity

Characteristics	Rheumatic Heart Disease Severity (n=73)			p
	Mild	Moderate	Severe	
Age (years)				0.157
<20	0	1	2	
20–29	1	1	5	
30–39	1	4	20	
40–49	0	2	17	
50–59	0	5	4	
≥60	0	2	8	
Sex				0.235
Male	0	1	14	
Female	2	14	42	
Area of origin				0.138
Urban	0	11	40	
Rural	2	4	16	
Economic status				0.025
Low (<1,500,000 IDR)	0	11	46	
Middle (1,500,000–2,500,000 IDR)	1	0	4	
High (2,500,000–3,500,000 IDR)	0	2	4	
Very high (>3,500,000 IDR)	1	2	2	
Occupancy density				0.065
>4 people	2	4	12	
≤4 people	0	11	44	
The number of valve lesions				0.003
Univalvular	2	2	3	
Multivalvular	0	13	53	

conditions, affecting the risk of RHD.[20] The results showed that the original area has no statistical significance in RHD severity (p=0.135). Other parameters, for instance, access to healthcare facilities and prophylaxis adherence, need to be measured.[21,22] According to Melani,[9] the area of origin has no association with RHD severity. It is stated that patients who come from outside the city of Medan have difficulty accessing complete health services to enter the Adam Malik General Hospital in a severe condition.

Low economic status was associated with RHD severity (p=0.025). The majority of patients have low economic status. RHD mostly occurs in developing countries with low socioeconomic status.[23] Monthly income was associated with RHD risk.[24] Low economic status is tied to living conditions and lack of access to health services.[20]

Occupancy density was not associated with RHD severity (p=0.065). The results showed that 75% of patients living with more or four people in the same house. Some parameters must be measured: house area, area per person, number of siblings, number of beds, and number of people in one room.[25]

Statistical analysis showed that multivalvular lesions were associated with more severe RHD (p=0.003). Heart valve lesions are progressive and cause various complications such as congestive heart failure, arrhythmia, stroke, atrial fibrillation, endocarditis, and death.[4,5] Initially, RHD showed no symptoms, causing the patient to be late for echocardiography screening. The difficulty in getting to health facilities in developing countries, and the fact that Dr. Soetomo is a type A referral hospital where most patients are already in multivalvular valve lesions.

Conclusions

Rheumatic heart disease (RHD) patients' clinical characteristics at Dr. Soetomo Regional General

Hospital Surabaya were on 30–39 years age group, female, living in rural area, having low economic status, and living with four people or more on the same roof. The majority of RHD patients had multivalve and severe RHD. Low economic status and multivalvular lesions were associated with RHD severity.

Conflict of Interest

There is no conflict of interest.

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RESEARCH ARTICLE

Food Safety Training for Food Handlers in the Canteen Elementary School, Yogyakarta

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Abstract

The knowledge in terms of food security affects the attitude that can influence the practice. A low level of knowledge can increase the prevalence of food-borne disease, especially among children. This study aimed to examine the effect of food security training on increasing knowledge, attitude, and practice. This study retrieved 87 food handlers (intervention=43, control=44) from 60 school canteens in Yogyakarta city, 2018. The quasi-experimental study used a non-equivalent design control group by using pretest and posttest. The given intervention was an interactive speech, presentation, and food security module. The statistical test used in this study were normality tests and independent t test. The majority of respondents for intervention and control groups were female (93% each). A significant increase in knowledge, attitude, and practice has been found after training (p value<0.05). There was no difference in knowledge, attitude, and practice among the control group (p value>0.05). This research finding the intervention effectively increases the knowledge, creates the positive, and increases food handlers and consumers' food security practices in the school. Public health providers should design health programs to conduct practical food security training. It is to be conducted continuously for food handlers in the school canteen. They need to remind them that knowledge is essential. Related stakeholders like schools should also provide adequate sanitation facilities and increasing supervision at the school canteen.

Key words: Food handlers, food safety training, school canteen

Pelatihan Keamanan Pangan untuk Penjamah Makanan di Kantin Sekolah Dasar, Yogyakarta

Abstrak

Pengetahuan dalam hal ketahanan pangan memengaruhi sikap yang dapat memengaruhi praktik. Tingkat pengetahuan yang rendah dapat meningkatkan prevalensi penyakit bawaan makanan, terutama pada anak. Penelitian ini bertujuan menguji pengaruh pelatihan ketahanan pangan terhadap peningkatan pengetahuan, sikap, dan praktik. Penelitian ini mengambil 87 penjamah makanan (intervensi=43, kontrol=44) dari 60 kantin sekolah di Kota Yogyakarta tahun 2018. Penelitian kuasi eksperimental menggunakan desain kelompok kontrol non-ekuivalen dengan menggunakan *pretest* dan *posttest*. Intervensi yang diberikan adalah pidato interaktif, presentasi, dan modul ketahanan pangan. Uji statistik yang digunakan dalam penelitian ini adalah uji normalitas dan uji t independen. Responden kelompok intervensi dan kontrol mayoritas berjenis kelamin perempuan (masing-masing 93%). Peningkatan signifikan pada pengetahuan, sikap, dan praktik ditemukan setelah pelatihan (p<0,05). Tidak ada perbedaan pengetahuan, sikap, dan praktik antara kelompok kontrol (p>0,05). Penelitian ini menemukan bahwa intervensi efektif untuk meningkatkan pengetahuan, menciptakan sisi positif, dan meningkatkan praktik ketahanan pangan antara penjamah makanan dan konsumen di sekolah. Petugas kesehatan masyarakat harus merancang program kesehatan untuk melakukan pelatihan ketahanan pangan yang efektif untuk dilakukan secara terus menerus bagi penjamah makanan di kantin sekolah, mengingatkan mereka tentang pengetahuan yang diberikan untuk setiap sesi dan pemangku kepentingan terkait seperti sekolah juga harus menyediakan fasilitas sanitasi yang memadai dan meningkatkan pengawasan di kantin sekolah.

Kata kunci: Kantin sekolah, pelatihan keamanan pangan, penjamah makanan

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Introduction

Diseases and infections caused by contaminated food (foodborne disease) remain a threat to public health globally.[1] The World Health Organization (WHO) reported one in ten people become sick because of foodborne disease. More than 91 million people in developing countries are affected even though several types of research and intervention towards food security have been conducted.[2] Awareness of food safety remains a significant problem among consumers in both developed and developing countries.[3,4] However, foodborne diseases are more common in developing countries due to poor hygiene sanitation, lack of clean water, inappropriate food storage facilities, and lack of food safety education.[5]

Food handlers and consumers take the most crucial role in foodborne disease outbreaks due to the mishandling of preparation, processing, and store food.[6] Many factors influence foodborne diseases in developing countries that, if handled properly, can reduce the incidence of this disease.[1] In Jordanian, there is a low level of knowledge in food safety among food handlers and customers.[7] Inadequate knowledge about food safety, awareness, and practice among consumers, lack of government regulations, and food safety initiatives have been reported in Cameroon.[8]

The improvement of food safety practices could be given by training, which is the most widely used.[9] The 2018 *Riskesdas* results showed that the prevalence of diarrhea based on diagnosis by health professionals and diagnosis based on symptoms for all age groups in Yogyakarta city was 6.11% and 8.47%. The prevalence of diarrhea based on health professionals' diagnosis in the under-five age group was 8.47%, while for school-age children (5 to 14 years), it was 6.38 percent.[10] The proper and standardized cooking process and food and good personal hygiene will protect against foodborne diseases among children in school-age.[11] The study's primary background is the importance of training to improve the knowledge, attitude, and practice among food handlers. This study aimed to compare the level of knowledge, attitude, and practice between the trained and control groups among food handlers in the elementary school canteen in Yogyakarta.

Methods

This study is quasi-experimental research with a non-equivalent control group design by pretest and post-test. The pretest was done using a questionnaire to determine the level of knowledge and attitude of food handlers and the checklist table to inspect their behavior during food processing. This research got ethical clearance from the Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada (Certificate approval No. KE/FK/297/EC/2017).

The sample of this study was 87 food handlers from 60 elementary schools in Yogyakarta. Notably, the sample was divided into two groups consisting of 43 food handlers who were become intervened and 44 others who became control groups.

The dependent variable of this study was food security. The independent variables were knowledge, attitude, and practice. The questionnaire used in this study is consisting of three parts: demographic characteristics of respondents (5 questions), knowledge of food security (11 questions), attitude in terms of food security (22 questions). The questionnaire has been validated and passed the reliability test by 38 food handlers in elementary school in Bantul regency. The checklist point (11 items) has been made to observe by the research assistant directly following each step during food processing.

The questions related to knowledge consisted of favorable and unfavorable questions with true or false choices. The questions related to attitude consisted of favorable and unfavorable, agree, disagree, and disagreeable choices. The point in the checklist of practices during food processing was adopted from the Regulation of Minister of Health Republic of Indonesia Number 1096 in 2011 with yes (if related) and no (if did not relate) choices. The respondents were informed that those questionnaires are not to grade their knowledge of food security because all of the information is secret. The respondents have been signed informed consent to ensure their participation. This study's intervention, namely food security training, has been done for one month in collaboration with the District of Health Office in Yogyakarta city. The training used interactive speaking with presentation and module of food security (four parts: introductions, food security and food-borne disease, food and drink hygiene sanitation, and hazard analysis critical control point (HACCP).

The data were tested for normalization then examined using an independent t test with

a significant level $\alpha<0.05$. Each variable has been tested by a paired t test and independent t test using Stata software for data science and statistical analysis.

Results

Demographic characteristic of respondents in this study describing by age, sex, educational level, participation in training, and duration of work) was presented in Table 1.

Table 1 describes the mean age of the intervention and control group, which is almost the same by 42.23±8.315 and 42.00±7.728, respectively. Intervened respondents were aged 23 to 60 years, and the controlled respondents were age 20 to 56 years. Regarding the sex, most respondents for the intervention and control groups were female (93% each). More than half of them graduated from senior high school, 65.1% and 72.7%, respectively. In terms of participating in food security training, more than half of them, both for intervention and control group, had had food security training. Around half of the respondents in this study have been work as food handlers varied from 0 to 5 years, particularly 55.8% for the intervention group and 50.0% for the control group.

Table 2 shows the significant differences between pre and post-test for intervention groups

Table 1 Demographic Characteristic of Respondents

Characteristics	Intervention		Control	
	n=43	Percentage	n=44	Percentage
Age				
Mean	42.23±8.315		42.00±7.728	
Median (min–max)	23–60		20–56	
Sex				
Female	40	93	40	93
Male	3	7	4	7
Level of education				
Elementary school	4	9	3	7
Junior high school	7	17	5	11
Senior high school	28	65	32	723
University	4	9	4	9
Participating in food handler training				
No	14	33	15	34
Yes	29	67	25	66
Duration of work (year)				
0–5	24	56	22	50
6–10	10	24	13	30
11–15	7	16	2	4
16–20	0	0	6	14
21–25	1	2	1	2
26–30	1	2	0	0

Table 2 Mean Differences of Knowledge between Intervention and Control Group

Variables	Pre-test	Post-test	Mean (Pre-Post)	95% CI		p
				Lower	Upper	
Control (n=44)	9.61	9.66	0.045	−0.413	0.504	0.842
Intervention (n=43)	9.07	10.05	0.976	0.539	1.414	0.000
The mean differences between control and intervention group			−0.931	−1.557	−0.306	0.004

Table 3 Mean Differences of Attitude between Intervention and Control Group

Variables	Pre-test	Post-test	Mean (Pre-Post)	95% CI		p
				Lower	Upper	
Control (n=44)	77.86	78.66	0.795	−2.538	4.129	0.633
Intervention (n=43)	73.69	79.67	5.977	3.231	8.722	0.000
The mean differences between control and intervention group			−5.181	−9.448	−0.915	0.018

Table 4 Mean Differences of Practice between Intervention and Control Group

Variables	Pre-test	Post-test	Mean (Pre-Post)	95% CI		p
				Lower	Upper	
Control (n=44)	9.64	9.68	0.045	−0.639	0.729	0.894
Intervention (n=43)	9.44	10.30	0.860	0.571	1.149	0.000
The mean differences between control and intervention group			−0.815	−1.554	−0.076	0.031

compared with the control group. After trained for one month, the knowledge of intervention respondents increases 0.0976 while the control respondents are only 0.045. It can be concluded that the food security training is significant to increase the knowledge of food handlers (p value=0.004).

Table 3 explains the mean differences in the attitude of respondents before and after given food security training. For the control group, the mean is 0.795, and for the intervention group, the mean is 5.977. It can be concluded that the food security training is strongly significant to increase the attitude of food handlers (p value=0.018).

The practice of food security by respondents before and after training shows significant value (Table 4). The mean for control groups is 0.045 and for the intervention groups is 0.860. Overall, the food security training is significant to increases the practice of food security of food handlers (p value=0.031).

Discussion

The t test result revealed that knowledge was a significant intervention to increase the knowledge of food security. The food handlers in the school canteen were required to understand the prevention of food contamination to ensure the food is proper to consume. Food handlers need to have knowledge and skill to produce hygiene food.[12] This study is in line with the previous study, which stated that training as an intervention could increase food handlers' knowledge.[13] The training is an effective model to increase the understanding of food handlers about food security.[14] It is essential to have proper practices, so training is needed continuously for food handlers.[15] The training in this study consisted of the knowledge of food processing and the prevention of contaminated food and ensure the food handlers have their proper knowledge to fulfill food hygiene requirements.[16]

Attitude is the scale of people to evaluate the strength and weakness of the practice.[17] The positive attitude is needed to transform knowledge to be proper practices, and it can be a mediator between knowledge and practice.[18] This study found that training significantly increased the attitude of food handlers. This result is supported by the previous study in Lebanon that reported intervention can positively improve attitude through food security.[13] Attitude is an essential factor besides knowledge for indicating the tendency of decreasing food-borne disease. The excellent attitude of food handlers will lead to the safe food serving which is consumed. The study in South Africa also found the same result, the education and training about food security must be given to food handlers to strengthen their knowledge, attitude, and skill so the outbreak can be prevented.[19,20]

The result of this study found that the practice of food security can be increased by training. Children are one vulnerable age group.[21] To prevent foodborne diseases among children, food security is essential to ensure healthy food for children.[22] The finding in this study is supported by the study in Turkey, which found the training is essential in increasing food handlers' food security practice.[23]

Food security training is an effective model to increase knowledge which the basic to implement the proper hygiene attitude.[24] The association between knowledge, attitude, and practice is also reported by other studies in Malaysia, Nigeria, and India.[13,25,26] The food security practice can be increased by training food handlers. The refreshment of training is also needed to train and renew their skill. The duration of training needs to be shorter, no more than two weeks respectively.[26]

From the findings, there were two important issues for public health programs; 1) public health providers should design health programs to conduct effective food security training is to be conducted continuously for food handlers in the school canteen and remind them about the given knowledge for each session. 2) related stakeholders like school should also provide adequate sanitation facilities and Increasing supervision at the school canteen.

Conclusion

The result of this research finding the intervention (training: interactive speech, presentation, and food security module) is effective to increase the knowledge, create the positive and increase the food security practices between food handlers and consumers in the school.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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This study has been conducted by participants from the school canteen and food handlers.

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RESEARCH ARTICLE

Sigi's Response to the Disaster Program: Nutrition, Sanitation, and Food Fulfillment

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Abstract

The earthquake occurring in Indonesia caused various problems, especially the decreased degree of human health caused by insufficient food availability. It makes victims of natural disasters need assistance from government programs. One case of a natural disaster in 2018 was an earthquake in Central Sulawesi. Unfortunately, disaster survival has the challenge to continue their living caused by program absences from the government in post-disaster. This study aimed to explore disaster survival's resilience after one year of the earthquake disaster in Sidera village, Sigi regency, in response to government programs, especially nutrition, sanitation, and food fulfillment response. This research used mixed-method approaches with a cross-sectional design. Data collection was done with questionnaires, in-depth interviews, and anthropometric measurements. The study was conducted from February to April 2020 with 30 refugees in the temporary shelter in Sidera village as respondents. The study results found that people still need to initiate countermeasures related to sanitation and fulfillment of food. Government programs in health services produce good conditions related to normal nutritional status at the age of 5 years (40%), 5–18 years (100%), and 18 years (41%). The conclusion is that the government program is not sustainable, which makes the community still need to initiate countermeasures related to sanitation and food fulfillment. The program recommendations are to ensure clean water availability and guide the community to fulfill their food need.

Key words: Community resilience, nutrition, post-disaster, program recommendations, sanitation

Tanggapan Sigi terhadap Program Bencana: Gizi, Sanitasi, dan Pemenuhan Pangan

Abstrak

Gempa bumi yang terjadi di Indonesia menyebabkan berbagai masalah, secara khusus penurunan derajat kesehatan manusia dikarenakan oleh ketersediaan pangan yang tidak tercukupi. Hal tersebut membuat korban bencana alam membutuhkan bantuan dari program pemerintah. Salah satu kasus bencana alam tahun 2018 adalah gempa bumi di Sulawesi Tengah. Namun, hingga pascabencana, korban bencana alam masih tinggal di pengungsian tanpa program. Tujuan penelitian ini adalah mengeksplorasi daya lenting pengungsi pasca-satu tahun bencana gempa bumi di Desa Sidera, Kabupaten Sigi sebagai tanggapan dari program pemerintah khususnya gizi, air bersih, dan pemenuhan pangan. Penelitian ini menggunakan pendekatan *mixed-method* dengan desain *cross-sectional*. Teknik pengumpulan data dilakukan melalui pengisian kuesioner, *in depth interview*, serta pengukuran antropometri. Penelitian dilakukan pada 30 responden yang merupakan pengungsi di hunian sementara (huntara) Desa Sidera. Penelitian dilakukan selama bulan Februari hingga April 2020. Hasil penelitian menemukan bahwa masyarakat masih perlu melakukan inisiasi penanggulangan terkait air bersih dan pemenuhan pangan. Program pemerintah terkait pelayanan kesehatan menghasilkan kondisi yang baik terkait status gizi normal pada usia ≤5 tahun (40%), 5–18 tahun (100%), dan ≥18 tahun (41%). Simpulan dari penelitian ini adalah program pemerintah tidak memiliki keberlanjutan, hal tersebut membuat masyarakat masih perlu melakukan inisiasi penanggulangan terkait air bersih dan pemenuhan pangan. Rekomendasi program yang dapat diberikan adalah ketersediaan air bersih serta pemantauan program dalam membimbing masyarakat agar dapat memenuhi kebutuhan pangan.

Kata kunci: Daya lenting masyarakat, gizi, pascabencana, rekomendasi program, sanitasi

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Introduction

Natural disasters are natural phenomena that threaten human survival, whether earthquakes, floods, or tsunamis, which can cause various risks that threaten human resources' quality, especially health.[1] In the event of an earthquake, there is a significant risk of injury that requires medical treatment.[2] When it turns to be a flood or tsunami, it can simply cause damage to the environmental sanitation system and decrease the quality of clean water, so that it will cause various types of diseases such as diarrhea.[3] Those cases are like what happened in the 2008 Sichuan earthquake and the 2004 Indian Ocean tsunami.[4,5]

One of the countries classified as having a high risk of natural disasters, especially for the earthquake, is Indonesia.[6] The earthquake that occurred in Indonesia caused various problems, especially the decreased degree of human health caused by insufficient food availability. It makes victims of natural disasters need assistance from government programs.[7,8] One case of the natural disasters in 2018 was an earthquake followed by liquefaction and tsunami in Central Sulawesi. This natural disaster was appalling since it was also the first time it occurred in Indonesia.[9,10] Natural disasters in Central Sulawesi caused various emergencies that affected health, including damage to areas covering residents' houses to agricultural land, especially in the Sigi area, which is the local community's source.[11] Besides, various health problems have also arisen related to food fulfillment, like diarrhea.[12] Due to this emergency condition, the local government of Palu made temporary shelters and implemented various programs to deal with the health problems.[13]

Various government programs have been implemented in the Central Sulawesi region, particularly in Sigi regency.[14] Various evaluations were also carried out by focusing on the achievement of the program being implemented. Unfortunately, the disaster survivors were still living in a temporary shelter without any program until the post-disaster. Therefore this study aimed to explore the resilience of disaster survival after one year of the earthquake in Sidera village, Sigi regency, in response to government programs, especially nutrition, sanitation, and food fulfillment response.

Methods

This research used a descriptive study with mixed-method approaches having a cross-sectional design. This study's population were the disaster survivors in Palu taking refuge in the temporary shelter of Sidera village, Biromaru district, Sigi regency, which was 38 households. The number of respondents in the study was 17 families or as many as 30 people. The sample was selected using the random sampling technique divided into three groups: toddlers, pregnant women, and groups of women and men of all age distribution. Each group met the inclusion criteria, namely: 1) toddlers were included in the pre- and postnatal health care and information center (*posyandu*) data; 2) covers all age groups, including the elderly; 3) receiving disaster programs from the government. Meanwhile, the exclusion criteria were: respondents still received assistance from their families and did not participate in government disaster programs. The research was conducted from February to April 2020 in a temporary shelter established by *aksi cepat tanggap* (ACT) in Sidera village.

In collecting the data in the field, first, the observations to monitor the situation and the total number of refugees in the temporary shelter of Sidera village. After having the data, respondents from each family were asked to fill in a questionnaire compiled by researchers based on Ncube et al.[15] It contained 29 questions about demographics, health, environmental sanitation, and emergency nutrition programs received from the government for pregnant women and other age groups, including the elderly. Also, in-depth interviews were conducted using new interviews related to government programs that have been implemented. The questionnaire was filled in by all respondents, which, especially for infants and toddlers, were represented by parents. Meanwhile, in-depth interviews were conducted with adult respondents (aged 18 years and over). Furthermore, the anthropometric measurement method was carried out on all respondents to determine the Z-score and body mass index (BMI). It served as an indicator for determining respondents' nutritional status relating to the health condition of respondents who depend entirely on government programs. The BMI classification according to the Ministry of Health Republic of Indonesia are severely underweight <17.0 kg/m^2, underweight 17.0–18.5 kg/m^2, normal >18.5–25.0 kg/m^2, overweight >25.0–27.0 kg/m^2, and obese >27.0 kg/m^2.[16] While the Z-score category to weight-for-length or height is severely wasted <−3 SD, wasted −3 SD to

<−2 SD, normal −2 SD to +1 SD, possible risk of overweight >+1 SD to 2 SD, overweight >+2 SD to +3 SD, and obese >+3 SD. Meanwhile, BMI for age are thinness −3 SD to <−2 SD, normal −2 SD to +1 SD, overweight +1 SD to +2 SD, and obese >+2 SD.[17]

After the questionnaire data has been collected, it is processed using Microsoft Excel. Meanwhile, the Z-score and BMI results were calculated using the following formula bodyweight = bodyweight weighing according to age; median, standard weight = standard bodyweight according to age; the standard deviation of bodyweight = standard deviation of bodyweight for age.

$$\text{BMI} = \frac{\text{Weight (kilogram)}}{\text{Height (meter)}^2}$$

$$\text{Z-score} = \frac{\text{Bodyweight} - \text{Median standard weight}}{\text{Standard deviation of bodyweight}}$$

The research was conducted during the COVID-19 pandemic so that contact with respondents can still be carried out by paying attention to health protocols. However, this research has weaknesses due to the COVID-19 pandemic in the middle of the research. The data collection was only carried out for families who still live in the temporary shelter while the researcher was out in the field.

Ethical clearance for this study was obtained from the Health Ethics Committee of Faculty of Medicine and Health Sciences in Universitas Kristen Satya Wacana. The issuance of the ethical clearance No. 262/PE/KEPK.UKSW/2020.

Results

This research succeeded in taking the respondent data of 30 people from temporary shelter in Sidera village. These respondents came from Petobo and Jono Oge village, which were affected by the earthquake and liquefaction. All respondents moved to Sidera village in 2018 and lived in a temporary shelter for more than one year.

This research found that 20 of 30 refugees were dominated by women (Table 1). However, men's fulfillment of needs was carried out, whom most of them (9 of 30) worked as laborers or informal workers. The most age distribution in the temporary shelter was the group over 18 years old, which is 17 of 30 refugees, while the highest level of education was the senior high school.

Based on the results of interviews with all respondents, especially in detail conveyed by the deputy head of the temporary shelter, it was found that the refugees have received assistance from the government while living in a temporary shelter in Sidera Village. Assistance channeled through the health department to provide

Table 1 Respondents Demographic Data in the Sidera Village Temporary Shelter

Descriptions	n=30
Gender	
Male	10
Female	20
Age (years)	
≤5	5
5–18	8
≥18	17
Level of education	
No schooling	5
Elementary school	8
Junior high school	6
Senior high school	11
Marital Status	
Single	15
Married	13
Divorced	0
Widowed	2
Religion	
Muslim	30
Ethnicity	
Palu city	13
Sidera village	11
Toraja	4
South Sulawesi	1
Solawe	1
Language Spoken	
Bahasa	13
Ledo Kaili	0
Bahasa and Ledo Kaili	17
Length of living in a temporary shelter (month)	
≤1	0
1–3	2
6–12	0
≥12	28
Family livelihood	
Formal workers	6
Small trader	6
Farmer	3
Labor	9
Driver	3
Office guard	3

Table 2 Distribution of Nutritional Status of Children Aged 0–5 Years

Nutritional Status Variable	n=5
Severely wasted	0
Wasted	1
Normal	2
Risk of overweight possibility	1
Overweight	1
Obese	0

Table 4 BMI Frequency Distribution (Over 18 Years)

BMI (kg/m²)	n=17
Severely underweight (<17.0)	0
Underweight (17.0–18.5)	0
Normal (18.5–25.0)	7
Overweight (>25.0–27,0)	4
Obese (>27.0)	6

two reservoirs of clean water and provision of *pemberian makan bayi dan anak* (PMBA) or infant and toddler feeding kitchen equipped with health examinations and counseling for toddlers. The provision of a public kitchen served to process ordinary food for adult groups. This assistance came up because of the difficulty in fulfilling clean water and local food, which causes vomiting and allergies in toddlers. Unfortunately, the assistance provided could not solve all the community's problems, especially water problems due to dry conditions. Finally, the refugees were moved to a temporary shelter established by *aksi cepat tanggap* (ACT) in Sidera village. In the new residential area, the community received assistance in clean water and provided PMBA kitchens and shared kitchens for two months. However, the time interval for the distribution of this assistance could not be ascertained. Besides, the amount of water given was minimal compared to the number of refugees.

Another problem arose when the PMBA and shared kitchen procurement program stopped after running it for eight months. These conditions eventually encouraged refugees to look for work. The loss of some of their main livelihoods as farmers made most people looked for new jobs as casual daily laborers. Responding to this, an NGO called Islamic Relief supported economic improvement activities through empowering women. This empowerment is engaged in processing food from fish, mushrooms, and onions. The activities included providing training for women and providing funds to purchase raw materials. Processed food products were sold in the area of temporary shelters and during exhibition activities in Palu city. Unfortunately, the community admitted that they had difficulty in marketing those preparations. Not fixed and insufficient demand and difficulties in obtaining raw materials were still obstacles to fulfill sales targets.

The economic problems related to food fulfillment ultimately encouraged other people, especially women, to help fulfill the economy by trading on a small scale based on their knowledge. The work as small traders carried out by the community including selling cooked vegetables in the temporary shelter yard, snacks, and accessories through social media.

Apart from the community's problems, respondent's health conditions related to nutritional status at the age of 0–5 years are shown in Table 2. It can be seen that most status shown (2 of 5 subjects) has normal nutritional status, while the other 1 of 5 has wasted, possible risk of overweight, and overweight.

Respondent's health conditions related to health history are shown in Table 3. It can be seen that most of them (22 of 30) have no history of certain diseases. In comparison, several other respondents have cholesterol as much as 4 of 30, diabetes mellitus, gout, hypertension, gout complications, and cholesterol 1 of 30, respectively.

All respondents' health conditions related to nutritional status at the age of 5–18 are normal. While the nutritional status at the age over 18 years in Table 4 showed, 7 of 17 have normal

Table 3 Health History of Respondents in the Temporary Shelter of Sidera

Medical History	n=30
Do not have certain diseases	22
Cholesterol	4
Diabetes mellitus	1
Gout	1
Hypertension	1
Gouty arthritis and cholesterol	1

nutritional status, overweight (4 of 17), and obese (6 of 17).

Discussion

Based on the interviews, it was found that the condition of the sanitation problem encourages the community to initiate the acquisition of clean water by collecting rainwater. Unfortunately, the absence of directives from the government through the health department has resulted in this initiation harming health due to contaminants in the rainwater consumed. According to Yulistyorini,[18] although rainwater can be an alternative source of clean water during emergency conditions, such as natural disasters, one must pay attention to the absence of contact with the rainwater surface catchment, rainwater drainage areas, and rainwater storage tanks. It is done to prevent rainwater from carrying contaminants, physically, chemically, and microbiologically. Another problem coming up was the unpredictable weather so that people cannot entirely rely on rainwater.

The resilience that emerges from the community showed that government programs were not sustainable. For example, clean water assistance, which was stopped when people moved to temporary shelters, shared kitchen programs terminated without holding training programs for people to meet their food needs. The provision of programs that ran temporarily without any clear information made people lose trust in the government. It is following research conducted by Surtiari,[19] which revealed that the lack of information disclosure would trigger public distrust of leaders due to aid issues.

Government programs that only focus on babies and children through the PMBA kitchen and special health services for toddlers have good conditions (Table 2). It can be seen from the majority (40%) of under-five respondents who have a normal nutritional status, while the other 20% have wasted, possible risk of overweight, and overweight.

In other age groups, even children and adults, it can be seen that most of them had no history of certain diseases (Table 3). Meanwhile, several other respondents had cholesterol as much as 13%, diabetes mellitus, gout, hypertension, gout complications, and cholesterol 3%, respectively. Based on the nutritional status data, all groups aged 5–18 years had a normal nutritional status, and the group aged over 18 years, while most of which (41%) had normal nutritional status (Table 4). From this data, it can be identified that there were no major health problems in the community, especially those aged over five years. Although the government's focus on the fulfillment of food accompanied by health services specifically aimed at toddlers, the availability of shared kitchens and local health facilities has played a significant role in maintaining children's health conditions to adults.

Unfortunately, people need not only good health conditions to survive but also sanitation and guaranteed food. Article 53 of Law No. 24 of 2007 shows that, apart from health services, other basic needs, such as clean water and sanitation, food, clothing, psychosocial services, and shelter, also need to be fulfilled.[20] This is because when only health services are fulfilled, diseases that were previously neglected will emerge. In this study, to fulfill water needs, the community took the initiative to collect rainwater that may be contaminated and a potential means of generating, carrying, and spreading diseases, such as cholera and dysentery.[21,22] On the other hand, the lack of programs in other fields, such as the level of nutritional adequacy, can also decrease endurance. If it is not immediately addressed, it will cause problems in the health sector.[23]

Moreover, although the initial aid provision is following Article 53 of Law No. 24 of 2007 concerning disaster management, the program's continuation was not prepared, especially in the sanitation and food fulfillment program during the post-disaster period.[20] The programs provided to assist the post-disaster community's economic recovery do not come from the government but NGOs. The program also only provided training related to food processing. The community's condition is not stable yet, and experiencing social pressure due to the formation of new communities. According to Ridwan,[24] rapid changes in society can bring complexity to problems and challenges.Under the research results conducted by Yulianto,[25] the negative impact of disaster-affected community groups is damage in the social, economic, and environmental fields, which causes problems to include psychological aspects. In the end, this made the community unable to implement the training system that had been equipped and, in fact, made the community burden even more.

The government needs to pay attention to the program's instability for the people affected by the disaster. According to Awalia et al.,[26] the government must have the ability to control disasters that occur through disaster response planning and preparation, coordination assistance, reconstruction policies, and overcoming population problems. This refers to Law Number 24 of 2007 concerning disaster management, which emphasizes government efforts including mitigation, placing victims of natural disasters in a safe place, forming a disaster management team, providing counseling, and relocating victims gradually. In its implementation, the government needs to collaborate with related agencies so that disaster management can run well.[27]

This study found that there were some problems related to sanitation and food fulfillment in Sigi regency. Responding to this situation, the program recommendation that can be given is the availability of clean water provided by the government through Municipal Waterworks (PDAM). Dryland conditions should also be a concern in using municipal waterworks. Besides, the government also needs to monitor the programs carried out by nutrition workers from the health department and *puskesmas* (public health center) in providing direction and guidance to the community. The directive is related to the fulfillment of food to be being able to use vacant land to plant and cultivate freshwater fish. Its implementation still needs to be monitored and evaluated through the health department and the public health care. It is done for follow-up planning and program improvement if there are problems and obstacles in its implementation.

Conclusions

It can be concluded that the government program is not sustainable, which makes the community still need to initiate countermeasures related to sanitation and fulfillment of food. The program recommendations that can be given are the availability of clean water and guiding the community to fulfill their food.

Conflict of Interest

All authors stated that there is no conflict of interest in this study.

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RESEARCH ARTICLE

Effectiveness of Al-Qur'an *Tadabbur* Therapy on Nulliparous Women's Anxiety Level during Labor

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Abstract

The process of pregnancy affects psychological aspects significantly. This process may lead to anxiety and inconveniences. The previous research showed that anxiety might be contributing to the labor process. These can decreased baby birth weight and increased hypothalamic-pituitary-adrenal (HPA) hormone levels which cause changes in steroid hormone production and adult fertility rates. Besides, anxiety during pregnancy impacts emotional problems, disorders of hyperactivity, decentralization, and impaired child development. Nonpharmacological therapy that has been approved in reducing anxiety is religious copings. One of the religious copings is to contemplate (*tadabbur*) Al-Qur'an. Al-Qur'an can treat all kinds of diseases, including anxiety disorders. This study aims to examine the effect of Al-Qur'an *tadabbur* therapy on reducing nulliparous anxiety levels during labor. A quasi-experimental study was conducted with a pretest-postest control group design. The sampling technique was consecutive sampling. The number of 30 third trimester nulliparous women experienced moderate-severe anxiety levels in Tanjung Karang Public Health Center, Mataram was selected to participate in this study during July–September 2020. They were divided into two groups, the intervention and control groups. The intervention group received Al-Qur'an *tadabbur* therapy, while the control group was not. The statistical analysis used was univariate and bivariate analyses with an independent t-test. The instrument was used as the Zung Self-Rating Anxiety Scale (SAS) questionnaire. This study showed that the decrease of anxiety score in the intervention group was 26.1% higher than the control group p=0.001 (p<0.05). In conclusion, Al-Qur'an *tadabbur* therapy is effective in reducing anxiety levels during labor.

Key words: Al-Qur'an *tadabbur*, anxiety, labor, nulliparous women

Efektivitas Terapi *Tadabbur* Al-Qur'an untuk Menurunkan Tingkat Kecemasan Ibu Primigravida dalam Menghadapi Persalinan

Abstrak

Proses kehamilan sering kali memengaruhi aspek psikologis yang dapat menyebabkan berbagai permasalahan seperti kecemasan dan ketidaknyamanan. Selain berdampak pada proses persalinan, kecemasan juga dapat mengakibatkan penurunan berat lahir dan peningkatan aktivitas hipotalamus-hipofisis-adrenal (HHA) yang menyebabkan perubahan produksi hormon steroid dan angka fertilitas saat dewasa. Selain itu, kecemasan pada masa kehamilan berkaitan dengan masalah emosional, gangguan hiperaktivitas, desentralisasi, dan gangguan perkembangan kognitif pada anak. Terapi nonfarmakologis yang telah disetujui dalam mengurangi kecemasan adalah *religious coping*. Salah satu *religious coping* adalah dengan *tadabbur* Al-Qur'an. Al-Qur'an merupakan pedoman yang mampu mengobati segala macam penyakit termasuk gangguan kecemasan. Penelitian ini bertujuan menguji efek terapi *tadabbur* Al-Qur'an terhadap penurunan kecemasan ibu hamil primigravida dalam menghadapi persalinan. Penelitian *quasi-experiment* dilakukan dengan *pretest-postest with control group design*, teknik pengambilan sampel dengan *consecutive sampling*. Subjek penelitian adalah 30 ibu hamil primigravida trimester III yang mengalami kecemasan sedang–berat di wilayah kerja Puskesmas Tanjung Karang, Kota Mataram pada bulan Juli–September 2020 yang terbagi dalam 2 kelompok, yaitu kelompok intervensi yang mendapat terapi Al-Qur'an dan kelompok kontrol. Analisis data menggunakan uji t independen. Penilaian tingkat kecemasan dilakukan menggunakan kuisioner *Zung Self-Rating Anxiety Scale* (ZSAS). Berdasar atas hasil penelitian, penurunan tingkat kecemasan kelompok intervensi 26,1% lebih tinggi dibanding dengan kelompok kontrol dengan taraf signifikasi p=0,001 (p<0,05). Simpulan, terapi *tadabbur* Al-Qur'an cukup efektif untuk menurunkan kecemasan pada ibu hamil dalam menghadapi persalinan.

Kata kunci: Ibu primigravida, kecemasan, persalinan, *tadabbur* Al-Qur'an

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Introduction

The labor process often affects psychological aspects, which might contribute to psychological problems in terms of anxiety.[1] Anxiety is an unclear and pervasive worry which is related to feelings of uncertainty and helplessness. This emotional state has no specific object.[2] It has been reported that 54% of women experience anxiety at least during the first trimester, and it increases more frequently in the second and third trimesters.[3] During this period, pregnant women feel anxious about many things, such as whether they can deliver the baby normally or abnormally, preparing for delivery, and postnatal care.[3] Anxiety that occurs mainly in the third trimester might be another risk factor for releasing stress hormones. It contributes to uterine contractility disorders, prolonged labor, birth weight loss, and increased hypothalamic-pituitary-adrenal (HPA) hormone levels which causes changes in steroid hormone production.[4] Another risk of anxiety during pregnancy is emotional problems, decentralization, and cognitive development disorders.[5]

Factors during pregnancy that might contribute to anxiety during pregnancy include hormonal changes, physical changes, and accepting the role of parenthood.[6] Another factor is changes in neurotransmitters, a stressful life, low socioeconomic status, a history of stillbirth, miscarriages, congenital malformations, and unwanted pregnancy.[7] Research has shown anxiety is more common in nulliparous/ primiparous women.[8] Furthermore, according to Larasati and Wibowo,[9] the incidence of premature delivery is higher on women who often feel anxious facing labor. In Indonesia, there are 107,000 (28.7%) pregnant women who experience anxiety in facing labor.[10] In a study conducted by Astria et al.,[11] shows that anxiety is more experienced in primigravida (first pregnancy) as much as 66.2%, compared with anxiety in multigravida 42.2%. With the number of negative impacts of anxiety, it is essential to provide an intervention to reduce anxiety in primigravida in facing labor.

There are two methods to reduce anxiety, such as pharmacological and nonpharmacological therapies. One of the nonpharmacological therapy is using Islamic psychotherapy.[12] Islamic psychotherapy makes one's beliefs last longer. According to Maimunah and Retnowati,[13] nonpharmacological therapy that has been approved in reducing anxiety is religious coping. One of the religious copings is Al-Qur'an *tadabbur*. *Tadabbur* meant any observation, examination, reflection on the meaning of the Al-Qur'an. The reality of *tadabbur* occurs during the process of understanding the verses of the Al-Qur'an. However, an in-depth understanding is closely related to the extent of a person's capability to understand a verse's interpretation.[14]

Tadabbur is a recitation of the Al-Qur'an by meditating on the verses of the Al-Qur'an that can delete, calm change to soothe. The Al-Qur'an is essentially a book that can change one's mind, desires, and behavior. If the pregnant women do *tadabbur* Al-Qur'an, they are expected to be able to open their minds and hearts to be more positive in processing anxiety. Humans are essentially God's creatures who must have a close relationship with their creator. The Al-Qur'an is a guide that can treat or treat anxiety, including anxiety disorders. The Al-Qur'an is a guide and a solution for all kinds of problems. By frequently getting closer to Allah SWT through recitation and recitation of the Al-Qur'an, the heart and the mind becomes more positive, and the soul feels spacious.[15]

Based on the explanation above, the objective of this study aims to examine the effect of Al-Qur'an *tadabbur* therapy in reducing nulliparous anxiety levels during labor.

Methods

It was a quasi-experimental study with the pretest and posttest control group design. This study was conducted in the Tanjung Karang Public Health Center work area, Mataram city, from July to October 2020. The study population was all Muslim primigravida pregnant women (nulliparous) who entered their third trimester of pregnancy (28–40 weeks). The procedure and sampling technique used was consecutive sampling. The instrument used to measure anxiety level is the Zung Self-Rating Anxiety Scale (ZSAS) questionnaire. Participants were selected and divided into two groups: intervention and control groups. First, both groups conducted a pretest to determine anxiety levels, just those who have moderate to severe anxiety levels measured. The intervention group received Al-Qur'an *tadabbur* therapy, while the control group received standard health education leaflets about labor preparation. The Al-Qur'an *tadabbur* therapy refers to the module developed by Prapto et al.[12]

The Al-Qur'an *tadabbur* therapy is designed for three meetings. Each session is approximately 60–100 minutes, accompanied by a researcher and an *ustadzah* who certified in *Mahad Qur'an wal hadits*. The Al-Qur'an *tadabbur* discussed the meaning of Al-Qur'an *tadabbur*, introductions between participants and facilitators, expressing anxieties during childbirth, hadits explanation, and *Asbabun nuzul* of Al-Qur'an verse about pregnancy and labor. The intervention group's sample size given Al-Qur'an *tadabbur* therapy is 15 respondents, and the control group is 15 respondents.

Ethical clearance for this study was obtained from the Health Research Ethics Committee of the Universitas Mataram with no potential for ethical violations with the issuance of the ethical clearance number 110/UN18.F7/ETIK/2020.

Results

The characteristics of the two groups were similar that the groups were considered comparable (Table 1).

In the pretest, the same results from the intervention group and the control group, 13 of 15 respondents had moderate anxiety levels, and 2 of 15 respondents had severe anxiety levels. After the intervention, the results showed that in the intervention group, 8 of 15 respondents did not experience anxiety/normal range, 6 of 15 respondents experienced mild anxiety, and 1 of 15 respondents experienced moderate anxiety. In contrast, the control group found 2 of 15 respondents had no anxiety, 5 of 15 people had mild anxiety, and 8 of 15 had moderate anxiety (Table 2).

There was a difference in the mean of the anxiety score between the control and intervention groups after therapy. The decrease in the mean of the intervention group's anxiety scores was 26.1% higher than the control group (Table 3). The statistical results showed that Al-Qur'an *tadabbur* therapy effectively reduces the anxiety level of nulliparous during labor with a p value=0.001.

Discussion

This study's novel aspect was Al-Qur'an *tadabbur* method as one of the religious copings to reduce nulliparous anxiety during pregnancy.

Table 1 Respondent Characteristics

Characteristics	Groups		p Value*
	Intervention (n=15)	Control (n=15)	
Age (years)			0.537
<25	6	9	
25–30	5	3	
>30	4	3	
Education			0.143
<High School	6	10	
≥High School	9	5	
Occupation			0.704
Employed	5	6	
Unemployed	10	9	

Note: *chi-square test

Table 2 Comparison of Anxiety Score before and after Therapy

Anxiety Level	Intervention Group (n=15)		Control Group (n=15)	
	Pretest	Posttest	Pretest	Posttest
Normal range	0	8	0	2
Mild	0	6	0	5
Moderate	13	1	13	8
Severe	2	0	2	0

Table 3 Effect of Al-Qur'an *Tadabbur* Therapy on Anxiety Level of Third Trimester Nulliparous

Variable (Scale 100)	Groups		p Value*
	Intervention (n=15)	Control (n=15)	
Anxiety score			0.001
Pretest			
Mean (SD)	67.5 (6.8)	66.2 (7.6)	
Medium	61–83	61–88	
Posttest			
Mean (SD)	41.3 (11.6)	57.8 (13.02)	
Medium	24–63	29–78	
Differences			
Pretest vs posttest**	p=0.001	p=0.002	
% decrease (mean)	38.8	12.7	

Note: *Willcoxon test, **independent t test

Nulliparous women in labor tend to experience anxiety and inconveniences before and during labor. Other studies have been performed to see the effect of this therapy. It is according to Qadri's theory,[16] which states that Al-Qur'an provides directions and solutions to everything problem. All of the diseases can be treated with the Al-Qur'an.

Anxiety is a mixture of unpleasant emotions dominated by fear, worry, and uncontrollable anxiety about threatening conditions that are not clear in the future.[17] In pregnant women, especially in the third trimester, maternal psychology changes seem more complex and increased compared to the previous trimester, which is due to the increasing condition of pregnancy. This study results in line with Rinata and Andayani's[18] study that indicated there is a relationship between anxiety and gestational age, primigravida women in labor tend to experience anxiety and restlessness before and during labor. Research conducted by Berle et al.[19] also states that gestational age affects pregnant women's level of anxiety. Due to the closer to childbirth, the level of anxiety will increase in line with concerns about abnormal childbirth, pain, and baby delivery preparation.

This study aims to determine the effectiveness of Al-Qur'an *tadabbur* therapy to reduce anxiety in primigravida. This study pointed out that the intervention group's level of anxiety was lower compared to the control group after getting Al-Qur'an *tadabbur* therapy. There are differences in the level of anxiety scores between the intervention group and the control group. The test result in the intervention group is p=0.001, and in the control group is p=0.002. It can be concluded that there is a difference in the level of anxiety in the intervention group compared to the control group. It can be concluded that Al-Qur'an *tadabbur* therapy is effective in reducing anxiety levels. The finding is consistent with the theory of Najati,[20] which states that the Al-Qur'an can soothe one's heart and mind, can change thoughts, desires, and behavior. Someone who performs Al-Qur'an *tadabbur*, it is hoped that it will be able to open mind and heart to be more positive in processing anxiety.

Factors that influence labor include the passage, passenger, power, maternal psychology, and birth attendance. Pregnancy is a crisis involving deep psychological factors, which occur because of a very large somatic change.[21,22] The findings were similar to Prapto et al.[12] on the Al-Qur'an *tadabbur* therapy to reduce anxiety on the first delivery. The group who followed the Al-Qur'an *tadabbur* therapy has a lower anxiety score, p=0.032 (p<0.05), compared with the control group. This study shows that Al-Qur'an *tadabbur* therapy can be used as one of the ways to reduce anxiety before delivery.[13,23]

According to Mulyadi et al.[24] that Islamic psychotherapy is the capability of reducing anxiety. This research also shows that the Al-Qur'an *tadabbur* therapy improves mental health by reducing anxiety before the delivery process. This research stated that religion-based therapy (mainly Islamic) could also reduce anxiety and improve mental health. The finding is consistent with Zakaria et al.[25] that the contents of the Al-

Qur'an become a source of principles for humans as the way of life in the world and the hereafter.

Fifteen respondents who received the Al-Qur'an *tadabbur* and 15 respondents who received the health education leaflet felt comfort and reducing anxiety before the delivery process. The Al-Qur'an *tadabbur* is beneficial in the early third trimester of the labor process to prevent unnecessary interventions.

Conclusion

Al-Qur'an *tadabbur* therapy was effective in reducing the anxiety level of nulliparous women during labor.

Conflict of Interest

All authors state there is no conflict of interest in this article.

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RESEARCH ARTICLE

Android-based Stunting Child Nutrition Application (GiAS) to Assess Macro-nutrients, Zinc, and Calcium in Stunting and Non-stunting Under Two Children

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Abstract

Stunted children will have normal cognitive ability if nutrition is improved. The rapid brain growth in the first 1,000 days of life means that children should not be malnourished. Stunting is generally caused by a lack of macronutrients (carbohydrates, protein, and fat) and micronutrients (calcium and zinc). The mobile application called stunting child nutrition (GiAS) has features that can detect stunting, monitor toddler growth, recommend daily menus for toddlers, nutritional adequacy rate (RDA) in 2019, and others. The purpose of this study was to make it easier to distinguish macronutrients, zinc, and calcium from stunting and non-stunting children aged 12–24 months using the GiAS android application. It is conducted at the Citeureup Public Health Center, Cimahi city, for June–July 2020. The sampling technique was a simple random sampling of 88 respondents. This type of research is an observational analytic with a statistical test is a cross-sectional design. The results of the study using the Mann-Whitney test showed differences in carbohydrates (84.99±26.31 vs 151.16±68.43, p=0.001), protein (30.81±11.03 vs 60.55±38.43, p=0.001), fat (32.80±15.39 vs 64.84±47.81, p=0.001), and calcium (0.55±0.40 vs 1.43±1.16, p=0.001) and there is similarity of zinc (0.005±0.004 vs 0.010±0.016, p=0.084) after 7 days of using the GiAS application between stunting and non-stunting children. The probability value <0.05 means that the application can compare macronutrients, zinc, and calcium between stunted and non-stunted children on the 7th day. Chi-square analysis showed an increase in children's weight and height under five at two weeks and one month (p=0.001). In conclusion, the comparison of macronutrients, zinc, calcium in stunting and non-stunting children aged 12–24 months can be differentiated using the GiAS application.

Key words: GiAS application, nutrient, stunting, toddlers

Aplikasi Gizi Anak Stunting (GiAS) berbasis Android untuk Menilai Zat Gizi Makro, *Zinc*, dan Kalsium pada Anak *Stunting* dan *Non-stunting*

Abstrak

Anak *stunting* akan memiliki kognitif yang normal jika dilakukan perbaikan gizi yang optimal. Pertumbuhan otak yang pesat di 1.000 hari pertama kehidupan menjadikan anak tidak boleh kekurangan nutrisi. Stunting umumnya kekurangan zat gizi makro (karbohidrat, protein, dan lemak) serta zat gizi mikro (kalsium dan *zinc*). Aplikasi mobile bernama gizi anak *stunting* (GiAS) memiliki fitur yang dapat mendeteksi *stunting*, memantau pertumbuhan balita, merekomendasikan menu harian untuk balita, angka kecukupan gizi (AKG) tahun 2019, dan lainnya. Tujuan penelitian ini adalah kemudahan membedakan zat gizi makro, *zinc*, dan kalsium anak *stunting* dengan *non-stunting* usia 12–24 bulan menggunakan aplikasi android GiAS di Puskesmas Citeureup Kota Cimahi periode Juni–Juli 2020. Teknik pengambilan sampel adalah simpel *random sampling* sebanyak 88 responden. Jenis penelitian ini adalah analitik observasional dengan uji statistik adalah desain *cross-sectional* (α=0,05). Hasil penelitian menggunakan Uji Mann-Whitney terdapat perbedaan karbohidrat (84,99±26,31 vs 151,16±68,43; p=0,001), protein (30,81±11,03 vs 60,55±38,43; p=0,001), lemak (32,80±15,39 vs 64,84±47,81; p=0,001), dan kalsium (0,55±0,40 vs 1,43±1,16; p=0,001) serta ada persamaan *zinc* (0,005±0,004 vs 0,010±0,016; p=0,084) sesudah 7 hari penggunaan aplikasi GiAS antara anak *stunting* dan *non-stunting*. Nilai probabilitas <0,05 berarti aplikasi dapat membandingkan zat gizi makro, *zinc*, dan kalsium antara anak *stunting* dan *non-stunting* pada hari ke-7. Analisis *chi-square* terlihat peningkatan berat badan dan tinggi badan balita pada 2 minggu dan 1 bulan (p=0,001). Simpulan, komparasi zat gizi makro, *zinc*, kalsium anak *stunting* dan *non-stunting* usia 12–24 bulan dapat dibedakan menggunakan aplikasi GiAS.

Kata kunci: Aplikasi GiAS, balita, *stunting*, zat gizi

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Introduction

Micronutrient deficiency contributes seriously to child morbidity and mortality. Fulfilling the needs of micronutrients or micronutrients can be obtained from food, fortified foods, and direct supplementation. The low food quality causes the deficiency of various vitamins and minerals in toddlerhood.[1]

Carbohydrates are the primary source of energy needed for activities, while excess carbohydrates in the body will be stored in fat as a reserve source of energy. Fat in the body is useful as a source of energy and dissolves vitamins so that it can be easily absorbed by the intestine.[2]

Protein is a macro substance that plays a vital role in the body's metabolic processes and enzyme function. Excess or lack of protein in the first 1,000 days will negatively affect the body, such as growth and maintenance of disturbed tissue, disturbed fluid balance regulation, reduced antibodies, and disturbed nutrient transport.[3]

Zinc is a micromineral that has a role in the enzyme part of the body's metabolism, growth hormone production, and as an antioxidant for immune function.[4]

Lack of calcium in children causes adaptation to bone formation controlled by growth hormone, thyroid, calcitonin, parathyroid hormone (PTH), sex hormones, calcium, phosphorus, vitamin A, and D.[5]

Stunting describes chronic undernutrition status during growth and development since early life.[6] Editing detection needs to be done, especially by parents in daily nutritional intake, especially in children who have received complementary foods. There are differences in macronutrients and micro-nutrient intake for stunted and non-stunted children, both in terms of the amount and diversity of food consumption.[7]

According to the World Health Organization (WHO), if breast milk is not sufficient to meet children's nutritional needs, complementary foods must be added to their diet. In general, breast milk alone does not meet the nutritional needs of children at the age of 6 months to 18–24 months because the supply of breast milk has decreased and must be supplemented with complementary foods and is a period that is prone to malnutrition.[8]

Consumption of food or nutrients that enter can be known by carrying out food recall or food memories to determine that macro and micronutrients are in the body. So far, recall food has only been carried out by health workers or nutritionists to determine a person's nutritional status by recording the type and amount of food consumed during the past 24 hours. The mother or caregiver is asked to describe everything she ate and drank during the past 24 hours. Usually, it starts from when the child woke up yesterday until the child went to sleep again.[9]

The development of digital technology is increasingly rapid. The GiAS application designed by researchers is an application or digitization of children's nutrition records. Parents can observe the past nutrition when their child having development problem, and it can also be used to prevent development problem because parents can recall food intake easier with the help of an android cellphone.[9,10]

Another advantage of the GiAS application is that it is easy to fill in children's anthropometric data. Streamlines the time that midwives, nutrition officers, cadres, and parents use to detect stunting and monitor children's growth. It also provides food ingredients that will increase children's growth in the GiAS application.[11,12]

The purpose of this study was to observe the application of nutrition for stunting children (GiAS) whether it is easier to distinguish macronutrients, zinc, and calcium from stunting and non-stunting children aged 12–24 months.

Methods

This research is an analytical study with a cross-sectional study design with two groups of stunting and non-stunting toddlers aged 12–24 months in the working area of the Citeureup Public Health Center, Cimahi city, from June to July 2020. Random sampling was carried out to obtain a total sample of 88 subjects, 45 stunted children and 43 non-stunting children. Subjects were parents and toddlers aged 12–24 months who met the inclusion criteria and stated their willingness to participate in the study after obtaining information by signing informed consent.

The instruments used to collect data measured children's weight and height and an android-based application of stunting child nutrition (GiAS). Both groups of children under five were measured for weight and height at the start of the study after two weeks and a month. GiAS application to determine macronutrients (carbohydrates, protein, and fat), zinc, and calcium from the recommended dietary allowance (RDA) in 2019

and determine stunted and non-stunted children seen from the graph of height growth per age, as well as food menu recommendations contained in the menu application according to the 2020 mother and child health (MCH) book draft.[13] The inclusion criteria are parents and toddlers aged 12–24 months. The exclusion criteria for children aged 12–24 months who were sick.

This research's ethical permission was obtained from the Health Ethics Committee of STIKes Dharma Husada Bandung Number 01/ KEPK/SDHB/B/VII/2020.

Results

Respondents were almost the same number of males with females (51% vs 49%), while more females were non-stunting (56% vs 44%. The mean mean age of non-stunting children with stunting (16.72 months vs 17.77 months, p=0.234). The weight gain of non-stunting children after 2 weeks was heavier than stunted children (260 g vs 50 g, p=0.001), as well as after one month (410 g vs 160 g, p=0.001). The height increase in stunting children was greater than that of non-stunting children after two weeks (2.69 cm vs 0.6 cm, p=0.001) and after one month (2.89 cm vs 0.84 cm, p=0.001). The increase in body weight of non-stunting and stunting children at two weeks (p=0.446 vs p=0.001) and at one month (p=0.001 vs p=0.001), the increase in height for non-stunting and stunting children after two weeks (p=0.001 vs p=0.001), and after one month (p=0.001 vs p=0.001).

Table 2 describes the results of normality tests and comparison of micronutrients, zinc, and calcium in stunted and non-stunted children using the GiAS application. The normality test shows that the data distribution is not normally distributed with a probability value below 0.05, so the comparison test uses the Mann-Whitney test.

Table 1 Stunting and Non-stunting Respondents Characteristics

Children Characteristic	Non-stunting		Stunting		p*
	n=43 (%)	Gained	n=45 (%)	Gained	
Gender					0.516
Male	19 (44)		23 (51)		
Female	24 (56)		22 (49)		
Aged (month) (mean±SD)	16.72±4.36		17.77±4.12		0.234
Weight (kg) (mean±SD)					0.001
Start	9.91±2.00		8.88±1.10		0.001
After 2 weeks	10.17±1.79	260 g, p*=0.446	8.93±1.27	50 g, p*=0.001	
After 1 months	10.32±1.80	410 g, p*=0.001	9.04±1.26	160 g, p*=0.001	
Height (cm) (mean±SD)					0.001
Start	78.02±5.3		71.56±6.45		
After 2 weeks	78.62±5.07	0.6 cm, p*=0.001	74.25±4.23	2.69 cm, p*=0.001	0.001
After 1 months	78.86±50.04	0.84 cm, p*=0.001	74.45±4.16	2.89 cm, p*=0.001	

Note: *chi-square test

Table 2 Normality Test of Macro Nutrients, Zinc, and Calcium in Stunting Children and Non-stunting Ages of 12–24 Months Using the GiAS Application

Variables	p Value Normality Test		Explanation
	Stunting	Non-stunting	
Carbohydrate	0.007	0.001	Not normal
Fat	0.000	0.001	Not normal
Protein	0.047	0.001	Not normal
Calcium	0.000	0.001	Not normal
Zinc	0.000	0.001	Not normal

Note: Mann-Whitney test

Table 3 Comparative Test of Macro Nutrients, Zinc, and Calcium After 2 Weeks of Stunted Children with Non-stunting Ages of 12–24 Months Using the GiAS Application

Nutrients (grams)	Stunting Mean±SD	Non-stunting Mean±SD	p Value
Carbohydrate	84.99±26.31	151.16±68.43	0.001
Fat	32.80±15.39	64.84±47.81	0.001
Protein	30.81±11.03	60.55±38.43	0.001
Calcium	0.55±0.40	1.43±1.16	0.001
Zinc	0.005±0.004	0.010±0.016	0.084

Note: Mann-Whitney test

Based on Table 3, it can be seen that the comparative test of research variables after two weeks in stunted and non-stunted children the mean of carbohydrate, fat, protein, calcium and zinc nutrients as follows: carbohydrate (84.99 g vs 151.16 g, p=0.001), as well as fat (32.80 g vs 64.84 g, p=0.001), protein (30.81 g vs 60.55 g, p=0.001), and calcium (0.55 mg vs 1.43 mg, p=0.001) and zinc (0.005 mg vs 0.01 mg, p=0.084).

Discussion

The respondents' characteristics in this study included the child's characteristics (gender, age of the child at the time of the study, initial body weight, two weeks and one month, and initial height, two weeks and one month). There was an increase in body weight and height in both groups after using the GiAS application. The application can detect macronutrients (protein, carbohydrates, and fats) and zinc, and calcium.

The analysis results showed a difference between the nutrition of stunting and non-stunting children using the GiAS application. The GiAS application is an application that can help parents, cadres, and health workers to quickly detect nutrition problems in toddlers—nutrients such as carbohydrates, protein, fat, zinc, and calcium. This application can also detect stunting or non-stunting toddlers by looking at the growth chart, one of the features in the GiAS application. According to their age, nutritional needs can be seen from the nutritional graph and adjusting the calories needed by toddlers. This application lists food ingredients adjusted to the type of nutrition and food ingredients that can be searched according to the Indonesian food composition table issued by the Ministry of Health Republic of Indonesia. This application also includes recommendations for food menus whose composition is almost the same as the 2020 MCH book draft, and there are other information needs regarding health.[14,15]

The GiAS application can compare macro-nutrients, zinc, and calcium, that the nutrients of stunting and non-stunting children are different with a probability value of $p<0.05$. There were differences in carbohydrate, protein, fat, and calcium nutrients between non-stunting and stunting children after two weeks, except for zinc.

The results showed that the carbohydrate needs of stunted and non-stunted children had a significant difference in that the carbohydrate needs of children aged 12–24 months were 215 grams/day according to the 2019 RDA. while 84.99 grams of stunting children only half the RDA.[14,15]

Research according to Ayuningtyas et al.,[16] there is a relationship between energy intake and the incidence of stunting in children under five. It is found that less energy intake primarily affects the incidence of stunting.

Astutik et al.[17] stated a significant relationship between energy intake and stunting because almost 50% of energy needs are adequately met.

Carbohydrates in the human body are useful as the primary source of energy needed for activities. In contrast, excess carbohydrates in the body will be stored in fat as a reserve source of energy. When the body lacks energy intake, the body will change these fat reserves. This will affect a person's nutritional status.[3,17,18]

Fat nutrition for stunted children and non-stunted children was significantly different. Fat for stunted children is 32.80 grams, and non-stunting is 64.84 grams. Fat for non-stunting children exceed the recommended fat nutrients

for the 2019 RDA, which is 45 grams/day while stunting needs fat is still less than the RDA.[14] Fat in the body is useful as a source of energy and dissolves the carrying vitamins A, D, E, and K so that the intestines can easily absorb it.[2] Lack of fat in the body will reduce energy availability and lead to catabolism (overhaul) of protein, while a lack of fatty acids will interfere with growth.[3] The highest sources of fat are oil, butter, margarine, and others. It is better if providing food for children, especially in the first 1,000 days of life, is accompanied by a source of fat.[15] Ayuningtyas et al.[16] stated an association of less fat intake with the incidence of stunting. It is inversely proportional to the research of Isnainy et al.,[18] the average daily fat needs of stunted children are well fulfilled or following the 2013 RDA.

The results showed that the children's nutritional intake for seven days, on average, only a few stunted children ate daily fats such as margarine, butter, coconut milk, cooking oil, and others. In contrast, non-stunting children eat more foods that contain lots of high fat, such as fried and fast food (sausages, nuggets, and others). Only a few eat unsaturated fats such as soybeans and fish. Following the research of Isnainy et al.,[18] the consumption of unsaturated fats, especially polyunsaturated fatty acids (PUFA), plays an essential role in fat transport and metabolism, immune function, and maintains the function and integrity of cell membranes. One of the consequences of less fat consumption is a lack of vitamin A because vitamin A is a vitamin that helps absorb carotenoids. It also functions for immunity, epithelial cell integrity, growth and development, vision, and reproduction.

Based on the comparative test of protein nutrition variables in stunted children 30.80 grams and non-stunted children 60.55 grams, both have a significant difference. However, if seen from the 2019 RDA, the protein needed for toddlers aged 12–24 months is 20 grams per day, the daily protein needs of both stunting and non-stunting children have met the RDA standard.

Following the research of Ayuningtyas et al.,[16] there is a significant relationship between the level of protein intake and the incidence of stunting. In contrast to Aryati et al.[4] the average protein intake from 2×24 hours recalls food processing was 23.38 grams. This figure is still below the RDA, the RDA for 2013, the daily protein intake for under-five children is 26 grams when viewed from the RDA for 2019, the protein intake is sufficient because the protein requirement for the 2019 RDA is 20 grams/day.

The study of daily nutritional intake at 12–24 months of stunting and non-stunting children consumed an excellent average of protein by including vegetable protein and animal protein. The average of parents of stunted children after using the application included animal protein every time the child ate to meet the needs of essential amino acids.

Based on the research results on calcium nutrition, there is a significant difference between stunting and non-stunting children, with the mean value of stunted children with 0.55 grams and non-stunting children 1.43 grams. In contrast, the normal value of calcium nutrition according to the 2019 RDA is 650 mg or 0,65 grams, which means that non-stunted children have average calcium exceeding the child's daily calcium needs, while for stunting it is still insufficient, but almost fulfills their daily needs because children aged 12–24 months on average are still breastfeeding or receiving formula milk.

In contrast to the study, Kurniasari et al.[5] stated that most of the calcium levels in the subjects were categorized as high, and there was no significant difference in serum calcium levels between stunting and non-stunting children. Febria et al.[19] stated that there was no significant relationship between calcium levels in breast milk, breastmilk substitute, and complementary foods with the incidence of stunting. According to Astutik et al.,[17] there is no significant difference between stunting and non-stunting calcium intake. Calcium is not associated with the incidence of stunting.

Based on the results of the analysis of the comparative test of zinc nutrition, the average stunted children were 0.005 grams, and 0.010 grams of non-stunted children had shown zinc adequacy because their needs were 3 mg/day or 0.003 grams/day. In contrast to the research of Aryati et al.[4] the average zinc intake was 2.89 mg, and this figure is still far from the RDA in 2013, which was 4 mg.

Zinc is a micromineral that has a role in the enzyme part of the body's metabolism. Zinc also functions in the production of growth hormone and as an antioxidant needed for immune function. Zinc deficiency is associated with suboptimal growth, diarrheal disease, and decreased immune function.[4] Chronic zinc deficiency can interfere with the central nervous

system and brain function, interfere with vitamin A metabolism, thyroid gland function, and metabolic rate, can also interfere with appetite, decrease a sense of taste, and slows wound healing.[20] Food sources high in zinc nutrients are soybeans, red beans, palm sugar, fish, etc.

This study is not following the study by Astutik et al.,[17] which states a relationship between zinc intake and stunting in children under five. Less zinc intake for children under five had a 4.241 times risk of stunting compared to adequate zinc intake for children under five. Leo et al.[21] stated that the analysis results showed that a low zinc adequacy level was a risk factor for the incidence of stunting in mountain areas with a risk of being stunted 2.827 times.

The GiAS application is following the Purbaningtyas[22] research states that the application is a mobile-based application that can help independent early detection of the nutritional status of each individual and provide recommendations for daily nutritional intake based on the user's daily calorie needs. The nutritional intake recommendations refer to the standard RDA set by the Ministry of Health of the Republic of Indonesia.[14,15] Afiana and Yuanita[23] stated that their application is called GIZIe to monitor anthropometry and nutritional status of children aged 0–60 months. Application design focuses on developing health nutrition studies by monitoring the digital mobile nutritional status development using anthropometry. It also provides advice following the development of infant's and toddlers' nutritional status and age. The application follows the GiAS application but differs from the RDA and the graph of macronutrients, zinc, and calcium.

Rahmawati et al.[24] stated that the toddler nutrition monitoring application could help posyandu officers and toddlers' parents monitor the development of toddler nutrition. Officers enter toddler data from the server application, and parents can monitor the development of toddlers in the form of data and graphics. The information displayed is in the form of the nutritional status of toddlers, whether it is more, less, or significantly less. There is a slight difference in the research, namely in the GiAS application of parents or cadres that enter food memories and the weight and height of children under five.

Hendra and Rahmad[25] stated that in the study of the nutritional status monitoring application (PSG) for toddlers, it positively impacted and significantly affected the quality and nutrition, especially the nutritional status data for toddlers. The value of the application of the android-based PSG application for toddlers has a better quality value than the conventional use of PSG books.[26] Also, using an electronic-based system (PSG application for toddlers based on android) is of better quality. Data analysis is more for planning, monitoring, and evaluating nutrition programs and supporting decision-making in dealing with nutritional problems.

Nutrients of carbohydrates, fat, protein, calcium, and zinc were known from filling the recalled food by mothers of stunting and non-stunting children filled in for seven days on application. For parents who do not have a quota or wifi, researchers provide pocketbooks to write children's daily recall food to help mothers not forget. The indicator of success can be seen from the increase in body weight and height in stunting and non-stunting toddlers. On average, there is an increase in body weight and height in both groups after being given the GiAS application at two weeks and one month.

The GiAS application shows menu recommendations that can help mothers or parents of stunting and non-stunting toddlers to prepare food menus for their toddlers so that parents can make food and their nutritional needs are met.

Conclusions

The GiAS application is an application with the latest 2019 RDA. There are menu recommendations with a composition that is following the 2020 MCH recommendation. The GiAS application makes it easy for parents to know how to compare macronutrients, zinc, and calcium in their children.

Conflict of Interest

All authors state that there is no conflict of interest in this study.

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RESEARCH ARTICLE

The Potential of Strawberry, Rome Beauty Apple, and New Combination on Fasting Blood as Supporting Diet Therapy in Patients with Type II Diabetes Mellitus

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Abstract

Strawberries (*Fragaria x ananassa*) and Rome beauty apples (*Malus sylvestris*) contain high antioxidants to reduce oxidative stress and improve the glycemic response with diabetes mellitus (DM) type II. This study aimed to analyze the effect of strawberries, Rome beauty apples, and their combination to decrease fasting blood glucose levels of type II DM patients. This experimental research used a randomized pre-post-test with a control group design. Subjects were 44 patients divided into four groups: K (control, not given the fruit), P1 (strawberries 200 g/day), P2 (apples 300 g/day), and P3 (the combination strawberries 100 g/day, apples 150 g/day) for 14 days in February–March 2019 in Surakarta. The inclusion criteria were DM patients aged 40–55 years who received oral antidiabetic drug therapy. Exclusion criteria were type II DM patients who, based on the doctor's diagnosis, had complications such as gastritis, hypertension, chronic renal failure, heart disease, liver disease, stroke, cancer, patients with consumption of antioxidant supplements. Mean of blood glucose level on K (149.82±39.125), P1 (141.00±38.079), P2 (128.73±31.841), and P3 (177.73±50.176). Analysis paired t test results showed a significant difference between before and after treatment (p=0.045). To find out which group was the most meaningful, the Bonferroni post hoc test. Decrease of fasting blood glucose levels with group K (–10.55, p>0.05), P1 (–30.18, p<0.05), P2 (40.27, p<0.05), and P3 (–32.91, p<0.05). ANOVA test was used to analyze, and the post hoc Bonferroni test with a control comparison was performed, resulting in p=0.028, in P2 and p>0.05 in P1 and P3. In conclusion, strawberries, Rome beauty apples, and their combination significantly decreased fasting blood glucose levels of type II DM patients, with the highest and most significant decrease in Rome beauty apples.

Key words: Apple, diabetes mellitus, diet therapy, strawberry

Potensi Stroberi, Apel *Rome Beauty*, dan Kombinasi Baru terhadap Gula Darah Puasa sebagai Penunjang Terapi Diet pada Pasien Diabetes Melitus Tipe II

Abstrak

Stroberi (*Fragaria x ananassa*) dan apel *Rome beauty* (*Malus sylvestris*) mengandung antioksidan tinggi untuk mengurangi stres oksidatif dan memperbaiki respons glikemik pada diabetes melitus (DM) tipe II. Tujuan penelitian adalah menganalisis pengaruh buah stroberi, apel *Rome beauty*, dan kombinasinya terhadap penurunan kadar glukosa darah puasa pasien DM tipe II. Jenis penelitian eksperimen dengan rancangan *randomized pre-post-test* dengan *control group design*. Subjek penelitian 44 pasien yang dibagi empat kelompok, yaitu K (kontrol tidak diberi buah), P1 (stroberi 200 g/hari), P2 (apel *Rome beauty* 300 g/hari) dan P3 (kombinasi stroberi 100 g/hari dan apel 150 g/hari) selama 14 hari pada bulan Februari–Maret 2020 di Surakarta. Kriteria inklusi adalah pasien DM usia 40–55 tahun yang mendapat terapi obat antidiabetik oral. Kriteria eksklusi adalah pasien DM tipe II yang berdasar atas diagnosis dokter mengalami komplikasi seperti mag, hipertensi, gagal ginjal kronik, penyakit jantung, penyakit hati, strok, kanker, dan pasien dengan konsumsi suplemen antioksidan. Kadar glukosa darah rerata pada K (149,82±39,125), P1 (141,00±38,079), P2 (128,73±31,841), dan P3 (177,73±50,176). Hasil analisis *paired t test* menunjukkan terdapat perbedaan yang signifikan antara sebelum dan sesudah perlakuan (p=0,045). Untuk mengetahui kelompok mana yang paling bermakna, dilakukan Uji *Post Hoc* Bonferroni. Penurunan kadar glukosa darah puasa pada K (–10,55; p>0,05), P1 (–30,18; p<0,05), P2 (–40,27; p<0,05), dan P3 (–32,91; p<0,05). Analisis dilakukan dengan uji ANOVA dan Uji *Post Hoc* Bonferroni dengan perbandingan kontrol menghasilkan p=0,028 pada P2 serta p>0,05 pada P1 dan P3. Simpulan, pemberian stroberi, apel *Rome beauty*, dan kombinasinya signifikan menurunkan kadar glukosa darah puasa pasien DM tipe II dengan penurunan tertinggi dan bermakna pada apel *Rome beauty*.

Kata kunci: Apel, diabetes melitus, stroberi, terapi diet

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Introduction

Diabetes mellitus defined as a syndrome of metabolic disease.[1] Metabolism of carbohydrates, proteins, and fats that are impaired in diabetes is caused by decreased insulin sensitivity, insulin secretion, or both, characterized by chronic hyperglycemia.[2] The number of people with diabetes mellitus in 2017 was 425 million. This number is expected to increase every year, and in 2045 it will reach 629 million.[3] Based on the report on *Riskesdas* 2018,[4] the prevalence of diabetes mellitus in Indonesia increased to 10.9% in 2018. This number increased by 4% compared to 2013. Prevention and management of diabetes mellitus are very important, that the necessary complementary alternative.[5]

Diabetes mellitus type II is caused by decreased insulin secretion or increase insulin resistance. It is related to reduced insulin secretion due to inflammation, metabolic stress, and genetic factors where β-cell dysfunction is the underlying factor.[6] Insulin resistance is caused by many factors, one of which is the oxidative stress factor. Oxidative stress is a condition of imbalance between free radicals and antioxidant systems, which causes decreased peripheral insulin sensitivity.[7] Reactive oxygen species (ROS) in the mitochondria result in increased endoplasmic reticulum stress and oxidative stress on β-cells that cannot respond to hyperglycemia. ROS also damages cellular components such as lipids, proteins, and DNA and triggers changes in transcription that increase insulin resistance.[8] Oxidative stress has an essential role in the development of DM type II. An external source of antioxidants can help address this oxidative stress. Therefore, the use of antioxidant agents is a promising new therapeutic strategy. Functional foods that have the effect of reducing blood glucose (hypoglycemic) levels are now considered as alternatives to drugs.[9] Routine intake of functional foods can improve sustained or chronic stress in diabetes mellitus through the antioxidant properties contained in functional foods.[10]

Strawberry antioxidants have hypoglycemic activity in diabetes mellitus.[11] Anthocyanins are part of the antioxidants in strawberries with very high levels, which affect lowering blood glucose levels, reducing blood vessel inflammation, and endothelial dysfunction.[12] Quercetin is a flavonoid found in fruits such as apples which functions as an anti-diabetic. This powerful antioxidant can prevent oxidative stress and delay the development of complications of diabetes mellitus.[13] This study was conducted to analyze the effect of administering strawberries, Rome beauty apples, and their combination to decrease patients' fasting blood glucose levels with diabetes mellitus type II.

Methods

This experimental study used a pretest-posttest control-group design. The subjects were diabetes mellitus type II patients at the public health centers in Kratonan, Ngoresan, Sangkrah, and Banyuanyar in the area of Surakarta city from February to March 2020. The inclusion criteria were patients with diabetes mellitus type II, male and female, aged 40–55 years who took only oral antidiabetic drugs, consumed fresh fruit, and communicated well. The subjects were measured for their body weight and height to determine their body mass index (BMI), a statistical index that describes nutritional status. The formula for calculating BMI is the weight (kg) divided by height (m^2). BMI is classified as underweight (under 18.5 kg/m^2), normal (greater than or equal to 18.5 to less than 24.9 kg/m^2), overweight (greater than or equal to 25.0 to less than 27.0 kg/m^2), and obesity (greater than 27.0 kg/m^2).[14] BMI is used as a weight correction factor in calculating energy requirements. Then, the subjects were given education about the benefits of physical activity and a therapeutic diet using the diabetes mellitus leaflet and a list of food exchanges. The subjects who met the inclusion criteria at four health centers were randomized, which resulted in 44 people. Respondents were divided into four groups, group K (control group, not given the fruit), group P1 (given strawberries 200 g/day, obtained directly from farmers in Cemoro Kandang Strawberry Farm, Gondosuli village, Tawangmangu subdistrict, Karanganyar regency, Central Java province).

Group P2 (given Rome beauty apples 300 g/day) from farmers in Andonosari village, Pasuruan regency and group P3 (given the combination of strawberries 100 g/day and apples 150 g/day) for 14 days. The researcher explained the purpose and the procedure of the study before the subjects signed an informed consent form. The subjects fasted at least 8–12 hours before checking their fasting blood glucose levels. The

hexokinase method was used to measure fasting blood glucose levels on the first day before the intervention and the fifteenth day after the intervention. The examination was at the CITO Surakarta laboratory. It received accreditation from the National Accreditation Committee (KAN) LM-060-IDN using the INDIKO tool. The fruits were delivered to the respondents' houses every day for two times snacks, in the morning and afternoon for 14 days.

The SPSS version 22 program was used to process and analyze the data. The descriptive analysis of research variables was performed with frequency distribution tables and percentages, including gender, age, education, and BMI. The analysis of mean differences before and after the intervention was conducted using Paired t test. The analyses of differences in the effect of administering strawberries, Rome beauty apples, and their combinations were carried out using the one-way ANOVA test because the data were normally distributed. There was a significant difference ($p<0.05$) between the groups, so the posthoc test was performed to analyze the most different interventions in the strawberry, Rome apple, and combination groups with a confidence level of 95%. The Health Research Ethics Committee of Dr. Moewardi Regional General Hospital Surakarta has approved this research, with the ethical clearance number: 087/I/HREC/2020.

Table 1 Characteristics of Subjects

Characteristics	n=44 (%)
Gender	
Female	10 (23)
Male	34 (77)
Age (years)	
40–45	9 (21)
46–50	11 (25)
51–55	24 (54)
Education	
Elementary school	16 (36)
Junior high school	10 (23)
Senior high school	16 (36)
University	2 (5)
Profession	
Housewife	22 (50)
Private employee	11 (25)
Entrepreneur	11 (25)
Body mass index	
Normal	24 (54)
Overweight	6 (14)
Obesity	14 (32)

Results

Statistically, the distribution of research subjects between the control group, strawberries, Rome beauty apples, and the combination based on the characteristics of gender, age, education, occupation, nutritional status showed no difference ($p>0.05$). Table 1 showed the female patients dominate the distribution of research subjects (34/77%). The subjects' age range in this study was 51–55 years (54%). The education was an elementary school and high school respectively(36%). Half of the subjects were a housewife. The average BMI scores in the control group were 25.28 kg/m^2, the strawberry group 24.65 kg/m^2, the Rome beauty apple group 25.50

Table 2 Comparison of Average Blood Glucose Levels Between Groups Before and After Intervention

Groups	Duration		Decrease Fasting Blood Glucose (Mean±SD) mg/dL	p[a]
	On Day-0 (Mean±SD) mg/dL	On Day-15 (Mean±SD) mg/dL		
K	160.36±46.807	149.82±39.125	10.55±16.263	0.057
P1	172.09±28.648	141.00±38.079	30.18±29.253	0.004*
P2	170.73±41.478	128.73±31.841	40.27±23.018	<0.001*
P3	209.64±61.112	177.73±50.176	32.91±22.488	0.001*
p[b]	0.080	0.045*	0.030*	

Note: *significant differences ($p<0.05$), K=control, P1=strawberries, P2=Rome beauty apples, P3=combination of strawberries and apples, a=paired t test, b=one-way ANOVA

Table 3 Comparison of Mean Difference of Fasting Blood Glucose Levels Based on Bonferroni Post Hoc Test

Groups		Mean Difference	p
K	P1	19.636	0.325
K	P2	29.727	0.028*
K	P3	22.364	0.176
P1	P2	10.091	1.000
P1	P3	2.727	1.000
P2	P3	7.364	1.000

Note: *significant differences (p<0.05), K=control, P1=strawberries, P2=Rome beauty apples, P3=combination of strawberries and apples, a=paired t test, b=one-way ANOVA

kg/m², and 25.36 kg/m². Most of the total study subjects had 54% normal nutritional status.

The results of the paired t test based on Table 2 showed that the groups administered with strawberries, Rome beauty apples, and their combination experienced a significant decrease in fasting blood sugar levels (p<0.05) after the intervention, but the control group experienced an insignificant decrease (p=0.057). A one-way ANOVA test was conducted to determine the presence or absence of significant differences between treatment groups. Table 2 showed that based on the one-way ANOVA test, there were no significant differences in the fasting blood glucose levels between the four groups at the pretest (p=0.080). Meanwhile, at the posttest, there were significant differences in each group (p=0.045).

Based on Table 3, it is known that the Bonferroni post hoc test results showed a significant decrease in fasting blood glucose levels between the control group and the Rome beauty apple group (p=0.028). On the one hand, the strawberry and the combination groups did not show significantly different results with p=0.325 and p=0.176, respectively. Based on the Bonferroni posthoc test results compared to the combination group, the strawberry group and the Rome beauty apple group obtained the same result (p=1.000).

Discussion

The results of the paired t test showed that the average decrease in blood glucose levels in Q patients with DM who were given 200 grams of strawberries per day for 14 days was 30.18 mg/dL (Table 2). It is caused by anthocyanin content in strawberries which can reduce fasting blood glucose levels.[12] Strawberries contain anthocyanins 171.4 ppm wb/100 grams.[14] Anthocyanins control carbohydrate metabolism in the body by increasing PPARγ activation in the skeletal muscle and tissue, translocation, expression of glucose transporter 4 (GLUT4), and the secretion of adiponectin and leptin. Anthocyanins activate AMP-activated protein kinase (AMPK), which stimulates glucose uptake and insulin secretion by pancreatic β-cells and decreases RBP4 to increase insulin sensitivity. Anthocyanins also have the effect of inhibiting intestinal α-glucosidase, pancreatic α-amylase which slows glucose absorption after meals, thereby preventing postprandial hyperglycemia and increasing insulin secretion.[15]

The results of this study are similar to those of Putri et al.'s[12] study, which states that the administration of strawberry juice with a dose of 3.6 mL/200 gBW/day (made from 274 g of strawberries) for 14 days decreases fasting blood glucose levels of rats with DM type II (mean=163.82 mg/dL, p<0.05).[15] Administering a strawberry powder drink of 20 g/day (made from 330 g of strawberries) in obese subjects with insulin-resistant abdomen for 14 days did not significantly decrease blood glucose levels (mean=35 mg/dL, p>0.05). Increasing the dose to 40 g/day (440 g of strawberries) had a significant result (p<0.05) with an average decrease of 84 mg/dL.[16]

The average decrease in fasting blood glucose levels of patients with DM who were given 300 g of Rome beauty apples per day in this study was 40.27 mg/dL (Table 3). Apple Rome beauty contains quercetin 4,189/100 mg/kg.[17] This is caused by the content of quercetin in apples which has the effect of lowering blood glucose levels. Quercetin reduces blood sugar levels by inducing PPARγ expression, reducing intestinal glucose absorption, increasing glucose uptake in organs and tissues as well as insulin resistance, and reducing oxidative stress.[13] Quercetin has anti-hyperglycemic activity in diabetes.[18,19] Apple consumption has beneficial health effects, one of which is to manage diabetes mellitus type II. Polyphenols in apples have antioxidant activity and inhibit the activity of α-glucosidase.[20] Quercetin reduces oxidative stress by increasing

antioxidants, decreasing malondialdehyde and nitric oxide levels, and activating gene expression related to the PI3K/PKB signaling pathway, which modulates glucose metabolism resulting in a decrease in fasting blood sugar levels.[21]

The results of this study are in line with the study in the chronic disease management program (*program pengelolaan penyakit* kronis, *Prolanis*) group at the Genuk Public Health Center in Semarang with the intervention of *manalagi* apples for 14 days. They found that there were decreased fasting blood sugar levels in patients with diabetes mellitus type II who were given 200 g, 300 g, and 400 g of *manalagi* apples per day with a higher average decrease of 98.83 mg/dL, 124.33 mg/dL, and 150.16 mg/dL, respectively. The higher the dose of fruit is given, the higher its effect on decreasing fasting blood glucose levels will be.[22] Another similar study in hyperglycemic rats. The study found that giving *manalagi* apples to hyperglycemia rats at a dose of 20 g/200 gBW for 28 days could decrease their fasting blood glucose levels with an average decrease of 130.87 mg/dL.[23]

Giving a combination of 100 grams of strawberries and 150 grams of Rome beauty apples per day for 14 days in this study could significantly decrease fasting blood glucose levels of diabetes mellitus type II patients with $p<0.05$ and an average decrease of 32.91 mg/dL. The results of the Bonferroni post hoc test showed that the differences in the average fasting blood glucose levels between the P1 & P3 and P2 & P3 groups were not significantly different. It means that the P3 dose had the same effectiveness as P1 and P2 doses in reducing fasting blood glucose levels in patients with diabetes mellitus type II.

The decrease in fasting blood glucose levels in P3 is lower than P2. It is possible because the strawberries and Rome beauty apples are served in the form of fresh fruits so that the combination of fruit cannot be mixed evenly. As a result, the interaction of the phenolic compounds of the two fruits that produce a more hypoglycemic effect height cannot take place to the maximum. Besides, the composition of strawberries and apples is 50% of the P1 dose and 50% of the P2 dose. In comparison, the P3 dose was with a combination of 40% of strawberries and 60% of Rome beauty apples. The proportion of fruit combinations may not produce a more significant hypoglycemic effect than the effect of fruit without a combination (P1 and P2). The result showed a combination of juice with 20% of strawberries and 80% of apples had the highest hypoglycemia effect compared to other proportions, which are 30/70, 40/60, and 50/50.[24]

Phenols in apples contribute more to the α-amylase and α-glucosidase inhibitory activity in a combination of blueberries and apples. Fruit combination processing to juice has the potential to produce a more optimal interaction of phenolic compounds. It can release phenols bound into the water while maintaining antioxidant activity in the fruit to produce a higher hypoglycemic effect. The phenolic composition and pH influence the hypoglycemic effect of the fruit combination, and enzymes contained in the proportion of fruit combinations.[24]

Conclusions

Provision of strawberries and Rome beauty apples and their combination significantly decrease fasting blood glucose levels of patients with diabetes mellitus type II. Giving Rome beauty apples had a significant effect on reducing fasting blood glucose and LDL-C levels.

Conflict of Interest

There is no conflict of interest in this study.

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RESEARCH ARTICLE

The Source of Stress of Students During Pandemic COVID-19: a Qualitative Study

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Abstract

The COVID-19 pandemic can lead to students' mental health problems, such as anxiety, depression, and stress. The government's policy for study from home exacerbates mental health problems. This study aimed to determine the source of student stress during the study from home during the COVID-19 pandemic. The study design used qualitative research. The sampling technique uses consecutive sampling. This research involved 36 students from the faculty of psychology and the faculty of medicine in Bandung city who underwent study from home (SFH). Data were collected by interviewing participants in May 2020. This study's results are the dominant thing that participants feel during the study from home is boredom, relaxed, and complicated; the things they missed during learning at home were friends, playing, and chatting; and they feel fear, sadness, and anxiety when heard the news about COVID-19. This study concludes that the source of student stress was being far from friends, limited communication and face-to-face contact with friends and lecturers, and did not get direct attention from friends or lecturers.

Key words: COVID-19 pandemic, mental health, source of stress, students, study from home

Sumber Stres Mahasiswa Selama Masa Pandemik COVID-19: Penelitian Kualitatif

Abstrak

Pandemik COVID-19 dapat mengakibatkan masalah kesehatan mental untuk mahasiswa seperti kecemasan, depresi, dan stres. Kebijakan pemerintah untuk melakukan pendidikan jarak jauh (PJJ) telah memperburuk masalah kesehatan mental. Tujuan dari penelitian ini adalah untuk menentukan sumber stres mahasiswa selama menjalani PJJ di masa pandemik COVID-19. Desain penelitian ini adalah penelitian kualitatif. Metode pengambilan sampel adalah *consecutive sampling* dengan melibatkan 36 mahasiswa dari fakultas psikologi dan fakultas kedokteran di Kota Bandung yang menjalani PJJ. Data diambil dengan melakukan wawancara kepada partisipan pada bulan Mei 2020. Hasil dari penelitian ini adalah perasaan dominan yang dirasakan partisipan selama PJJ adalah bosan, santai dan tidak praktis; hal yang dirindukan selama masa kuliah di rumah adalah teman, bermain dan mengobrol; dan hal yang terlintas ketika mendengar berita tentang COVID-19 adalah takut, sedih dan cemas. Kesimpulan dari penelitian ini adalah sumber stres mahasiswa adalah merasa jauh dengan teman, komunikasi dan kontak tatap muka dengan teman dan dosen yang terbatas serta tidak mendapatkan perhatian langsung dari teman dan dosen.

Kata kunci: Kesehatan mental, mahasiswa, pandemik COVID-19, pendidikan jarak jauh, sumber stres

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Introduction

In early 2020, COVID-19, originating from China, began to spread throughout the world.[1] This disease is very easily transmitted through the air and can cause death. In a short time, COVID-19 increased the number of confirmed cases and deaths worldwide.[2] COVID-19 first entered Indonesia around March 2020. Based on the worldometer on April 11, 2021, Indonesia ranks fourth in Asia with the number of COVID-19 cases reaching 1,566,996 cases with a death toll of 154 cases per 1 million population.[3]

The increase in confirmed cases of COVID-19 and death rapidly has resulted in the emergence of psychological problems, such as anxiety, depression, and stress.[2] Government policies to limit mobility and community social activities through the work from home (WFH) and school from home (SFH) programs worsen people's mental health.[4] One of the groups that are prone to experiencing psychological problems is students. The student world is a transitional period from adolescence to adulthood. At this time, students have not yet experienced emotional maturity.[5–7]

An online survey of students in Guangzhou, China, showed that 7.5% of students showed anxiety symptoms and 12.2% experienced symptoms of depression. Besides, students who have family members infected with the coronavirus, both confirmed and suspected, are more at risk of experiencing symptoms of depression.[1] The results of an online survey of students in Bangladesh showed that 26.6% of students experienced mild to very severe anxiety symptoms, 61.97% of students experienced symptoms of depression, and around 57.05% of students experienced mild to very extreme stress levels. Negative perceptions of pandemics, disruption of education, inadequate health service systems, and existing physical health conditions causing mental health problems to students.[8]

Researchers developed a game-based learning program that aims to reduce anxiety in students during the corona pandemic. At the beginning of the program, the researchers conducted an initial mapping to determine the source of participant stress. The purpose of this study was to determine the source of participant stress during the pandemic. This study's results have been presented in the 2020 Social and Humanities Research Symposium (SoRES) activities.

Methods

The design of this study is qualitative research conducted at the Universitas Islam Bandung (Unisba). The research sample used consecutive sampling involving 36 students from the Faculty of Psychology and the Faculty of Medicine Unisba who underwent study from home (SFH), each from the Faculty of Psychology and the Faculty of Medicine totaling 18 people. Participants come from students in semesters 2, 4, 6, and 8.

Data were collected by interviewing participants in May 2020. Researchers asked three questions to explore the sources of stress for respondents, consisting of: (1) what did participants feel during the study from home? (2) what is the most missed (feel lost) during the study from home? (3) what comes to mind when participants heard about COVID-19?

Results

We carried out a qualitative mapping of the sources of stress. Participants answer three questions about what participants felt during the study from home, things they missed during college, and what comes to mind when they heard about COVID-19. The first question is what the participants felt during the study from home. The dominant thing that participants feel during the study from home is boredom, relaxed, and complicated. For the second question, the things they missed during learning at home were friends, playing, and chatting. For the third question, they feel fear, sadness, and anxiety when they heard the news about COVID-19. The results of qualitative mapping can be seen in Figure.

Discussion

Figure shows the initial mapping results to determine the source of the participants' stress. This figure shows that three things that respondents felt during the study from home were boredom, relaxed and complicated. The things they missed during their study from home were friends, playing, and chatting. What comes to mind when hearing news about COVID-19 is fear, sadness, and anxiety.

The dominant thing that participants feel during the study from home is boredom, relaxed, and complicated. Bored shows the initial stage of saturation, which will have an impact on

Figure Qualitative Mapping of the Source of Stress

decreasing motivation to learn. The condition of being far from friends, limited communication, and face-to-face contact were situations that unusual for the participants to experience. They need time to adjust to the problem. Therefore they feel bored, sleepy (don't have the energy), lazy to move, and feel complicated using technological media. This condition is not in line with the character of their age, which should quickly adapt to technology. It turns out that the social support factor remains essential in the student learning process.

Students who carry out learning activities from home cause students too much using smartphones and access social media. Too much information flow from social media can cause (mis)information overload, leading to mental health problems. Based on the results of a cross-sectional study of Chinese residents over the age of 18 during the Covid-19outbreak in Wuhan, the research shows that 48.3% of respondents experienced depression, 22.6% experienced anxiety, and around 19.4% experienced a combination of depression and anxiety. Of these respondents, more than 80% are exposed to social media.[9–11]

For the second question, the things they missed during learning at home were friends, playing, and chatting. That word shows their need to socialize. This condition is in line with feeling bored, lazy, and not excited (sleepy). It turns out that it is more due to the high need for socialization, so meeting online limited by space becomes an obstacle that makes its primary needs remain unfulfilled. The two data above show that most students on high affiliation motives. The study from home requires that every student must be independent and understand the subject matter visually. However, it is not easy for some students who have high affiliation motivation, and they depend on the attention of friends, lecturers, and people around them. Therefore, it is essential to carry out an activity that requires interaction between individuals that stimulates them to give each other attention, empathy, and work together to achieve specific goals.

WHO recommends supporting each other, helping others when others need help can benefit both those who provide help or those who get help. The concept of please help can create social support. Numerous studies have shown that social support is essential for maintaining physical and psychological health.[12,13] Besides, sharing feelings with other people, especially other people who also feel the same emotions at the same time, can reduce stress. Research says that sharing feelings in a threatening situation with someone in the same emotional state holds someone back from experiencing severe stress levels.[14]

For the third question, they feel fear, sadness, and anxiety when they heard the news about COVID-19. Expressions of fear, sadness, anxiety when hearing the information of COVID-19 is a natural expression for students. This expression happens not only among students but also for most people in general. This feeling does not seem too excessive they were still able to think objectively and control themselves in reacting naturally. However, we can saw the impact in their unusual learning behavior. This impact is also another source of stressors that make students have their problems.

Anxiety and other psychiatric problems can result in decreased immunity,[18,19] which can increase the risk of being infected with COVID-19.[12] Also, unresolved student anxiety problems can reduce academic performance and increase the likelihood of dropping out of university. Increasingly severe anxiety will worsen academic performance, and worse academic performance will exacerbate anxiety. Anxiety was also positively correlated with depression and increased suicidal thoughts in college students.[7,15–17]

Based on this, mental health intervention for students during the COVID-19 pandemic is urgently needed.[20] An online survey of students in Guangzhou, China, states that students need knowledge of mental health problems and interventions to overcome mental health problems during the COVID-19 pandemic.[1] Recognizing this, the WHO launched guidelines for handling mental health, and the National Health Commission of China launched online mental health services to reduce mental health problems during the COVID-19 pandemic.[2,12] The results of the meta-analysis study stated that e-mental health intervention could overcome mental health problems in students and also improve academic performance.[7]

Conclusions

This study concludes the source of student stress during the study from home in the COVID-19 pandemic was a social limitation that results in mental health problems. Therefore adequate mental health treatment is needed.

Conflict of Interest

The authors declare no conflict of interest.

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