Bali Medical Journal (*Bali MedJ*) 2023, Volume 12, Number 3: 3392-3398 P-ISSN.2089-1180, E-ISSN: 2302-2914



Characterization and evaluation of the hepatoprotective activity of α-mangostin isolate in diabetic rats



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ABSTRACT

Introduction: Isolation of α -mangostin (α -MG) from the rind extract of *Garcinia mangostana L*. showed antioxidant, anticancer, antidiabetic, and cytotoxic activities. The effectivity of a-mangosteen isolate compounds on hepatoprotective bioactivity in diabetic conditions has not been found. This study aimed to characterize α -MG isolate and evaluate its hepatoprotective activity in diabetic rats.

Methods: This research was a post-test-only control group design. This study used 36 rats which were divided into 6 groups, namely positive control group, negative control group, standard drug group, and treatment group with α-MG dose of 10 mg/kg body weight, 30 mg/kg body weight, and 50 mg/kg body weight. Hepatoprotective evaluation parameters examined included levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin and liver histopathology observations. Test the effect on the study using the One-Way ANOVA test.

Results: The results showed that a-mangosteen compound has a significant effect on levels of AST (p-value<0.05), ALT (p-value<0.05), ALP (p-value<0.05), and total bilirubin (p-value<0, 05), but there were no necrotic cells on the histopathological observation of the liver.

Conclusion: a-mangostin isolate from *Garcinia mangostana L*. has the potential as hepatoprotective in diabetic rats.

Keywords: *a-mangostin, hepatoprotective, AST, ALT, total bilirubin.* **Cite This Article:** Wulandari, D.D., Santoso, A.P.R., Suciati, S.S., Wulansari, D.D., Andini, A., Khanifah, F. 2023. Characterization and evaluation of the hepatoprotective activity of α-mangostin isolate in diabetic rats. *Bali Medical Journal* 12(3): 3392-3398. DOI: 10.15562/bmj.v12i3.4453

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Received: 2023-05-27 Accepted: 2023-10-28 Published: 2023-11-30

INTRODUCTION

Mangosteen (Garcinia mangostana L.) is a species of the genus Garcinia. This plant can grow in Southeast Asian countries, one of which is Indonesia. This plant is also widely cultivated in several provinces in China. Previous studies reported that mangosteen contains various bioactive compounds such as xanthone compounds, pigments, polysaccharides, and phenolic acids. Xanthones are the main active substances in mangosteen, where the isolated compound a-mangostin (a-MG; 1,3,6-trihydroxy-7-methoxy-2,8-bis(3methyl-2-butenyl)-9H-xanthan-9-one) is the most dominant compound is found in the skin and trunk of the mangosteen fruit tree (Garcinia mangostana L.).¹ These compounds are reported to have many pharmacological activities such as antibacterial, antifungal, antidiabetic, anticancer, and several other bioactivities.²

Diabetes mellitus (DM) is still a health problem in the world with a high prevalence that continues to increase, especially in developing and newly industrialized countries. This disease can attack almost all systems of the human body, starting from the skin to the heart that can cause complications due to irreversible pathological conditions such as retinopathy, neuropathy, nephropathy, hepatopathy, vasculopathy, and cardiovascular disease.3 Elevated levels of chronic transaminase enzymes describe the occurrence of insulin resistance. Elevated serum aminotransferase levels, such as those of the enzymes hepatic intracellular circulation (ALT) and aspartate aminotransferase (AST), show the presence of the enzyme. This is a main indicator of non-screening alcoholic steatohepatitis (NASH), which causes hepatocellular damage. Non-alcoholic fatty liver disease (NAFLD), often known

as fat buildup or steatosis, is another condition that can impair liver function. NALFD affects 40 to 70 percent of DM patients.⁴

Based on research (Bath, 2018) reported that the aqueous extract of Bixa orellana was able to show a significant reduction in enzyme levels in the liver in rats induced by diabetes so this extract could be indicated as having hepatoprotective properties. According to studies conducted by Fu et al., 2018, the isolated compound -MG can reduce levels of liver malondialdehyde (MDA), serum alanine aminotransferase (ALT), aspartate transaminase (AST), tumor necrosis factor (TNF-), interleukin-1 and 6 levels (IL-1, IL-6), and hepatic glutathione recovery (GSH), superoxide dismutase (SOD).⁵

So far, studies on the effect of α -MG isolate compounds on hepatoprotective bioactivity in diabetic conditions have not been found. This study aimed to

characterize the isolated compound α-MG using 13C and 1H NMR (Nuclear Magnetic Resonance) and to find out whether this compound has bioactivity as hepatoprotective in diabetic rat models with parameters measured are alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), bilirubin, and liver histopathology.

METHODS

Study Design

The 36 rats used in this true-experimental study were divided into 6 groups: a positive control group, a negative control group, a standard drug group, and a treatment group that received -MG doses of 10 mg/kg body weight, 30 mg/kg body weight, and 50 mg/kg body weight after a week of acclimatization (adaptation) prior to treatment. The rats were taught in exposure cages while they were becoming acclimated.

Compound Characterization of a-MG

Garcinia mangostana L. rind is washed under running water to remove dirt and other foreign matter. Then it is dried in the open air, and the skin of the dried fruit is mashed then weighed 1 kg, and extracted using hexane at room temperature. Pure compound alpha mangosteen was isolated from mangosteen rind using column chromatography using n-hexane extract. The isolation process was carried out by a fractionation process using a mixture of n-hexane and ethyl acetate eluent with a gradual increase in polarity gradient. The isolates obtained were characterized by UV-Vis and infrared to determine the functional groups. The pure solid obtained was also characterized by 1H-NMR and 13C-NMR. It takes as much as 7-10 mg and is dissolved in 0.5 ml of proton-free solvent (CD3OD) which can dissolve completely. The sample solution was put in an injection tube and then placed in a Jeol INM ECA 500 MHz NMR tool to measure 1H-NMR and 13C-NMR.

Measurement of Blood Biochemical Parameters AST, ALT, ALP, and Bilirubin

Thirty-six experimental animals that met the inclusion and exclusion criteria, were divided into 6 treatment groups with 6 mice in each group, namely: (1) Positive control group (diabetic): given a 20% fructose solution orally at a dose of 1.86 g/kg BW, (2) Negative control: given equates, (3) Treatment group I: given a-MG compound at a dose of 50 mg/kg BW, (4) Treatment group II: given the compound a-MG at a dose of 100 mg/kg BW, (5) Treatment group III was given a-MG compound at a dose of 150 mg/kg BW, (6) Standard drug group: given orally with glibenclamide at a dose of 10 mg/kg BW.

The experimental animals were then adapted for 14 days. During the acclimatization process, normal food and drink were given. Hyperglycemia occurred on day 55 after the induction of a high-fructose diet. After 56 days of administration of 20% fructose solution orally at a dose of 1.86 g/kg BW, the rats were fasted overnight and then the rat's blood glucose level was measured first, then the rat's blood glucose level was measured again. Mice with blood glucose levels of more than 150 mg/dl were used for research in the treatment and control groups of diabetics. Furthermore, the treatment group was given a-MG compound according to the dose of each group for 14 days. Fasting blood glucose levels were checked using a glucometer.⁶

The rat serum was prepared, then determined using the enzymatic method with a photometric device. Then 250 mL of AST/ALT mono reagent was added with 25 mL of serum, then incubated for 50 seconds. Then measured with a wavelength of 340 nm at 37°C. then read the results of the AST/ALP readings that appear on the monitor of the device.7 4-nitrophenol phosphate is hydrolvzed by alkaline phosphatase to produce phosphate and free 4-nitrophenol, which is colorless in aqueous acid solution. Under fundamental circumstances, 4-nitrophenol is changed into the highly concentrated yellow 4-nitrophenoxide ion. A recording spectrophotometer was then used to measure the rate of production of 4-nitrophenol at 405nm after the addition of alkaline phosphatase to 4-nitrophenol at 37°C.8

Purple azo bilirubin is created by adding plasma to a sodium acetate and caffeine solution (sodium benzoate), which is then combined with diazotized sulfanilic acid. Acetate of sodium is resistant to diazotized sulfanilic acid's pH. When ascorbic acid is added, the process is stopped because the extra diazo reagent is destroyed. After that, blue azobilirubin was created by adding a strong base tartrate solution, and a spectrophotometer was used to detect the color intensity at 600 nm.⁸

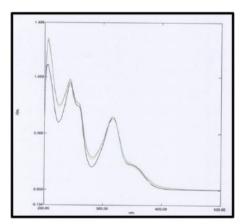
Data analysis

The output of this study was compiled, revised, input into a computer, coded, and cleaned. The collected data were entered using the Statistical Product for the Social Sciences (SPSS) data format version 20.0 (SPSS, Inc., Chicago, Illinois). The One-Way ANOVA test was used for analysis.

RESULTS

Characterization Results with UV-Vis

The UV spectrum of a compound with the x-axis is a wavelength (nm) and the y-axis is an absorbance (Figure 1) in methanol solvent shows the presence of 2 bands, namely band 1 shows a peak at a wavelength of 316 nm which indicates the presence of electron excitation $\pi \rightarrow \pi^*$, which is a typical chromophore for conjugated double bond systems (-C=CC=C-) or on the aromatic ring. The absorption in band 2 shows peaks at wavelengths of 204 and 243 nm which indicates the presence of conjugated heteroatoms with bonds (-C=CC=C). The



Information:

- : MeOH
- : MeOH+AlCl₃

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: MeOH+AlCl3+HCl
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Figure 1. The UV spectrum of the compound.

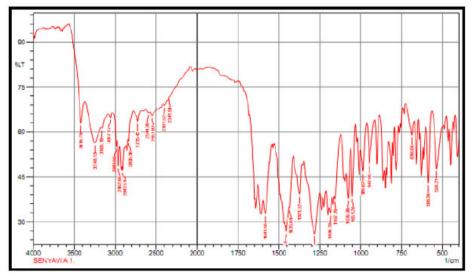


Figure 2. The IR spectrum of the compound.

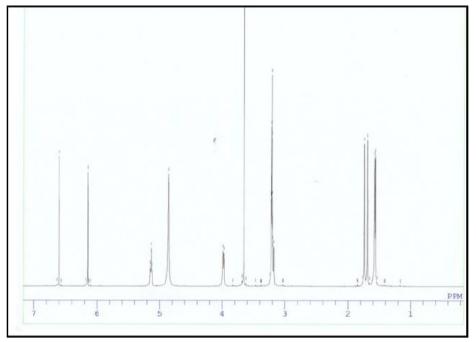


Figure 3. 1H-NMR spectrum data for compound.

addition of NaOH causes a bathochromic shift of 53 nm in band 1 from max 316 nm to 369 nm.

Characterization Results with Infra-Red

The IR spectrum of a compound with the x-axis as a wave number (cm-1) and the y-axis as a transmittance (Figure 2) shows typical absorptions for several functional groups, including the broad absorption at wave number (ν max) 3419.79 cm-1 indicating the presence of a hydroxy group which is amplified. with phenol CO absorption at wave number (ν max) 1280.73 cm-1. Furthermore, the carbonyl

group C=O is indicated by the presence of absorption in the wave number region (ν max) 1583.56 cm-1.⁹ Meanwhile, (ν max) 1454.33 cm-1 showed a typical absorption for double bonds in the aromatic system.¹⁰ The appearance of absorption in the wave number region (ν max) 2962.66 and 2912.51 cm-1 indicates the presence of C-H (aliphatic) originating from the methoxy or prenyl group.

Characterization Results with 1H NMR

Based on the 1H-NMR spectrum data for compounds with the x-axis as a chemical shift (ppm) and the y-axis as an intensity (Figure 3) it was shown that there were several signal groups consisting of 11 protons with different chemical environments. This spectrum shows 4 singlet signals at H 1.77; 1.75; 1.59 and 1.58 which indicated the presence of 4 methyl groups. The singlet signal at H 3.68 is a typical signal for the methoxy group. The doublet signal at H 3.23 and 3.90 indicates a signal from a methylene proton, while the multiplet signal at H 4.85 and 18 indicates a signal from a methine proton. The singlet signal at H 6.18 and 6.61 indicates the presence of a signal from isolated aromatic protons. From the 1H-NMR spectrum data for compound (1), information can be obtained that there are 2 prenyl groups, 1 methoxy group, 2 methylene groups, 2 methine groups, and 2 isolated aromatic protons.

Characterization Results with 13C NMR

Based on the 13C-NMR data with the x-axis as a chemical shift (ppm) and the y-axis as an intensity (Figure 4), it shows that there are 24 carbons, namely 183.3; 163.7; 161.7; 158.0; 156.8; 156.3; 144.9; 138.6; 131.9; 131.8; 125.2; 123.9; 112,3;111.5;103.8; 102.8; 93.2; 61.4; 49.3; 49.1; 48.9; 27.2; 26.0963 ppm, 22.3; 18.4; and 18.0. The spectrum at c 183.3 is carbon from carbonyl (C=O), absorption at c 131.9 and 131.8 indicates the presence of C=C in the prenyl group, at c 18.4 and 18.0 shows 2 methyl groups at the end of the prenyl group. From the data obtained from the 13C-NMR spectrum, it can be concluded that xanthone compounds are substituted by prenyl groups.

Characterization Results with 2D NMR Analysis of HMBC

To determine the position of the substituent, the 2D NMR analysis of HMBC was carried out as follows.

Analysis using 2D-HMBC NMR was carried out to strengthen the position of the carbon atom because it can explain the long-range correlation between protons and carbon as shown in Figure 5. From the above spectrum, it can be seen that the methylene proton at C-11 (3.23 ppm, m) correlates with C-12 (123.9 ppm), C-13 (131.8 ppm), and C-1 (161.7 ppm), and C-2 (111.5 ppm). It can be seen that 1 prenyl group is located at C-2. In addition,

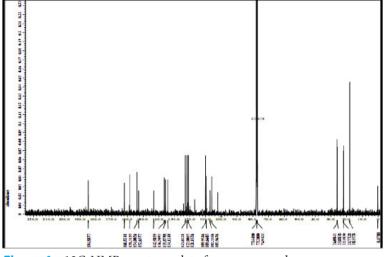


Figure 4. 13C-NMR spectrum data for a compound.

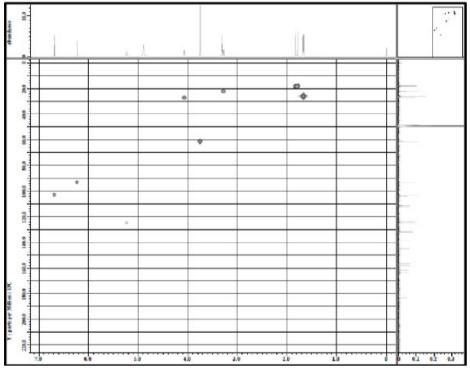


Figure 6. HMQC spectrum for the compound.

methylene protons at C-16 (3.39 ppm, m) correlated with C-9a (112.3 ppm), C-17 (125.2 ppm), C-18 (131.9 ppm), C -7 (144.9 ppm), and C-8 (138.5 ppm) which indicated that 1 other prenyl group was substituted at C-8. The methoxy proton at the proton shift of 3.38 is correlated with C-7 (144.9 ppm) so it can be suggested that at C-7 a methoxy group is substituted.

Characterization Results with 2D NMR Analysis of HMQC

Based on the HMQC analysis, it can be seen in Figure 6 the correlation between

protons and the carbon that binds them. From the HMQC data for compound (1), it can be seen that the carbon shift c C-4 102.9 has a correlation with aromatic protons at shift 6.28 ppm, and carbon shift c C-5 93.2 has a correlation with aromatic protons at shift 6.71 ppm. This indicates that there are 2 unsubstituted aromatic carbons, namely at C-4 and C-5.

Based on the analysis of UV, IR, 13C-NMR, HMBC, and HMQC data, it can be suggested that 2 prenyl groups are substituted at positions C-2 and C-8, 1 methoxy group at position C-7, 3 hydroxy

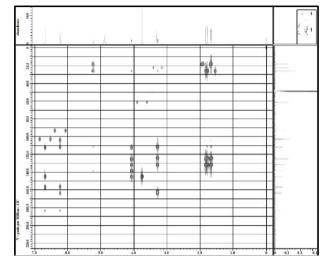


Figure 5. HMBC spectrum for the compound.

groups at position C-1, C-3, and C-6, and 2 aromatic protons at C-4 and C-5, so it can be suggested that compound (1) is α -MG compound.

Blood Chemical Analysis

In the positive control group, the negative control group, the standard glibenclamide drug group, and the α -MG treatment group at doses of 10, 30, and 50 mg which were stained with HE (Hematoxylin Eosin) showed no necrosis of hepatocytes. The following are the results of liver histopathology observations from each group which are presented in Table 2 below.

DISCUSSION

Previous research conducted by Fu, 2018 has identified the ability of a-MG compounds as hepatoprotectors in rats induced by lipopolysaccharide/Dgalactosamine in mice, but research on the ability of a-mangosteen compounds as hepatoprotectors in diabetic conditions has not been found.⁵ Research conducted by Wulandari, 2020 explains that a-MG compounds can reduce levels of malondialdehyde (MDA) which is a marker of lipid peroxidation due to diabetes conditions and this compound can repair cell necrosis in pancreatic tissue due to streptozotocin induction through antioxidant mechanisms.6 Diabetes can be a risk factor for the development and progression of liver disease. On the other hand, diabetes develops as a complication of cirrhosis known as "hepatogenic

diabetes".11

Excessive intake of fructose has been proven to enhance de novo lipogenesis. Conversion of fructose to glucose will increase the risk of type 2 diabetes. In addition, fructose can also contribute negatively to blood glucose homeostasis by causing insulin resistance. In human studies, fructose has been shown to cause insulin resistance. Insulin resistance is a condition in which cells fail to respond normally to insulin. Several studies have shown an increase in cases of insulin resistance with fructose consumption.

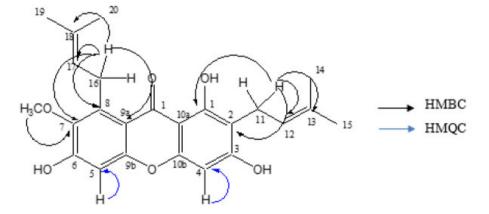


Figure 7. 2D NMR results of HMBC and HMQC compounds.

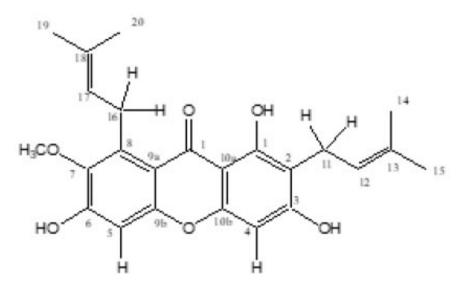


Figure 8. α-mangostin compound.

The administration of fructose in animal experiments induces insulin resistance and obesity caused by hyperinsulinemia.¹² Fructose can enter cells via GLUT5. membrane carrier enterocytes so that they are not dependent on sodium and do not require energy. Then most of the fructose will be absorbed through the intestines into the portal vein and the hepatocytes via GLUT2. In hepatocytes, fructose is rapidly converted by the enzymes fructokinase to fructose-1 phosphate and then converted to triose phosphate by the enzyme aldolase B. These two enzymes are not affected by both insulin and the energy status of the cells. An increase in the concentration of triose phosphate in hepatocytes will trigger a higher rate of hepatic ATP consumption high at the start of fructose phosphorylation, so that when high fructose intake triggers depletion ATP, AMP formation and adenosine degradation to uric acid. The accumulation of triose phosphate will be converted to lactate or glucose which will be released into the circulation (gluconeogenesis). Therefore, the accumulation of triose phosphate can stimulate the synthesis of glycogen and fatty acids from carbon dioxide on fructose through a metabolic pathway known as de novo lipogenesis. Process de novo lipogenesis will trigger the formation of adipose tissue in the abdomen which is an important component in the development of dyslipidemia, hyperglycemia, and hypertension.13

Liver function tests are used clinically in practice to detect liver failure, monitor the progress of liver disease, and monitor the potentially hepatotoxic effects of a substance. The most commonly used liver function parameters include ALT (alanine aminotransferase), AST (aspartate

Blood	Positive	Negative	Standard Drug		a-mangostin		_
Biochemical Parameter	Control	Control	Glibenclamide	10 mg/kg BB	30 mg/kg BB	50 mg/kg BB	p-value
SGOT/AST	244.27 ± 27.94	145.5 ± 22.86	186.5 ± 19.33	156.67 ± 16.45	183.33 ± 41.02	117.33 ± 25.7	<0.05*
SGPT/ALT	73.55 ± 10.48	67.33 ± 12.32	65.5 ± 14.93	58.54 ± 8.42	85.66 ± 6.04	72 ± 13.29	<0.05*
Bilirubin Total	4.77 ± 0.34	3.77 ± 0.77	2.735 ± 2.17	2.25 ± 0.76	1.90 ± 0.44	1.735 ± 0.51	< 0.05*
Alkaline Phosphatase	69.27 ± 6.15	41.5 ± 7.17	31.76 ± 10.73	29.78 ± 7.07	29.36 ± 4.48	27.08 ± 7.14	< 0.05*

*Analysis was carried out using the One-Way ANOVA test. Results were considered significant if the p-value≤0.05.

Table 2. Liver histopathology observations			
Animal Group	% Necrosis Cell	Scoring	Information
Positive control	0%	1	There was no necrosis
Negative control	0%	1	There was no necrosis
Standard drug	0%	1	There was no necrosis
Administration of α-MG dosage at 10 mg/kg BB	0%	1	There was no necrosis
Administration of α-MG dosage at 30 mg/kg BB	0%	1	There was no necrosis
Administration of α -MG dosage at 50 mg/kg BB	0%	1	There was no necrosis

Table 2. Liver histopathology observations

aminotransferase), alkaline phosphatase (ALP), and total bilirubin.¹⁴ Hepatocellular disease is indicated by a rise in ALT and AST that is out of proportion to ALP and bilirubin. On the other hand, a cholestatic pattern will appear if ALP and bilirubin levels increase disproportionately along with ALT and AST. AST and ALT are examples of aminotransferases. They serve as indicators of hepatocellular damage. By facilitating the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid to form oxaloacetic acid or pyruvic acid, respectively, they contribute to gluconeogenesis. Bilirubin, -glutamyl transpeptidase, and alkaline phosphatase are indicators of biliary function and cholestasis. In individuals with type-2 diabetes, elevated transaminases are frequently discovered.¹⁵

Proteins, lipids, and nucleic acids are among the cell components that experience damage from oxidative stress. The major hepatotoxicity mechanism of the toxicant is the generation of free radicals since the liver is constantly subjected to oxidative stress. The equilibrium between the production of reactive oxygen species and the levels of antioxidants is upset in oxidative stress. Because they can prevent and neutralize free radicals and have the potential to protect organs from hepatotoxins through their free radical scavenging activities, prevention of lipid peroxidation, and prevention of cell damage, -MG compounds are known as antioxidants with free radical scavenging activity. The antioxidants prevent reactive oxygen species (ROS) from damaging DNA, which is linked to carcinogenesis, coronary heart disease, and many other age-related health issues.¹⁶ The compound a-MG has been shown to reduce blood glucose levels, reduce malondialdehvde (MDA) levels, and can repair cell damage in pancreatic cells through its antioxidant mechanism.⁶ Antioxidants are substances

that when present in low concentration significantly delay or reduce the oxidation of the substrate.¹⁷

Observations made were microscopically on liver histopathological preparations of strain Rattus novergicus Wistarratsusing HE staining (Hematoxylin Eosin) in the negative control group, positive control group, standard drug, treatment with a-MG doses of 10, 30, 50 mg/kg BW, were found. There were no necrotic cells on the histopathological picture of the liver. Based on previous studies, administration of a diet with 25% sucrose (containing 50% fructose) can increase liver ALT and AST levels for 18 days. Fructose metabolism differs from glucose metabolism. Early fructose metabolism involves the phosphorylation of fructose to fructose-1-phosphate by fructokinase (keto hexokinase, KHK) utilizing the substrate ATP before entrance into the glycolytic pathway. Hepatic ATP depletion can occur as a result of fructokinase's increased activity in phosphorylating fructose to fructose-1-phosphate in the liver.¹⁸ However, another study showed that samples of Ossabaw pigs in which the pigs were fed a 6000 kcal/day moderate fructoserich diet (20% energy) with higher choline levels (1200 ppm) showed no difference between groups on histological observations or steatosis after 24 weeks. This shows that the administration of fructose, which is supported by dietary choline and the provision of foods with high-fat content, will produce changes in liver histopathology. This interpretation is supported by a recent study comparing a high-fructose diet alone and with a choline/methionine diet it was found that only a high-fructose and choline diet caused steatosis.¹⁹ This study still has several limitations such as still consists of compounding variables and uncontrollable variables during the experiment.

CONCLUSION

a-mangostin from Garcinia mangostana L. isolate in diabetic rats has a hepatoprotective through evaluation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, but there were no necrotic cells on the histopathological observation of the liver. In further research, it is recommended to establish clinical toxicity and safety tests as a follow-up to develop mangosteen compounds as an alternative treatment for impaired liver function due to diabetes mellitus.

FUNDING

This research was funded by Universitas Nahdlatul Ulama Surabaya.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

ETHICAL STATEMENT

This study has been declared ethically feasible by the Health Research Ethics Committee, the Universitas Nahdlatul Ulama Surabaya.

AUTHOR CONTRIBUTION

Thank you to Universitas Nahdlatul Ulama Surabaya for funding this research and providing easy access to research facilities.

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Firdaus, Andreas Putro Ragil Santoso, Annif Munjidah, Elly Dwi Masita, Ika Mardiyant, Wesiana Heris Santy
Online First: Sep 22, 2023
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chemotherap /view/4797) Hadian Rahmar	hip between the difference neutrophil to lymphocyte ratio before and after anthracycline-based neoadjuvant y with clinical response in locally advanced breast cancer (https://blog.balimedicaljournal.org/index.php/bmj/article n, Iskandar Ali, Dwi Hari Susilo
Online First: No	v 1, 2023
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Comparison of the histological features in the photoaged animal model (https://blog.balimedicaljournal.org/index.php/bmj/article /view/4410)
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Online First: Oct 25, 2023
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Online First: Nov 8, 2023
Abstract Abstract Pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4696/3115)
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Online First: Oct 27, 2023
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4826/3077)
ORIGINAL ARTICLE
Correlation between family support and self-efficacy in stroke survivors (https://blog.balimedicaljournal.org/index.php /bmj/article/view/4375)
Yurike Septianingrum, Ah Yusuf, Ika Yuni Widyawati, T Fahzilatul Zrechva, Lono Wijayanti, Nety Mawarda Hatmanti, Andikawati Fitriasari
Online First: Sep 20, 2023
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4375/3020)
ORIGINAL ARTICLE
Preliminary study of immunomodulatory effects of fermented garlic honey on non-specific immune responses (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4428)
Devyani Diah Wulansari, Aguslina Kirtishanti, Devyana Dyah Wulandari, Andreas Ragil Putro Santoso, Emanuela Alicia Christy, Lidiana Hiskiati, Dwi Winarni
Online First: Nov 30, 2023
Abstract Def (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4428/3172)
ORIGINAL ARTICLE Clinical practice supportive psychotherapy for borderline personality disorder (https://blog.balimedicaljournal.org/index.php
/bmj/article/view/4614)
Natalia Dewi Wardani, Maria Immaculata Widiastuti, Aris Sudiyanto, Hardian, Petrin Redayani Lukman, Hertanto Wahyu Subagio, Dodik Tugasworo
Online First: Aug 21, 2023
Abstract Ddf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4614/2957)

Effect of Aerobic Walking on Maximal Oxygen Consumption and Serum Vitamin D Levels in Patients with Mechanical Chronic Low Back Pain in Office Workers at Dr. Soetomo Hospital Surabaya (https://blog.balimedicaljournal.org/index.php/bmj/article /view/4702) I Nengah Bagus Surianta, Damayanti Tinduh, Andriati, Meisy Andriana, Sony Wibisono Mudjanarko, Soenarnatalina Melaniani Online First: Aug 24, 2023]
ORIGINAL ARTICLE Assessing keloid treatment efficacy: a comparison of intralesional umbilical-cord mesenchymal stem cells, their conditioned medium, and triamcinolone acetonide injection through macroscopic and microscopic examination (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4758) Anastasia Dessy Harsono, Ismail Hadisoebroto Dilogo, Theddeus Octavianus Hari Prasetyono, Marcel Prasetyo, Retno Asti Werdhani, Sri Widia Jusman, Nuryati Chairani Siregar, Hardisiswo Soedjana Online First: Sep 9, 2023
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Online First: Aug 22, 2023

Spiritual diabetes self-management health coaching on self-efficacy, self-care, and blood glucose levels in type 2 diabetes mellitus patients (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4440)

Ratna Yunita Sari, Nety Mawarda Hatmanti, Imamatul Faizah, Riska Rohmawati, Abdul Muhith, Raden Khairiyatul Afiyah
Online First: Sep 20, 2023
Abstract Ddf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4440/3017)
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fibroblasts counts and collagen density (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4631) Astri Taufi Ramadhani, Ariandi Setiawan, I Gusti Bagus Adria Hariastawa
Online First: Aug 15, 2023
Abstract Def (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4631/2946)
ORIGINAL ARTICLE Genomic characterization of reinfection cases in the first wave of the COVID-19 pandemic in Indonesia (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4713) Erike Anggraini Suwarsono, Laifa Annisa Hendarmin, Chris Adhiyanto
Online First: Nov 6, 2023
Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4713/3107)
ORIGINAL ARTICLE Apoptosis evasion and local recurrence after mastectomy and chemotherapy in lobular type breast cancer. A cross-sectional study in a single-center (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4766) Andreas Andrianto, I Ketut Sudiana, Desak Gede Agung Suprabawati Online First: Sep 12, 2023
Abstract Ddf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4766/3001)

Microbial inhibition test and optimization of temperature, aeration fermentation of endophytic Fusarium sp LBKURCC 41 from Dahlia tuber (Dahlia variabilis) (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4879)

Dewi Yudiana Shinta, Muhammmad Diki Juliandi, Widyastuti Widyastuti, Herix Sonata MS, Saryono Saryono

Online First: Nov 14, 2023
Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4879/3105)
ORIGINAL ARTICLE
Effectiveness of Yoga in reducing IL-6 serum level in elderly with knee osteoarthritis (https://blog.balimedicaljournal.org /index.php/bmj/article/view/3386)
Nila Wahyuni, Nyoman Adiputra, I Putu Gede Adiatmika, Luh Putu Ratna Sundari, Agung Wiwiek Indrayani
Online First: Aug 22, 2023
Abstract Ddf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/3386/2961)
ORIGINAL ARTICLE
The effect of tummy time and oral massage on infants' oral motor skills as an effort to receive complementary food (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4396)
Annif Munjidah, Nanik Handayani, Firdaus
Online First: Nov 21, 2023
Abstract Ddf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4396/3136)
ORIGINAL ARTICLE
Survival rate of children acute lymphoblastic leukemia at the end of therapy protocol with minimal residual disease (MRD) in Prof l G.N.G. Ngoerah General Hospital Denpasar (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4652)
Dewa Gde Windu Sanjaya, Ketut Ariawati, Putu Junara Putra, I Putu Gede Karyana, Eka Gunawijaya, I Made Gde Dwi Lingga Utama
Online First: Aug 18, 2023
Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4652/2952)
ORIGINAL ARTICLE
Clinical research capacity evaluation at Prof. I.G.N.G Ngoerah General Hospital, Denpasar: Teaching general hospital, Indonesia (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4720)
Tri Fajari Agustini, Amal Chalik Sjaaf, Wachyu Sulistiadi, Harimat Hendrawan

Online First: Sep 5, 2023 |

Abstract Def (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4720/3000)	
ORIGINAL ARTICLE Research trends on the use of ultrasound as neuromodulation-based therapy, based on bibliometric analysis (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4775)	
Arman Yurisaldi, Tony Setiobudi , Muhammad Shiddiq Sayyid Hashuro	
Online First: Nov 3, 2023	
Abstract Ddf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4775/3089)	
ORIGINAL ARTICLE Surgical management for ponto-mesencephalic cavernoma: determining the brainstem safe entry zone – a case report	
(https://blog.balimedicaljournal.org/index.php/bmj/article/view/4976) Fariz Eka Setiawan, Muhamad Thohar Arifin, Krisna Tsaniadi Prihastomo, Happy Kurnia Brotoarianto, Ajid Risdianto, Yuriz Bakhtiar, Dody Priambad Erie Bambang Priyono Setyabudi Andar, Zainal Muttaqin	a,
Online First: Nov 25, 2023	
Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4976/3141)	
ORIGINAL ARTICLE Hepatoprotective effect of ethanolic extract of Syzygium polyanthum in rats induced by rifampicin and isoniazid (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4327) Handayani, Renny Novi Puspitasari, Lysa Veterini, Devyana Dyah Wulandari Online First: Nov 30, 2023	
Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4327/3175)	
ORIGINAL ARTICLE	
Human interaction model analysis of factors influencing nurse-patient interaction in pregnancies at risk for preeclampsia (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4401)	
Faridah Umamah, Budi Santoso, Esti Yunitasari, Siti Nur Hasina, Raden Khairiyatul Afiyah	
Online First: Nov 17, 2023	
Abstract Ddf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4401/3135)	1

(https://blog.balimedicaljournal.org/index.php/bmj/article/view/4465)
Yanis Kartini, Nursalam, Ahsan, Imamatul Faizah, Abd Nasir
Online First: Nov 21, 2023
Abstract Ddf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4465/3139)
ORIGINAL ARTICLE
Association between Human Papilloma Virus (HPV) genotype and protein of retinoblastoma expression in cervical cancer (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4662)
Nadia Kurnia Wardani Rauf, Sharvianty Arifuddin, Elizabet Catherine Jusuf, Muhammad Husni Cangara, Firdaus Hamid, Nugraha Utama Pelupessy, Irnawaty Bahar
Online First: Sep 12, 2023
Abstract Def (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4662/3005)
ORIGINAL ARTICLE
Progesterone Serum Level on HCG Trigger Day among Frozen Embryo Transfer IVF Patients and Pregnancy Outcomes: A Systematic Review and Meta-Analysis (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4726) Alifia Ramadhani Herida, Muhamad Faza Soelaeman Online First: Aug 28, 2023
Systematic Review and Meta-Analysis (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4726) Alifia Ramadhani Herida, Muhamad Faza Soelaeman
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The association between C-reactive protein (CRP)/Albumin Ratio (CAR) and the outcomes of patients with Ludwig's Angina (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4804)
l Made Candra Purnama, Dwi Hari Susilo, Maryono Dwi Wibowo
Online First: Nov 8, 2023
Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4804/3113)
ORIGINAL ARTICLE
Implementation of COVID-19 prevention and control at PT. Port of indonesia III (PELINDO 3) (https://blog.balimedicaljournal.org
/index.php/bmj/article/view/4356)
Muslikha Nourma Rhomadhoni, Friska Ayu, Rizka Novembrianto, Octavianus Hutapea, Merry Sunaryo, Ratna Ayu Ratriwardhani, Moch. Sahri, Maya Nurahmadiana
Online First: Sep 22, 2023
Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4356/3028)
ORIGINAL ARTICLE
Role of liver damage on glucose metabolism in a lipopolysaccharide-induced mouse model (https://blog.balimedicaljournal.org /index.php/bmj/article/view/4416)
Hotimah Masdan Salim, Evi Sylvia Awwalia, Sobihatus Syifak, Danny Irawan, Ilham Putera Alam
Online First: Nov 29, 2021
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4416/3166)
The effect of additional transcutaneous auricular vagus nerve stimulation on back muscle endurance of chronic low back pain (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4689)
Retno Nirmala Kusumastuti, Raden Ayu Meisy Andriana, Yudith Dian Prawitri, Damayanti Tinduh, Paulus Sugianto, Soenarnatalina Melaniani
Online First: Aug 31, 2023
Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4689/2983)

halikul Razi,	Agi Satria Putranto, Wifanto Sadityo Jeo, Riana Pauline Tamba, Herqutanto
Online First: (Oct 12, 2023
Abstract	pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4820/3069)
ORIGINAL AF	साCLE the effect of nutritional status, workload and work period on worker productivity (https://blog.balimedicaljournal.or
index.php/	/bmj/article/view/4373)
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online First: S	Sep 20, 2023
Abstract	pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4373/3018)
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Lailatul Khusnul Rizki, Nur Masruroh, Esty Puji Rahayu
Online First: Sep 21, 2023
Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4378/3023)
ORIGINAL ARTICLE
Hazard identification with failure mode & effect analysis and fault tree analysis in the chemical industry (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4435)
Ratna Ayu Ratriwardhani, Merry Sunaryo
Online First: Sep 22, 2023
Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4435/3032)
ORIGINAL ARTICLE The immediate effects of Porang-processed rice (Amorphophallus muelleri) on triglyceride levels in patients with type 2 diabetes mellitus and dyslipidemia (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4622) Satrio Adi Wicaksono, Zahrien Assyifa Nur Palisma, Taufik Eko Nugroho, Sulistiyati Bayu Utami Online First: Aug 25, 2023
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r Ricky Ricardo Nalley, Nusratuddin Abdullah, Fatmawati Madya, St. Nur Asni, Sriwijaya, Susiawaty
Online First: Oct 7, 2023
Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4707/3062)
ORIGINAL ARTICLE
Diagnostic value of immature platelet fraction for detecting production disorders in thrombocytopenia (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4860)

I Wayan Losen Adnyana, Ni Made Renny Anggreni Rena, Nyoman Martha Chrismayana, Putu Gizha Satria Gautama

Online First: Nov 3, 2023	
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4860/3088)	
ORIGINAL ARTICLE Entrepreneur intention: bonding cognitive, bridging cognitive, entrepreneurial knowledge, subjective norms, perceived	
desirability, perceived feasibility, self-efficacy on student university in Surabaya (https://blog.balimedicaljournal.org/index.php /bmj/article/view/4394)	
Denis Fidita Karya, M. Yusak Anshori, Fily Irhamni, Rizqi Amalia Elfita	
Online First: Nov 17, 2023	
Abstract Def (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4394/3117)	
ORIGINAL ARTICLE Identification of customer satisfaction factors through the service quality dimensions: A case study of ABC Hospital, Surabaya (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4443) Rachma Rizqina Mardhotillah, Denis Fidita Karya, Reizano Amri Rasyid, Berto Mulia Wibawa Online First: Sep 23, 2023	
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4443/3036)	
ORIGINAL ARTICLE	
Characterization of ultraviolet B light emitting diodes (UVB LED) irradiation device for Wistar rats as an experimental animal model (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4639)	
Diah, Chiquita Prahasanti, Retno Puji Rahayu, Vani Rachmad Wijayanto	
Online First: Aug 28, 2023	
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4639/2976)	
ORIGINAL ARTICLE	

Effect of Blood Flow Restriction in Low-Intensity Resistance Training of the Quadriceps Femoris Muscle on Joint Position Sense and Threshold to Detect Passive Motion in Patients with Knee Osteoarthritis (https://blog.balimedicaljournal.org/index.php /bmj/article/view/4717)

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Online First: Au	ug 28, 2023
Abstract	pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4717/2977)
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Abstract	pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4769/3110)
sector (https	ership management and organizational work culture on electronic medical records in the Indonesian health service :://blog.balimedicaljournal.org/index.php/bmj/article/view/4399) a, Titin Wahyuni, Lilis Masyfufah, Alfina Aisatus Saadah, Diah Wijayanti Sutha, Anif Prasetyorini
Abstract	Pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4399/3133)
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Abstract	Pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4462/3165)
coronary into /bmj/article/	f major adverse cardiac events during 7-days period in acute coronary syndrome patients who had percutaneous ervention in Cipto Mangunkusumo National Hospital, Jakarta (https://blog.balimedicaljournal.org/index.php view/4658)
Djallalludin, lka Online First: Au	a Prasetya Wijaya, Sukamto Koesnoe, Idrus Alwi

Abstract Ddf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4658/2947)
ORIGINAL ARTICLE The association between postpartum perineal laceration with anatomy and pelvic floor muscle strength (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4724) Gledies Theresye Gosal, Trika Irianta, David Lotisna, Isharyah Sunarno, Imam Ahmadi Farid, Nugraha Utama Pelupessy Online First: Nov 6, 2023 Abstract Def (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4724/3108)
ORIGINAL ARTICLE Comparison of cerebrospinal fluid results analysis against meningitis pathogens in meningitis patients (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4781) Magendi Indra Mukti, Aryati, Paulus Sugianto Online First: Sep 18, 2023
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Mortality of COVID-19 patients with comorbidity of diabetes mellitus: a retrospective study (https://blog.balimedicaljournal.org /index.php/bmj/article/view/4406)

Difran Nobel Bistara, Susanti, Arif Helmi Setiawan, Umi Hanik, Thomas Ari Wibowo, Niken Setyaningrum, Satriya Pranata

Online First: Sep 29, 2023

🖹 Abstract

bdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4406/3049)

ORIGINAL ARTICLE	
Analysis of influence factors gentle birth labor preparedness self-efficacy in the third-trimester pregnant women (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4468)	
Fritria Dwi Anggraini, Yunik Windarti , Nanik Handayani	
Online First: Nov 21, 2023	
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4468/3140)	
ORIGINAL ARTICLE	
The relationship between the role of the family and the use of control card with the level of compliance with medication in elderly patients at UPTD Puskesmas Wonokromo (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4354)	
Siti Nurjanah, Yuni Sufyanti, Umdatus Soleha, Nety Mawarda Hatmanti, Nunik Purwanti, Santi Karlina	
Online First: Sep 21, 2023	
Abstract D pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4354/3026)	
ORIGINAL ARTICLE	
The association of Hypoxia-Inducible Factor-2α (HIF-2α) overexpression score with Germinal Center B-Cell Like (GCB) and Non-	
Germinal Center B-Cell Like (Non-GCB) subtypes of Diffuse Large B-cell Lymphoma (DLBCL) (https://blog.balimedicaljournal.org /index.php/bmj/article/view/4521)	
Eko Adhi Pangarsa, Desta Nur Ewika Ardini, Daniel Rizky, Kevin Tandarto, Hermawan Istiadi, Dik Puspasari, Budi Setiawan, Damai Santosa, Hertanto	
Wahyu Subagio, Tri Indah Winarni, Sofia Mubarika Haryana, Catharina Suharti	
Online First: Aug 22, 2023	
Abstract D pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4521/2963)	
ORIGINAL ARTICLE	
Comparison of Various Blood Vacuum Tubes Brand for Clinical Chemistry, Hormonal, Hematology, and Hemostasis Parameters	
(https://blog.balimedicaljournal.org/index.php/bmj/article/view/4685) Astuti Giantini, Nuri Dyah Indrasari, Siskawati Suparmin, Punjul Kasihanto, Arif Ramadhan Tansir	
Online First: Oct 7, 2023	
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4685/3061)	

ORIGINAL ARTICLE
Developing a Mobile Learning Virtual Nursing Diagnosis (VND) Media for Medical Surgical Nursing Course (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4744)
Bustami, Abdurrahman, Wirda Hayati, Erlangga Galih Zulva Nugroho, Cut Mutiah, Muhammad Nazar
Online First: Nov 7, 2023
Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4744/3101)
ORIGINAL ARTICLE Supplementation of vitamin D reduces energy intake and prevents body fatness accumulation in obese woman (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4811) Linda Sinto, I Wayan Weta, Komang Bagus Satriasa, Nyoman Mantik Astawa Online First: Nov 1, 2023
ORIGINAL ARTICLE D-dimer level differences in pneumonia patients than in COVID-19 (https://blog.balimedicaljournal.org/index.php/bmj/article /view/4359) Rahayu Anggraini, Eppy Setiyowati, Difran Nobel Bistara, Firdaus, Budhi Setianto Online First: Sep 22, 2023
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4359/3030)
ORIGINAL ARTICLE Effectiveness of combination of benson relaxation and lavender aromatherapy relaxation towards changes in anxiety in the third trimester pregnant woman (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4419)
Siska Nurul Abidah, Hinda Novianti, Nur Masruroh
Online First: Nov 30, 2023
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4419/3174)

,	ngrum, Prananda Surya Airlangga, Wiwiek Indriyani Maskoep, Herdiani Sulistyo Putri
Online First: S	ep 9, 2023
Abstract	Pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4697/2998)
ORIGINAL AR	TICLE
ruit peel ex	the number of Streptococcus mutans and Staphylococcus aureus bacterial colonies after administration of sentul tract gel (Sandoricum koetjape) in gingivitis model of white Wistar rats (https://blog.balimedicaljournal.org/index.pł /view/4753)
Nyoman Wir	ata, Anak Agung Gede Agung, Ni Wayan Arini, I Gusti Ayu Raiyanti, I Ketut Aryana, I Nyoman Purna
Online First: S	ep 16, 2023
Abstract	D pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4753/3010)
ORIGINAL AR	TICLE
P rofile of an https://blog 1uhammad F	neloblastoma patients at Dr. Soetomo general hospital, Surabaya: A descriptive study g.balimedicaljournal.org/index.php/bmj/article/view/4829) urqan Hidayat, Marjono Dwi Wibowo
P rofile of an https://blog 1uhammad F	neloblastoma patients at Dr. Soetomo general hospital, Surabaya: A descriptive study g.balimedicaljournal.org/index.php/bmj/article/view/4829) urqan Hidayat, Marjono Dwi Wibowo
Profile of an https://blog /luhammad F Dnline First: N BAbstract ORIGINAL AR The aromat	heloblastoma patients at Dr. Soetomo general hospital, Surabaya: A descriptive study g.balimedicaljournal.org/index.php/bmj/article/view/4829) urqan Hidayat, Marjono Dwi Wibowo lov 1, 2023
Profile of an https://blog /luhammad F Dnline First: N @Abstract ORIGINAL AR The aromati tress in pat	heloblastoma patients at Dr. Soetomo general hospital, Surabaya: A descriptive study g.balimedicaljournal.org/index.php/bmj/article/view/4829) urqan Hidayat, Marjono Dwi Wibowo lov 1, 2023
https://blog Auhammad F Dnline First: N Abstract Abstract ORIGINAL AR The aromat	heloblastoma patients at Dr. Soetomo general hospital, Surabaya: A descriptive study g.balimedicaljournal.org/index.php/bmj/article/view/4829) urqan Hidayat, Marjono Dwi Wibowo lov 1, 2023

Preventive behaviors toward transmission of pulmonary tuberculosis (https://blog.balimedicaljournal.org/index.php/bmj/article 个 /view/4429)

Eppy Setiyowati, Khamidah, Nurul Khamariyah, I	Budhi Setianto, Nety Mawarda Hatmanti,	, Difran Nobel Bistara, Erika Martining Wardani
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Online First: Nov 30, 2023	
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4429/3173)	
ORIGINAL ARTICLE The relationship between traditional postnatal care (TPC) and sociodemographic of postnatal mothers in Kota Kinabalu, Sabah,	
Malaysia (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4616)	
Patricia Sator, Nicholas Tze Ping Pang, Rekaya Anak Vincent Balang, Rosalia Saimon, Mohammad Saffree Bin Jeffree, Norkiah Arsat	
Online First: Aug 16, 2023	
Abstract Ddf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4616/2948)	
ORIGINAL ARTICLE	
The effect of blood flow restriction in low-intensity load exercise on isokinetic strength of the quadriceps muscles in knee osteoarthritis (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4703)	
Priscilla Anastasia Fitriany Dugis, Damayanti Tinduh, I Putu Alit Pawana, Dwikora Novembri Utomo, Soenarnatalina Melaniani	
Online First: Aug 26, 2023	
Abstract D pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4703/2967)	
ORIGINAL ARTICLE	
Ascorbic acid and calcitriol reduce TNF-α to improve the viability of modified McFarlane random skin flaps in Wistar rats (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4843)	
Rosalyn Devina Santoso, Agus Roy Rusly Hariantana Hamid, Tjokorda Gde Bagus Mahadewa , I Gusti Putu Hendra Sanjaya, Ni Nyoman Sri Budayanti, Sianny Herawati	
Online First: Oct 9, 2023	
Abstract Ddf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4843/3071)	
ORIGINAL ARTICLE	
The practice of distance lactation counseling by peer counselors (https://blog.balimedicaljournal.org/index.php/bmj/article /view/4391)	

Uke Maharani Dewi, Ratna Ariesta Dwi Andriani, Yunik Windarti, Fauziyatun Nisa, Fritria Dwi Anggraini, Nanik Handayani

Online First: Oct 26, 2023		
Abstract Ddf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4391/3083)		
ORIGINAL ARTICLE		
A survey and conceptual of Internet of Things system for remote healthcare monitoring (https://blog.balimedicaljournal.org /index.php/bmj/article/view/4441)		
Sritrusta Sukaridhoto, Agus Prayudi, M Udin Harun Al Rasyid, Rizqi Putri Nourma Budiarti Online First: Sep 23, 2023		
Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4441/3034)		
ORIGINAL ARTICLE		
Clinical epidemiology pattern of orthopedic trauma during the COVID-19 pandemic: A multicenter study (Dr. Hasan Sadikin General Hospital and Ulin General Hospital) (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4634)		
Agus Hadian Rahim, Nucki Nursjamsi Hidajat, Yoyos Dias Ismiarto, Dicky Mulyadi, Widya Arsa, Ahmad Ramdhan, Ghuna Arioharjo Utoyo, Muhamad Naseh Sajadi Budi Irawan, Raden Andri Primadhi, Herry Herman, Gibran Tristan Alpharian, Renaldi Prasetia, Abdul Kadir Hadar, Realita Malik, Zairin Noor, Izaak Zulkarnaen, Andreas Siagian, Husna Dharma Putra, Essy Dwi Danayanti, Wongso Kesuma, Muhammad Iqbal, Cristian Setiadi Online First: Sep 12, 2023		
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4634/3004)		
ORIGINAL ARTICLE		
Maternal distress during pregnancy related to preeclampsia (https://blog.balimedicaljournal.org/index.php/bmj/article /view/4715)		
Siti Nur Khalida, Erry Gumilar Dachlan, Gatot Soegiarto		
Online First: Aug 22, 2023		
Abstract Ddf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4715/2959)		
ORIGINAL ARTICLE		
Role of cardiac marker troponin I in chronic periodontitis with coronary artery disease (https://blog.balimedicaljournal.org /index.php/bmj/article/view/4891)		
Sanggap Indra Sitompul, Aryati, Budi Susetyo Pikir, Monika Estherlita Sinta		

Online First: Nov 11, 2023 |

Abstract Df pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4891/3104)
ORIGINAL ARTICLE
The black cumin seeds oil with Curcuma xanthorrhiza extract preparation increases IFN-y expression
(https://blog.balimedicaljournal.org/index.php/bmj/article/view/3781)
Titiek Hidayati, Sunny Sun, Akrom
Online First: Aug 19, 2023
自Abstract D pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/3781/2953)
E put (https://blog.baimedicajournal.org/index.php/bhij/article/view/5781/2555)
ORIGINAL ARTICLE
Skin response to lipopolysaccharide-induced sepsis based on histology (https://blog.balimedicaljournal.org/index.php/bmj/article
/view/4397)
Maria Ulfa, Hotimah Masdan Salim, Winawati Eka Putri, Irmawan Farindra
Online First: Nov 17, 2023
Abstract D pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4397/3118)
ORIGINAL ARTICLE
Characterization and evaluation of the hepatoprotective activity of α -mangostin isolate in diabetic rats
(https://blog.balimedicaljournal.org/index.php/bmj/article/view/4453)
Devyana Dyah Wulandari, Andreas Putro Ragil Santoso, Sri Surya Suciati, Devyani Diah Wulansari, Ary Andini, Farach Khanifah
Online First: Nov 30, 2023
Abstract Def (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4453/3176)
ORIGINAL ARTICLE
The effect of adding exergame boxing to balance functions of elderly women (https://blog.balimedicaljournal.org/index.php
/bmj/article/view/4722)
Rizky Diah Kartikasari, l Putu Alit Pawana, Ditaruni Asrina Utami, Soenarnatalina Melaniani

ORIGINAL ARTICLE

ORIGINAL ARTICLE		
Combination diabetic foot spa, rubber band resistance exercise and music therapy in patients with diabetes mellitus (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4346)		
Erika Martining Wardani, Riezky Faisal Nugroho, Lono Wijayanti, Nur Ainiyah, Difran Nobel Bistara		
Online First: Sep 25, 2023		
Abstract Ddf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4346/3041)		
ORIGINAL ARTICLE		
Stroke self-management program based on health behavior theory on neurological function and quality of life of post-ischemic stroke patients (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4404)		
Imamatul Faizah, Yanis Kartini, Ratna Yunita Sari, Riska Rohmawati, Abdul Muhith		
Online First: Sep 23, 2023		
Abstract Ddf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4404/3038)		
ORIGINAL ARTICLE		
Knowledge About COVID-19, perceived behavior control, subjective norm, and health consciousness on vitamin purchase intention during COVID-19 (https://blog.balimedicaliournal.org/index.php/hmi/article/view//466)		
intention during COVID-19 (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4466) Dwi Handayani, Denis Fidita Karya, Atik Qurrota A'Yunin Al Isyrofi		
Online First: Nov 22, 2023		
Abstract Def (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4466/3163)		
ORIGINAL ARTICLE		
Hyperglycemia may induce a decrease in myoblast viability: A Preliminary Study (https://blog.balimedicaljournal.org/index.php		
/bmj/article/view/4663)		
Dicky Moch Rizal, Jajar Setiawan, Susi Susanti, Laksmita Dewi Adzillina, Nandia Septiyorini		
Online First: Sep 12, 2023		
Abstract D pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4663/3006)		

ORIGINAL ARTICLE Clinical outcomes of Descemet's stripping endothelial keratoplasty using a manual or automated procedure at Und Hospital Surabaya, Indonesia: with one-year follow up (https://blog.balimedicaljournal.org/index.php/bmj/article/vi Azzahra Afifah, Dini Dharmawidiarini, Farida Moenir, Sahata P. H. Napitupulu Online First: Aug 30, 2023 Abstract Pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4727/2978)	
ORIGINAL ARTICLE Conventional Magnetic Resonance Imaging (MRI) and histopathology agreement for the diagnosis of intracranial m Dr. Soetomo General Hospital, Surabaya, January 2016–December 2021: a single center study (https://blog.balimedi /index.php/bmj/article/view/4790) Muhammad Wildan Hakim, Joni Wahyuhadi, Sri Andreani Utomo, Asra Al Fauzi, Muhammad Arifin Parenrengi, Budi Utomo Online First: Nov 7, 2023 Pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4790/3111)	
ORIGINAL ARTICLE Supportive educative system based on the integration of family centered care and family tasks towards family supp adolescents with smartphone addiction (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4351) Nety Mawarda Hatmanti, Ratna Yunita Sari, Yurike Septianingrum, Eppy Setiyowati, Siti Maimunah Online First: Sep 29, 2023 Abstract Methods: Abstract Methods: Abs	ort in
ORIGINAL ARTICLE The effectiveness of video-based learning media and skills practicum module in diploma nursing students during th pandemic at the Faculty of Nursing, Universitas Nahdlatul Ulama Surabaya (https://blog.balimedicaljournal.org/ind /bmj/article/view/4409) Sulistyorini Sulistyorini, lis Noventi, Priyo Mukti Pribadi Winoto, Siti Nur Hasina, Faridah Umamah Online First: Sep 29, 2023 Abstract Def (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4409/3052)	

ORIGINAL ARTICLE		
The effect of empowerment of the "Mahatta" Cadre on the health status of the first thousand days of birth		
(https://blog.balimedicaljournal.org/index.php/bmj/article/view/4478) Nurul Kamariyah, Rahmadaniar Aditya Putri, Siti Nurjannah, Faridah Umamah, Elke Amanda Indrika		
Online First: Nov 21, 2023		
Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4478/3158)		
ORIGINAL ARTICLE The effect of the type of thyroidectomy on the incidence of hypothyroidism (https://blog.balimedicaljournal.org/index.php /bmj/article/view/4677)		
Sagiran, M. Afif Jaffariz, Ardi Pramono		
Online First: Oct 4, 2023		
Abstract Def (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4677/3059)		
ORIGINAL ARTICLE The effect of free peritoneal patch on bursting pressure in primary gastric suturing with peritonitis conditions (study on New Zealand White rabbits) (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4741) Ferry Rusdiansaputra, Edwin Danardono, I Gusti Bagus Adria Hariastawa Online First: Oct 12, 2023		
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4741/3073)		
ORIGINAL ARTICLE		
Relationship of HER2 with age, gender, location of the tumor, and tumor budding in colorectal adenocarcinoma at H. Adam Malik General Hospital, Medan, Indonesia (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4805)		
Adi Muradi Muhar, Lidya Imelda Laksmi, Nova Sabrina Lubis		
Online First: Nov 8, 2023		
Pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4805/3114)		

Rahmadaniar Aditya Putri, Sarmi, Siti Nur Hasina, lis Noventi		
Online First: Sep 22, 2023		
Abstract	Def (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4357/3029)	
ORIGINAL AR	TICLE	
	value in foot stories from Surabaya to support character learning in school (https://blog.balimedicaljournal.org bmj/article/view/4417)	
uharmono K	asiyun, Pance Mariati, Syamsul Ghufron	
online First: N	Nov 29, 2023	
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	☑ pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4417/3167) TICLE	
ORIGINAL AR he applica egment re bmj/article gus Rizal Arc lurul Ratna, d	TICLE tion of enhanced recovery after surgery (ERAS) protocol in radical cystectomy and urinary diversion using bowel	
egment ree bmj/article gus Rizal Arc lurul Ratna, (TICLE tion of enhanced recovery after surgery (ERAS) protocol in radical cystectomy and urinary diversion using bowel duces the length of stay: A multicenter comparative study in Indonesia (https://blog.balimedicaljournal.org/index.ph //view/4530) dy Hariandy Hamid, Adhitama Alam Soeroto, Ahmad Zulfan Hendri, Sawkar Vijay Pramod, Faurisky Febrian Prapiska, Dita Aditianingsih, Chaidir Arif Mochtar, Rainy Umbas	
ORIGINAL AR he applica egment red bmj/article gus Rizal Arc lurul Ratna, o online First: Jo Dolline First: Jo Polici Abstract ORIGINAL AR Potential of view/4374) ndah Prayek	TICLE	
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Application of mixed reality and artificial intelligence to assist medical students in learning injection technique (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4425)

Online First: Nov 30, 2023	
Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4425/3171)	
ORIGINAL ARTICLE	
Transnasal Esophagoscopy (TNE) procedure in the outpatient clinic at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4610)	
Muhammad Qodri Anantomo, Rizka Fathoni Perdana	
Online First: Aug 10, 2023	
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4610/2949)	
ORIGINAL ARTICLE Utilization of Phyo Referral Score in assessing maternal referral quality in emergency cesarean section of maternal COVID-19	
cases in Pekanbaru, Indonesia (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4700) Ilham Sejahtera Lubis, Eugenius Phyowai Ganap	
Online First: Nov 3, 2023	
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4700/3090)	
ORIGINAL ARTICLE	
Analysis of CYP17 gene polymorphism on increasing androstenedione levels in polycystic ovary syndrome women with obesity in Surabaya (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4757)	
Muarrofah, Budi Santoso, I Wayan Tirthaningsih	
Online First: Sep 3, 2023	
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4757/2988)	

Dermoscopic features of melasma: a descriptive study in Bali (https://blog.balimedicaljournal.org/index.php/bmj/article /view/4839)

Ketut Kwartantaya Winaya, Pande Agung Mahariski, I Gusti Ayu Agung Praharsini, I Gusti Ayu Sattwika Pramita

Online First: Oct 13, 2023 Abstract Def (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4839/3066) ORIGINAL ARTICLE Perceptions of health service providers on postpartum depression (https://blog.balimedicaljournal.org/index.php/bmj/article/view/43379) Uliyatul Laili, Yati Isnaini, Rizki Amalia	
Online First: Sep 21, 2023	
ORIGINAL ARTICLE Effectiveness of the habit of using mask and not using mask on the carboxyhemoglobin (COHb) levels in public transportation workers in Bangkalan district (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4439) Ersalina Nidianti, Devyana Dyah Wulandari, Ary Andini, Adinda Priyanti Dewi Online First: Sep 20, 2023 Mabstract Mathematical provides and the set of	
ORIGINAL ARTICLE The relationship between the cycle threshold value of SARS CoV-2 with neutrophil lymphocyte ratio (NLR), absolute lymphocyte count (ALC), severity degrees, long of treatment, and outcomes COVID-19 patients (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4627) Aina Nurlaila, Mohamad Isa, Rahmiati Lao, Ira Nurrasyidah, Isa Ansori, Erna Kusumawardhani Online First: Aug 23, 2023 Mabstract Pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4627/3091)	
ORIGINAL ARTICLE Effect of epigallocatechin gallate (EGCG) on neutrophils count and interleukin-6 (IL-6) expression in Pseudomonas aeruginosa keratitis: an experimental study on Rattus norvegicus rat (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4764) Annisa Karima, Luki Indriaswati, Susy Fatmariyanti, Nila Kurniasari	

Online First: Sep 7, 2023 |

Abstract	pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4764/2993)	
Indonesia (https	of participatory action research: Midwife empowerment in improving the quality of maternal referral in ://blog.balimedicaljournal.org/index.php/bmj/article/view/2172) _estari, Ova Emilia, Detty Siti Nurdianti, Retna Siwi Padmawati	
Abstract	pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/2172/3058)	
/bmj/article/viev Yeti Eka Sispita Sar Online First: Nov 17	xelan leaves (Chromolaena odorata) on the growth of salmonella (https://blog.balimedicaljournal.org/index.php v/4395) i, Fitrotin Azizah, Dita Artanti, Anindita Riesti Retno Arimurti, Andreas Putro Ragil Santoso	
Nutrient analysi tricolor) (https:// Yauwan Tobing Luk Online First: Sep 23	ORIGINAL ARTICLE Nutrient analysis of fermented chicken feather fertilizer waste using Bacillus subtilis on the growth of green spinach (Amaranthus tricolor) (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4444) Yauwan Tobing Lukiyono, Edza Aria Wikurendra, Salfa Salsabilah Zain, Satriyo Siswo Utomo Online First: Sep 23, 2023 Abstract pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4444/3037)	
(https://blog.bal	erum ferritin with parathyroid hormone level in patients with transfusion-dependent thalassemia imedicaljournal.org/index.php/bmj/article/view/4772) i, S. Ugroseno Yudho Bintoro, Ami Ashariati, Hermina Novida, M. Noor Diansyah, Putu Niken Ayu Amrita, Merlyna Savitri, Pradana	

Online First: Oct 19, 2023

D pdf (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4772/3076)

ORIGINAL ARTICLE
Spiritual experience related to anxiety level in pre operational cesarean section patients (https://blog.balimedicaljournal.org /index.php/bmj/article/view/4322)
Chilyatiz Zahroh, Fin Safitri Widyagarini, Erika Martining Wardani, I Ketut Sudiana, Ah. Yusuf
Online First: Sep 25, 2023
Abstract Df (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4322/3040)
ORIGINAL ARTICLE
The relationship between potential ergonomic hazard with complaints of musculoskeletal disorders (https://blog.balimedicaljournal.org/index.php/bmj/article/view/4400)
Moch. Sahri, Indira Ratu Felia, Akas Yekti Pulih Asih, Budhi Setianto
Online First: Nov 17, 2023
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Rizki Nurmalya Kardina, Dini Setiarsih, Andreas Putro Ragil Santoso, Sa'bania Hari Raharjeng, Pratiwi Hariyani Putri, Indri Dwi Lestari
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ORIGINAL ARTICLE
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ORIGINAL AR	TICLE
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Agung Bhakti Wiratama, Reni Prastyani, Indri Wahyuni, Thomas Valentinus, Hari Basuki Notobroto
Online First: Aug 26, 2023
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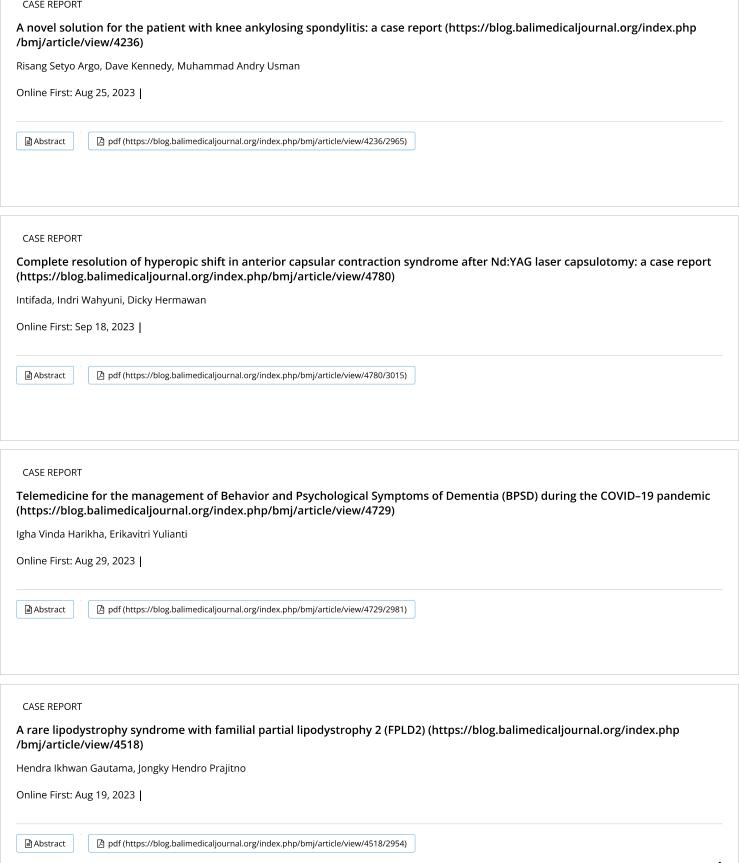
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