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Research Paper

Acute Toxicity Assessment of Ethyl Acetate Fraction of Melinjo Leaves (Gnetum gnemon L.) on Male Wistar White Rats and Liver Tissue Examination

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ABSTRACT

Background: The ethyl acetate fraction of melinjo (*Gnetum gnemon* L.) leaves has been reported to possess antihyperlipidemic and antidiarrheal activities, and phytochemical screening has identified tannins, phenolics, and flavonoids. However, the safety profile of this fraction remains unclear, necessitating toxicity evaluation. The purpose of this study was to use the fixed-dose method to examine the acute toxicity of the ethyl acetate fraction of melinjo leaves in male Wistar rats.

Methods: Based on preliminary testing, the main study used a single oral dose of 2000 mg/kg BW. In silico predictions using Swiss ADMET, Pro-Tox II, and pkCSM showed LD_{50} values ranging from 159 to 5000 mg/kg.

Results: There were no deaths or obvious symptoms of toxicity in either the treatment or control groups, according to observations. Additionally, biochemical analysis showed no significant differences across groups ($p > 0.05$). The treatment group's mean results were SGPT 76.01 ± 3.20 U/L, SGOT 144.31 ± 34.62 U/L, urea 42.12 ± 7.74 mg/dL, and creatinine 0.622 ± 0.066 mg/dL, while the control group showed SGPT 72.45 ± 11.64 U/L, SGOT 144.19 ± 19.90 U/L, urea 46.08 ± 6.32 mg/dL, and creatinine 0.618 ± 0.024 mg/dL. Macroscopic examination of the liver, kidneys, and heart revealed no pathological changes.

Conclusion: At a dose of 2000 mg/kg BW, the ethyl acetate fraction of melinjo leaves did not cause acute toxicity, indicating a good safety profile for further pharmacological research.

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Introduction

Medicine is an essential element in health maintenance efforts. One of the medicines used is a traditional medicine passed down from generation to generation for treatment [1]. One plant widely used in conventional medicine is the melinjo leaf (Gnetum gnemon L.), a member of the Gnetaceae family [2].

Melinjo leaves contain active compounds, including alkaloids, flavonoids, steroids, and tannins [3]. The melinjo plant has antidiarrheal, antibacterial, and antihyperglycemic properties, and can also lower uric acid levels [4, 5]. The ethyl acetate fraction of melinjo leaves exhibits antidiarrheal activity with an ED50 of 80.7123 mg/kg BW [6]. The ethanol extract of melinjo leaves is also effective in lowering blood glucose levels with an ED_{50} of 720.86 mg/kgBW [7]. The ethyl acetate fraction of melinjo leaves exhibited antihyperlipidemic effects with an ED_{50} of 34.74 mg/kgBW [8]. Ethanol extract of melinjo bark can exhibit an antihypercholesterolemic effect with an ED50 of 9.1 mg/kg BW [9].

The current paradigm holds that traditional medicines are harmless and have no side effects. This opinion is not entirely correct. Therefore, information is needed that provides safe limits for the use of traditional medicines. Understanding the potential for acute toxicity is one of the first criteria for evaluating a drug's safety [10].

This study aimed to determine the toxic dose range (LD_{50}) of the ethyl acetate fraction of melinjo leaves by macroscopic observation of the liver, kidney, and heart, as well as biochemical parameters including SGOT, SGPT, creatinine, and urea. This study used the fixed-dose procedure. Furthermore, observations of changes in the test animals' behavior indicated the emergence of toxic symptoms.

Materials and Methods

Materials and Animals

Daun melinjo (*Gnetum gnemon* L.) leaves were collected from Palembang, Indonesia, and authenticated in the herbarium of the Plant Conservation Center of the Purwodadi Botanical Gardens, LIPI (No. 0044/IPH.06/HM/I/2019). The test animals used in this research were 20 male white rats in good health, with normal behavior and no defects, aged 2-3 months and weighing 200-300 grams. An ethical approval letter was granted by the Research Ethics Committee of Ahmad Dahlan University (No. 022301013)

Research Methods

Melinjo leaves are macerated by extraction, then further processed with liquid-liquid fractionation. Phytochemical screening was carried out to detect flavonoids, saponins, tannins, alkaloids, and steroids/triterpenoids using standard test reagents [11-13].

In Silico Toxicity Prediction

Selected phytochemicals (Isoswertiajapon, Isoswertisin, Isovitexin, Swertiajapon, Swertisin) were analyzed using Swiss ADMET, Pro-Tox II, and pkCSM [14]. Parameters included ADMET profiles (absorption, distribution, metabolism, excretion, toxicity), LD50, hepatotoxicity, carcinogenicity, mutagenicity, and interactions with biological targets.

Preliminary Test

In the preliminary test, 1 test animal was used for each dose level. The dose is selected using the fixed-dose procedure: 5, 50, 300, and 2000 mg/kg BW. The initial dose chosen is 5 mg/kg BW, administered via oral sonde. If only symptoms of toxic effects occur in the test animals, the initial dose for the main test is set at 5 mg/kg BW. If there is no death in the test animals or toxic symptoms appear, the preliminary test is continued by increasing the dose to 50 mg/kg BW.

If, up to a dose of 2000 mg/kg BW, there is no mortality in the test animals, but they exhibit toxic effects, then the initial dose for the main test is set at 2000 mg/kg BW. After 24 hours of dosing, routine observations were continued for the first 4 hours in the test animals [1, 14-18].

Main Test

In the main test, 5 test animals are required per dose level during test preparation. One test animal was obtained from the preliminary test, and four additional test animals were used. The test procedure for the main test is the same as the primary test. After administration of the test preparation, observations were made for the first 30 minutes and periodically every 4 hours for the first 24 hours, then continued once a day for 2 weeks [1, 14-18].

Observation

Things observed during the observation period included the number of animals with toxic symptoms,

the number that died during the test, and behavioral changes in the test animals, such as diarrhea, salivation, weakness, tremors, walking on their stomachs, and walking backward. Observations continued by measuring body weight in test animals and examining macroscopic organs, including changes in color, shape, and weight, especially the liver, kidneys, and heart. In addition, biochemical parameters were observed by measuring the average levels of SGOT, SGPT, creatinine, and urea [14-18].

Data Analysis

Data analysis was carried out using Normality, Paired, and Independent T-tests. Normality test (Normality test) to determine differences in body weight in test animals before and after testing. The Paired T-test was based on the results of the normality test. At the same time, the Independent T-test was used to determine differences between the two treatment groups for organ weight, SGOT, SGPT, creatinine, and urea levels.

Results

Phytochemical Screening of Melinjo Leaves (*Gnetum gnemon L.*)

Phytochemical screening confirmed the content of alkaloids, steroids, triterpenoids, saponins, tannin, phenolic, and flavonoids in the ethyl acetate fraction of melinjo leaves (Table 1). This is in accordance with research that the phytochemical compounds of melinjo leaves contain saponins, tannins, phenolics, and flavonoids [19].

In Silico Toxicity Test Result

In this study, Isoswertiajapon, Isoswertisi, Isovitexi, Swertiajapon, and Swertisin were selected for in silico ADMET prediction using ProTox-II and pkCSM.

pkCSM results showed no AMES toxicity, hERG inhibition, hepatotoxicity, or skin sensitization. Predicted LD_{50} values indicated mild toxicity for isoswertiajaponin and isoswertisin, moderate toxicity for isovitexin, and practically non-toxicity for swertijaponin and swertisin (Tables 2 and 3). Chronic toxicity values (LOAEL) remained within acceptable ranges. Overall, the in silico evaluation suggested that these compounds have favorable pharmacokinetic profiles and low risks of mutagenicity, cardiotoxicity, and hepatotoxicity, supporting their use in pharmacological therapy.

Preliminary Test Results

Preliminary test results showed no toxic or fatal symptoms in the test animals. If the administration of the test preparation at a dose of 2000 mg/kgBW in the preliminary test does not show symptoms of toxicity and death to the test animals, then the dose of 2000 mg/kgBW is determined as the test dose in the main test [19]. The results of the preliminary test observations are shown in Table 4.

Main Test Results

The results of the main test showed that there were no toxic symptoms or deaths in the test animals in the normal group. The 2000 mg/kgBW group, the test preparation of the ethyl acetate fraction of melinjo leaves/kgBW did not affect toxicity, as shown in Table 5.

Results of Changes in Weight in the Animals

Table 6 states that the average weight of the test animals during the 14 days of observation increased and decreased by no more than 10%. If there is no decrease of more than 10% [20], the test preparation has no significant effect on body weight changes in the test animals. A substantial reduction in body weight can indicate poor health, such as inadequate food intake, illness, or toxic symptoms. Increases and decreases in test animals can be caused by erratic appetite, which can also be influenced by unfavorable environmental conditions or increased stress levels [21].

Biochemical Parameter Concentration Results

Based on the examination of biochemical parameters, the average values of SGOT and urea are outside the normal range, whereas SGPT and creatinine

Table 1. Phytochemical screening.

Compound Class	Result
Alkaloids	-
Steroids	-
Triterpenoids	-
Saponins	+
Tannin	+
Phenolic	+
Flavonoids	+

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Information: (+) positive (-) negative

Table 2. Toxicity Class Isoswertiajapon, Isoswertisin, Isovitexin, Swertiajapon, Swertisin (Protox Online).

Parameters	Isoswertiajaponin	Isoswertisin	Isovitexin	Swertiajaponin	Swertisin
Predicted LD50 (mg/kg)	832	832	159	5000	4000
Predicted Toxicity Class	**4**	**4**	**3**	**5**	5
Average Similarity (%)	59.25	60.23	63.84	62.72	61.85
Prediction Accuracy (%)	67.38	68.07	68.07	68.07	68.07
Molecular Weight (g/mol)	462.4	446.4	432.38	462.4	446.4
Number of Hydrogen Bond Acceptors	10	9	9	10	9
Number of Hydrogen Bond Donors	7	6	7	7	6
Number of Atoms	33	32	31	33	32
Number of Bonds	36	35	34	36	35
Number of Rotatable Bonds	4	4	3	4	4
Molecular Refractivity	113.1	111.08	106.61	113.1	111.08
Topological Polar Surface Area (Å²)	190.28	170.05	181.05	190.28	170.05
Octanol/Water Partition Coefficient (logP)	0.10	0.39	0.09	0.10	0.39

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Table 3. Toxicity Prediction of Isoswertiajapon, Isoswertisi, Isovitexi, Swertiajapon, Swertisin (pkCSM).

Toxicity Parameter	Isoswertiajaponin	Isoswertisin	Isovitexin	Swertiajaponin	Swertisin	Unit / Type
AMES Toxicity	No	No	No	No	No	Categorical (Yes/No)
Max. Tolerated Dose (Human)	0.613	0.603	0.649	0.661	0.644	log mg/kg/day
hERG I Inhibitor	No	No	No	No	No	Categorical (Yes/No)
hERG II Inhibitor	Yes	Yes	No	No	No	Categorical (Yes/No)
Oral Rat Acute Toxicity (LD_{50})	2.569	2.515	2.558	2.590	2.555	mol/kg
Oral Rat Chronic Toxicity (LOAEL)	3.795	3.673	5.370	4.232	3.971	log mg/kg_bw/day
Hepatotoxicity	No	No	No	No	No	Categorical (Yes/No)
Skin Sensitization	No	No	No	No	No	Categorical (Yes/No)
Tetrahymena pyriformis Toxicity	0.285	0.285	0.285	0.285	0.285	log µg/L
Minnow Toxicity	5.540	4.508	5.180	4.304	3.469	log mM

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Table 4. Results of Preliminary Test.

Group	Treatment	Number of Rats	Number of Dead Rats	Toxicity Symptoms 1	2	3	4	5	6
Normal	Na-CMC 0.5%	1	0	-	-	-	-	-	-
5 mg/kgBW	Dose 5 mg/kgBW	1	0	-	-	-	-	-	-
50 mg/kgBW	Dose 50 mg/kgBW	1	0	-	-	-	-	-	-
300 mg/kgBW	Dose 300 mg/kgBW	1	0	-	-	-	-	-	-
2000 mg/kgBW	Dose 2000 mg/kgBW	1	0	-	-	-	-	-	-

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Description: 1. Walk with stomach, 2. Walk backwards, 3. Weakness, 4. Tremor, 5. Diarrhea, 6. Salivation
(-) : No symptoms of toxicity occur

are within the normal range. The production of enzymes can cause increased levels of AST due to the development of test animals, increased muscle mass, red blood cell production, and increased liver metabolic activity. It has been shown that SGOT is found in the liver and blood plasma, as well as in muscle cells, pancreas, and heart [22].

The examination of biochemical parameters also showed that urea levels had increased. This is most likely caused by the feed given during the test, which has a high protein content that can improve the release of amino acids into the blood [19].

The results of the biochemical parameter examination were statistically analyzed using an independent T-test to compare the 2000 mg/kgBW dose group with the normal group. The analysis showed no significant difference ($p>0.05$), indicating that administering the test preparation in the form of the ethyl acetate fraction of melinjo leaves did not affect the levels of the biochemical parameters SGOT, SGPT, creatinine, and urea. Biochemical parameters showed no toxicity at the 2000 mg/kgBW dose.

Table 5. Main test results.

Group		Treatment	Dead Rats	Toxicity Symptoms					
				1	2	3	4	5	6
Normal	Rats 1	Na-CMC 0,5%	0	-	-	-	-	-	-
	Rats 2	Na-CMC 0,5%	0	-	-	-	-	-	-
	Rats 3	Na-CMC 0,5%	0	-	-	-	-	-	-
	Rats 4	Na-CMC 0,5%	0	-	-	-	-	-	-
	Rats 5	Na-CMC 0,5%	0	-	-	-	-	-	-
Dose 2000 mg/kg BW	Rats 1	Dose 2000 mg/kg BW	0	-	-	-	-	-	-
	Rats 2	Dose 2000 mg/kg BW	0	-	-	-	-	-	-
	Rats 3	Dose 2000 mg/kg BW	0	-	-	-	-	-	-
	Rats 4	Dose 2000 mg/kg BW	0	-	-	-	-	-	-
	Rats 5	Dose 2000 mg/kg BW	0	-	-	-	-	-	-

Description: 1. Walk with stomach, 2. Walk backwards, 3. Weakness, 4. Tremor, 5. Diarrhea, 6. Salivation
(-) : No symptoms of toxicity occur

Table 6. The average weight of the test animals.

Day-to-day Rats Weight	Group	
	Normal	Dose 2000 mg/kgBW
0	177,488±6,723	174,634±14,332
1	178,66±6,669	175,774±14,269
2	178,4±6,039	175,484±13,406
3	177,676±5,453	173,6±13,771
4	176,982±6,094	174,34±13,880
5	176,816±7,437	173,836±13,367
6	177,194±6,814	174,352±12,912
7	176,39±6,444	174,672±11,804
8	176,598±7,195	174,88±11,577
9	177,252±6,826	175,282±13,164
10	177,532±5,586	174,924±13,841
11	177,294±6,493	173,476±14,399
12	176,268±6,553	173,11±13,352
13	175,926±6,487	172,532±12,679
14	175,996±5,795	173,142±12,950

Organ Macroscopic Results

Based on the macroscopic data for the test animal organs in Table 8, there were no changes in the shape or color of the liver, kidneys, or heart in the normal group. Still, liver abnormalities were observed in the 2000 mg/kg BW group. Macroscopically, a normal liver is brownish-red in color with a smooth, flat surface and has a rubbery consistency, while an

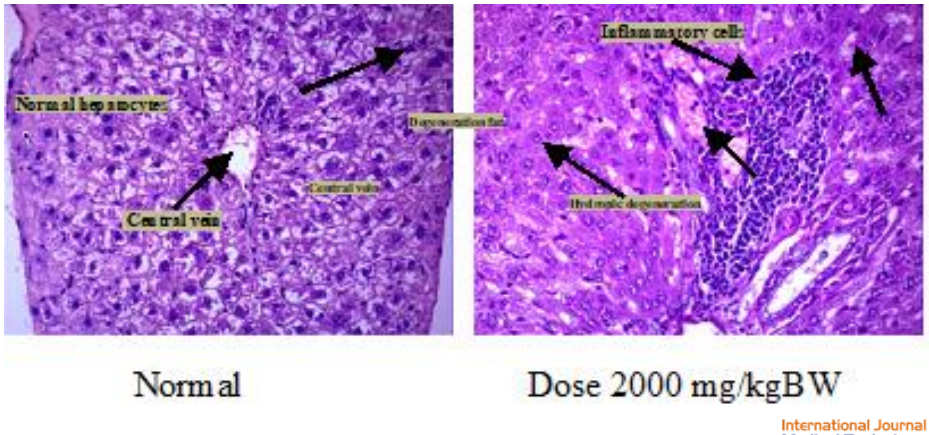

Figure 1. Histopathology of rat liver at 400 x magnification.

Table 7. Results of SGOT, SGPT, creatinine, and urea levels.

Group		Biochemical Parameters			
		SGOT (U/L)	SGPT (U/L)	Creatinin (mg/dL)	Ureum (mg/dL)
Normal	Rats 1	151,15	54,58	0,64	53,83
	Rats 2	169,74	77,97	0,64	41,32
	Rats 3	114,56	68,38	0,58	40,72
	Rats 4	142,15	76,17	0,61	52,04
	Rats 5	143,35	85,17	0,62	42,51
	Average	144,19±19,90	72,45±11,64	0,61±0,02	46,08±6,31
Dose 2000 mg/kgBW	Rats 1	131,96	79,17	0,55	30,54
	Rats 2	196,73	76,17	0,68	48,74
	Rats 3	159,55	72,58	0,61	38,62
	Rats 4	108,56	79,17	0,57	43,71
	Rats 5	124,76	72,98	0,70	48,98
	Average	144,31±34,61	76,01±3,19	0,62±0,06	42,11±7,74

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Table 8. Main organ macroscopic data.

Group	Rat	Liver – Shape	Liver – Color	Liver – Organ index (%)	Kidney – Shape	Kidney – Color	Kidney – Organ index (%)	Heart – Shape	Heart – Color	Heart – Organ index (%)
Normal	1	Normal	Brownish red	4.63	Normal	tanned	0.96	Normal	Dark red	0.58
	2	Normal	Brownish red	4,11	Normal	tanned	0.87	Normal	Dark red	0.52
	3	Normal	Brownish red	4.48	Normal	tanned	0.94	Normal	Dark red	0.54
	4	Normal	Brownish red	4.84	Normal	tanned	1.02	Normal	Dark red	0.54
	5	Normal	Brownish red	5.55	Normal	tanned	0.96	Normal	Dark red	0.56
Dose 2000 mg/kg BW	1	Normal	Brownish red	3.67	Normal	tanned	0.90	Normal	Dark red	0.50
	2	Normal	Brownish red	4,17	Normal	tanned	1.21	Normal	Dark red	0.55
	3	Normal, there are small spots, yellowish white	Brownish red	5.28	Normal	tanned	0.96	Normal	Dark red	0.52
	4	Normal	Brownish red	4.73	Normal	tanned	0.95	Normal	Dark red	0.51
	5	Normal	Brownish red	3.88	Normal	tanned	0.83	Normal	Dark red	0.51

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abnormal liver has spots on its surface, cysts, and a black discoloration means cell death, or a yellow color means organ fat [23, 24].

Macroscopically, normal kidneys are red-brown, smooth, and have a chewy consistency. In contrast, abnormal kidneys are characterized by a paler organ color and show enlargement or swelling of the organs [25]. In this study, macroscopically, the kidneys showed normal results in the normal and 2000 mg/kgBW groups. The macroscopic results of the heart organs in the normal and 2000 mg/kgBW groups showed no organ abnormalities, namely a dark red color and a rubbery consistency. The heart organ is normal if it has a smooth surface, supple consistency, and is dark red to brownish red [26].

The statistical analysis results showed no significant differences ($p>0.05$) in the index of liver, kidney, and heart between the 2000 mg/kgBW group and the normal group. This shows that the ethyl acetate fraction of melinjo leaves at a dose of 2000 mg/kg BW

does not affect the liver, kidneys, or heart.

Liver Histopathological Examination Results

The results of liver histopathology are shown in Table 9 and Figure 1. The results of liver histopathological analysis in the normal group showed a score of 0 for hydropic degeneration, fatty degeneration, and necrosis. In contrast, in the 2000 mg/kgBB group, liver hepatocytes showed hydropic degeneration and necrosis. The percentage of hydropic degeneration in the 2000 mg/kgBW dose group was 1, indicating that the liver underwent 25-50% hydropic degeneration, parenchymal degeneration, and apoptosis extending to the middle area (midzone). Cell damage known as hydropic degeneration is characterized by cytoplasmic swelling. When active transport is disrupted, cells cannot pump Na+ out, leading to increased intracellular Na+. This causes hydropic degeneration to occur [27]. A necrosis score of 2 on liver histopathology indicates hepatocyte damage of 6–25% of the total observed area, which is considered moderate. Liver histopathology was non-toxic in the 2000 mg/kgBW dose group. This is consistent with Table 3's prediction of no hepatotoxicity.

Discussion

The phytochemical profile of the ethyl acetate fraction of Gnetum gnemon leaves indicates enrichment in semi-polar secondary metabolites, particularly saponins, tannins, phenolics, and flavonoids. This composition is pharmacologically relevant because phenolic–flavonoid constituents are commonly associated with antioxidant and anti-inflammatory activities, which can mitigate early toxicodynamic processes such as oxidative stress and inflammatory signaling. Therefore, the detected metabolite classes provide a plausible biochemical basis for the generally favorable safety signals observed in the acute setting and support the expectation of a relatively wide safety margin for the fraction at the tested exposure.

The convergence between in silico ADMET predictions and in vivo observations strengthens the interpretation that the selected marker compounds exhibit a low probability of major safety liabilities at the screening level. Specifically, the absence of predicted AMES toxicity, hepatotoxicity, and skin sensitization, together with the lack of clinically observable toxicity signs and mortality following high-dose administration, suggests a low acute risk of mutagenicity and overt systemic toxicity. Nevertheless, the predicted hERG II inhibition for some compounds warrants a cautious stance, as it highlights the need for targeted follow-up assays (e.g., ion-channel/electrophysiological testing) if the fraction or isolated constituents are advanced toward therapeutic development, particularly for repeated dosing or use in vulnerable populations.

Overall, biochemical and organ assessments indicate that the ethyl acetate fraction did not produce a statistically significant impairment of hepatic or renal function at the tested dose, despite variability in transaminase and urea values that may reflect physiological variation, diet composition, and metabolic adaptation during the observation period. Importantly, the focal macroscopic liver finding and histopathological evidence of hydropic degeneration with moderate necrosis suggest the possibility of localized, subclinical tissue responses that serum biomarkers may not fully capture in an acute timeframe. This underscores the value of an integrated safety evaluation combining clinical observations, biochemical markers, gross pathology, and histopathology, and it provides a clear rationale for subacute/subchronic studies to determine whether these microscopic changes remain transient or could progress under repeated exposure.

Conclusion

In silico predictions using SwissADME, Pro-Tox II, and pkCSM showed LD_{50} values ranging from 159 to 5000 mg/kg. The toxic dose range for the ethyl acetate fraction of melinjo leaves (*Gnetum gnemon* L.) was determined to be >2000 mg/kg BW, indicating it is practically non-toxic. The ethyl acetate fraction of melinjo leaves (*Gnetum gnemon* L.) at a dose of 2000 mg/kgBW does not affect the macroscopic organs of the liver, kidneys, and heart.

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Ethical Approval

The Research Ethics Committee of Universitas Ahmad Dahlan, Yogyakarta, Indonesia approved this study. Ethical clearance was granted under approval number 022301013, issued on 16 February 2023. All experimental procedures involving animal subjects were conducted in accordance with institutional ethical guidelines and relevant regulations for the care and use of laboratory animals.

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Conflicts of Interest

The authors report there are no competing interests to declare.

References

[1] BPOM RI. Peraturan BPOM Nomor 12 Tahun 2014: Persyaratan keamanan dan mutu obat tradisional. BPOM RI. 2014;11:1–16. [Link]

[2] Febriyanti RM, Saefullah K, Susanti RD, Lestari K. Knowledge, attitude, and utilization of traditional medicine within the plural medical system in West Java, Indonesia. BMC Complement Med Ther. 2024;24:64. [DOI: 10.1186/s12906-024-04264-0]

[3] Trisha MR, Gunawan VD, Wong JX, Pak Dek MS, Rukayadi Y, et al. Antibacterial effect of ethanolic Gnetum gnemon L. leaf extract on food-borne pathogens and its application as a natural preservative on raw quail eggs. Heliyon. 2024;10:e35691. [DOI: 10.1016/j.heliyon.2024.e35691]

[4] Sari DP, Fahriati AR, Maelaningsih FS. Formulation of instant granules from ethanolic extract of melinjo peel (Gnetum gnemon L.) as anti-hyperuricemia. J Kefarmasian Indones. 2023;13:140–9. [Link]

[5] Sari M, Murniasih T, Adiguna SP, Siwi OGR, Artika IM, Putra MY. Evaluation of quality parameters and antioxidant activity of commercial melinjo (Gnetum gnemon) for functional food. Pharm Sci Asia. 2023;50:296–306. [DOI: 10.29090/psa.2023.04.296]

[6] Herlina, Solihah I, Wijaya DP, Nurrahmah AN. Antidiarrheal activity of ethanolic extract of melinjo leaves (Gnetum gnemon L.) in male Wistar rats induced by Escherichia coli. Int J Appl Pharm. 2022;14:60–6. [DOI: 10.22159/ijap.2022.v14s3.25]

[7] Herlina, Wijaya DP, Starlista V, Amriani A, Noviarny H, et al. Hypoglycemic potential of ethanol extract of melinjo leaves (Gnetum gnemon L.) in male Wistar rats. Pharmacoscript. 2025;8:168–86. [Link]

[8] Herlina, Hayati F, Wijaya DP, Belinda R. Antihyperlipidemia activity of ethyl acetate fraction from melinjo (Gnetum gnemon Linn.) leaf in male Wistar rats induced by propylthiouracil. Proc 2nd Int Conf Contemp Sci Clin Pharm. 2022;40:110–9. [DOI: 10.2991/ahsr.k.220303.018]

[9] Wardani VR, Fatimah S, Nadia, Cahyani IM. Antihypercholesterolemic activity of ethanolic extract of melinjo peel (Gnetum gnemon L.). Media Farmasi Indones. 2018;14:1466–70. [Link]

[10] Wardani VR, Fatimah S, Nadia, Cahyani IM. Antihypercholesterolemic activity of ethanolic extract of melinjo peel (Gnetum gnemon L.). Media Farmasi Indones. 2018;14:1466–70. [Link]

[11] Herlina H, Untari B, Solihah I. Antidiabetic activity test of ethanolic extract of Muntingia calabura leaves in alloxan-induced male rats. Sci Technol Indones. 2018;3:7–13. [DOI: 10.26554/sti.2018.3.1.7-13]

[12] Herlina H, Untari B, Solihah I, Santia M. Antihyperlipidemic activity of ethanol extract of Melia azedarach leaves in propylthiouracil-induced male Wistar rats. Sci Technol Indones. 2019;4:222–7. [DOI: 10.26554/sti.2019.4.1.24-30]

[13] Herlina H, Amriani A, Solihah I, Damayanti P, Rais SW, et al. Effectiveness of ethanolic extract of Senna alata leaves as antidiabetic in alloxan-induced male Wistar rats. J Phys Conf Ser. 2019;1282:012085. [DOI: 10.1088/1742-6596/1282/1/012085]

[14] Huong NT, Hop NQ, Duy DA, Son NT, et al. The genus Gnetum: Traditional use, phytochemistry, nutritional value, pharmacology, toxicology, and pharmacokinetics. Fitoterapia. 2025;168:106461. [DOI: 10.1016/j.fitote.2025.106461]

[15] Herlina H, Amriani A, Sari I, Apriani E. Acute toxicity test of ethanolic extract of Cosmos caudatus leaves in male Wistar rats using fixed dose procedure. J Adv Pharm Technol Res. 2021;12:157–61. [DOI: 10.4103/japtr.JAPTR_90_20]

[16] Herlina H, Amriani A, Wijaya DP, Lestari AA. Acute toxicity of melinjo (Gnetum gnemon L.) leaf

extract using fixed dose procedure. Indones J Pharm Sci Technol. 2022;9:140–6. [DOI: 10.24198/ijpst.v9i3.33683]

[17] Zhou JY. Innate immunity and early liver inflammation. Front Immunol. 2023;14:1–13. [DOI: 10.3389/fimmu.2023.1179341]

[18] Jensen VS, Fledelius C, Zachodnik C, Damgaard J, Nygaard H, Tornqvist KS, et al. Insulin treatment improves liver histopathology in a dyslipidemic hamster model of NAFLD. J Transl Med. 2021;19:1–21. [DOI: 10.1186/s12967-021-02729-1]

[19] Anisong N, Siripongvutikorn S, Wichienchot S, Puttarak P. Nutritional contents and functional properties of Gnetum gnemon Linn.: a comprehensive review. Food Sci Technol. 2022;42:e100121. [DOI: 10.1590/fst.100121]

[20] OECD. Test No. 407: Repeated dose 28-day oral toxicity study in rodents. OECD Guidelines. 2008. [Link]

[21] van Berlo D, Woutersen M, Muller A, Pronk M, Vriend J, Hakkert B. Body weight change as criterion for maximum tolerated dose. Regul Toxicol Pharmacol. 2022;134:105221. [DOI: 10.1016/j.yrtph.2022.105221]

[22] ECETOC. Guidance on dose selection. Technical Report No. 138. 2021. [Link]

[23] Bemidinezhad A, Zojaji SA, Taraz Jamshidi S, Mohammadi M, Alavi MS, Ghorbani A. Acute and subchronic toxicity evaluation of a hepatoprotective herbal formulation. Toxicol Rep. 2023;11:452–9. [DOI: 10.1016/j.toxrep.2023.11.002]

[24] Kundu SK, Khan MAHNA, Das SK. Beneficial role of mushroom in hypercholesterolemia. Indones J Pharm Clin Res. 2021;4:1–14. [Link]

[25] Ardana T, Yuandani, Satria D, Putra EDL, Muhammad M, Rosidah. Acute toxicity evaluation of Curcuma domestica rhizome extract. Int J Appl Pharm. 2024;16:44–51. [DOI: 10.22159/ijap.2024.v16s4.19]

[26] Fernando BNTW, Sudeshika TSH, Hettiarachchi TW, Badurdeen Z, Abeysekara TDJ, Abeysundara HTK, et al. Biochemical profile of chronic kidney disease of uncertain etiology in Sri Lanka. PLoS One. 2020;15:e0232522. [DOI: 10.1371/journal.pone.0232522]

[27] Sinkala M, Zulu M, Kaile T, Simakando M, Chileshe C, Kafita D, et al. Kidney injury molecule-1 performance in kidney disease diagnosis. Int J Appl Basic Med Res. 2017;7:94–9. [DOI: 10.4103/2229-516X.205813]

[28] Choi JH. Histological and molecular evaluation of liver biopsies: a practical review. Int J Mol Sci. 2025;26:1–22. [DOI: 10.3390/ijms26165234]