Trop J Nat Prod Res, July 2025; 9(7): 3092 - 3096 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Hepatoprotective Effect of Ethanol Extract of Chinese Betel Herb (*Peperomia pellucida (L.)*) On CCl_4-Induced Hepatotoxicity in Experimental Animals

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ARTICLE INFO

Article history:
Received 11 May 2025
Revised 29 May 2025
Accepted 13 June 2025
Published online 01 August 2025

ABSTRACT

Chinese betel herb (*Peperomia pellucida*) contains secondary metabolites, such as alkaloids, flavonoids, phenolics, tannins, saponins, and terpenoids with pharmacological potential. Therefore, this study aims to evaluate the hepatoprotective effect of ethanol extract of *P. pelluica* herb (EPP) on liver damage caused by CCl_4 toxicity. The test was conducted for twenty-one days on 30 male Wistar rats divided into six groups, namely normal control (Na CMC 0.5%), negative control (CCl_4 1 mL/kg BW), positive control (Silymarin 100 mg/kg BW), EPP test treatment group with doses of 100, 200, and 400 mg/kg BW. The protective effect of the extract was evaluated based on liver biochemical parameters, macroscopic analysis, and histopathology. The study showed that the EPP group experienced a significant decrease in SGPT, SGOT, ALP, and Total Bilirubin levels. Macroscopic observations and liver organ indices showed liver criteria that resembled normal. Meanwhile, histopathology analysis showed a linear improvement in liver tissue structure with increasing doses. EPP herb can potentially protect the liver from damage caused by CCl_4 hepatotoxicity.

Keywords: Hepatotoxicity, *Peperomia pellucida*, Protective effect.

Introduction

The liver is the center of metabolism and the main organ responsible for detoxifying various toxins that enter the body. Due to its complex functions, the liver has been reported to be highly susceptible to damage. Many risk factors can trigger oxidative stress, such as chemical compounds, biological agents, radiation, alcohol, and environmental pollutants. Untreated oxidative stress can trigger various types of liver diseases, including fatty liver, cirrhosis, fibrosis, and cancer, which cause community health problems worldwide.[1–3] In addition, liver dysfunction is a prevalent issue in the world of health. Based on World Health Statistics (2024), it is estimated that approximately 304 million people in the world suffer from chronic hepatitis B and C. In 2022, there were 2.2 million new cases of hepatitis, of which 1.2 million were hepatitis B and 1 million were hepatitis C.[4] In Indonesia, data from the 2023 Indonesian Health Survey showed that the prevalence of hepatitis was 0.12% or 877.531 people.[5] This figure is relatively large, demonstrating the need for preventive measures against diseases related to liver function.In recent years, several hepatoprotective agents have been developed from natural products to prevent and treat various diseases.[1] Medicinal plants and their secondary metabolite content, including lignans, alkaloids, phenolics, and flavonoids, play an essential role as hepatoprotective agents.

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Citation: Fitrya F, Elfita E, Kurniawaty NF, Ahsan AN, Amriani A, Agustiarini V, Novita RP. Hepatoprotective effect of ethanol extract of Chinese betel herb (*Peperomia pellucida* (L.)) on CCl_4-induced hepatotoxicity in experimental animals. Trop J Nat Prod Res. 2025; 9(7): 3092 – 3096 https://doi.org/10.26538/tjnpr/v9i7.21

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

The use of products from medicinal plants in treating liver injury is increasingly popular due to their effectiveness, efficacy, and safety. Several *in vitro* and clinical studies have shown that medicinal plant extracts can reduce liver damage caused by chemicals.[6] A plant that has the potential to protect liver function or serve as a hepatoprotector is *Peperomia pellucida L.* (Piperaceae), also known as sirih cina in Indonesia. *P. pellucida* is traditionally used by various cultures worldwide to treat various diseases, such as conjunctivitis, seizures, fatigue, fever, headache, gout, rheumatoid arthritis, skin diseases, high blood cholesterol levels, and breast cancer.[7,8] In addition, the plant is an annual weed that is widespread in tropical and subtropical regions, including Central and South America, Africa, Southeast Asia, and Australia. Due to its wide distribution, this species is not threatened with extinction. *P. pellucida* has been reported to contain diverse phytochemicals, namely terpenes, polyphenols, flavonoids, and alkaloids. These phytochemicals contain various pharmacological potentials that produce different pharmacological activities, such as antibacterial, antihypertensive, antioxidant, anti-inflammatory, antihyperglycemic, antihypercholesterolemic, and antiangiogenic.[7–10] Therefore, this study aims to evaluate the protective effect of *P. pellucida* on test animals induced with hepatotoxicity using carbon tetrachloride (CCl_4).

Materials and Methods

Plant Material and Chemicals

Samples were obtained in the Indralaya area, Ogan Ilir Regency, South Sumatra. Plant identification was carried out at the Andalas University Herbarium with voucher number 407/K-1D/ANDA/2024. Subsequently, the Chinese betel herb was sorted, washed, and dried in indirect sunlight. The chemicals used included 96% ethanol (Brataco®), silymarin (Hubei Nokete Pharmaceutical, Singapore), CCl_4 (Sigma Aldrich®), Carboxymethylcellulose Sodium, olive oil, kit reagents for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin; hematoxylin-eosin (HE) staining.

Extract Preparation
P. pellucida powder was extracted using the maceration method with 96% ethanol solvent. A total of 500 g of *P. pellucida* powder was placed into a vessel and 96% ethanol (3 L) was added. Furthermore, it was filtered using filter paper, the residue was re-macerated (2 x 2 L). The extract was concentrated with a rotary evaporator (Biobase®) to obtain a thick *P. pellucida* extract (EPP).

Experimental animals
Thirty male Wistar rats (190-210 g) were acclimatized for one week to adapt to the laboratory environment. The Ahmad Dahlan University Ethics Committee approved the procedure for handling test animals with No. 022410140. Animals were randomly divided into six groups (n = 5). The extract was tested at three levels of EPP doses, namely 100, 200, and 400 mg/kg BW. The hepatoprotective effect of EPP was compared to normal control (Na-CMC 0.5% p.o), positive control (silymarin 25 mg/kg BW p.o), and negative control-induced hepatotoxic but not treated. This test was preventive; all test animals were treated according to their group for 21 days. On the 22nd day, the animals were induced using hepatotoxic with CCl_4 1 mL/kg BW (i.p), except for the normal group. The following day, blood was obtained through the retro-orbital vein to measure the levels of liver biochemical parameters. Furthermore, the animal dissection was performed for macroscopic observation and histopathology of the liver organ.[11,12]

Measurement of Liver Biochemical Parameter Levels
The liver biochemical parameters (SGPT, SGOT, ALP, and Total Bilirubin) were measured using a biochemical analyzer (BioSystem BA200®). A total of 4 mL of blood was centrifuged for 10 minutes (3000 rpm) until the serum was separated from the plasma. This serum was taken and used to measure SGPT, SGOT, ALP, and Total Bilirubin levels. A total of 100 µL of serum was added to 1000 µL of the reagent kit. The absorbance of SGPT and SGOT was read at a wavelength of 365 nm, ALP at 405 nm, and total bilirubin at 546 nm.[13]

Macroscopic Observation of the Liver
The liver sample was collected and washed using physiological NaCl 0.9% (b/v). Macroscopic observations of the liver were conducted on the color, texture, and weight. The liver weight ratio (Liver Index) was calculated using the equation: *(liver weight)/(rats body weight) x 100%*.

Histopathology Observation of the Liver
Histopathology observation was carried out using standard procedures through several stages, namely fixation, dehydration and clearing, embedding, sectioning, as well as mounting and staining. The liver organ was fixed in 10% formalin buffer solution, then dehydrated using alcohol with graded concentrations (70% to 10%), followed by clearing using xylol. Furthermore, the process was continued by embedding the liver into liquid paraffin and allowing it to harden. The sample was cut using a microtome with a thickness of 4 to 5 µm, then attached to a glass object and stained with HE.[12]

Data Analysis
The test result data was processed using SPSS 25.0 software, normality was tested with Shapiro-Wilk, and the value was considered to represent the population or normally distributed when p>0.05. This study's analysis was continued with a one-way ANOVA test for normally distributed data. Furthermore, the Post Hoc Tukey HSD test was used to determine the significance of the data between treatment groups.

Results and Discussion

Extraction of 500 grams of Chinese betel herb simplicia produced 99.04 g of EPP extract, with a yield percentage of 19.81%.

Biochemical Parameters
The test results showed that liver biochemical parameter data were normally and homogeneously distributed (p > 0.05) in all groups. The results of the one-way ANOVA test, data on ALT, AST, ALP, and total bilirubin levels showed a p-value <0.05, showing a significant difference between treatment groups. As shown in Figure 1, CCL_4 induction significantly caused hepatotoxic effects, marked by increased transaminase and ALP enzymes. The diseased group (CCL_4) showed high levels of ALT, AST, ALP, and total bilirubin, significantly different from the normal control and silymarin groups (p <0.05). EPP administration could not reach the same level as the silymarin group (0 <0.05), but at a dose of 400 mg/kg BW, it could reduce ALT levels by up to 50% (p <0.05). Meanwhile, EPP significantly reduced AST levels at a dose of 400 mg/kg, there was no difference from the silymarin control group (p>0.05), as well as ALP levels.

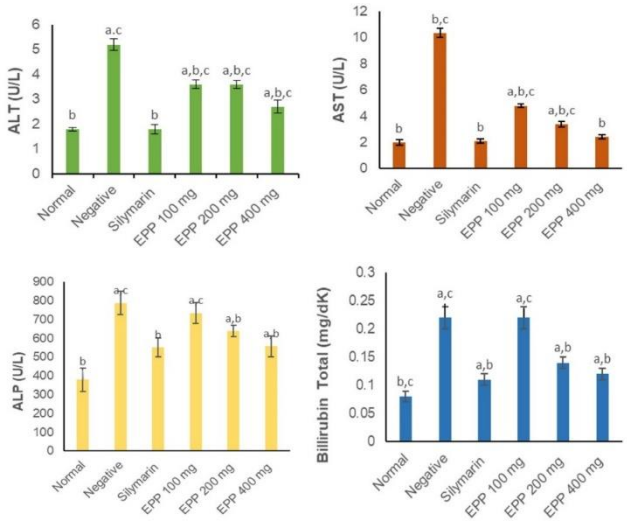

Figure 1: Effect of *P. pellucida* extract on changes in liver biochemical parameters. Description: Small letters above the graph showed significant differences (p<0.05) in (a) the normal group; (b) the negative control group; and (c) the positive control group.

The liver was the primary organ for the metabolism of several types of xenobiotics, involving various biochemical processes such as oxidation, reduction, hydrolysis, hydroxylation, sulfonation, acylation, and conjugation. In this study, the liver's metabolic function could be disrupted due to injury caused by various hepatotoxins such as CCl_4, acetaminophen, thioacetamide, and ethanol.[14] Among hepatotoxic compounds, CCl_4 is a common toxin used to evaluate the hepatoprotective properties of a substance, because it produces damage similar to the pathology involved in human liver disease.[2] Furthermore, CCL_4 hepatotoxicity produced metabolic and morphological changes with high reproducibility.[14,15] CCL_4 is metabolized in the liver endoplasmic reticulum by cytochrome P450 to trichloromethyl radical ($CCL_3^{\bullet}$), then forming trichloromethyl peroxyl radical ($CCl_3O_2^{\bullet}$).[14,16] This radical is highly reactive; as a result, it causes lipid peroxidation and inhibits antioxidant enzymes, leading to oxidative stress, inflammation, apoptosis, and necrosis.[17,18] Lipid peroxidation is characterized by leakage of liver enzymes such as transaminase and ALP into the blood. This study proved that CCL_4 caused an increase in ALT, AST, and ALP enzyme levels in the negative control group (Figure 1). An increase in these enzyme levels was a sign of liver injury due to loss of hepatocyte membrane integrity.[16,19] A significant decrease (p <0.05) in transaminase levels observed in mice treated with EPP doses of 200 and 400 mg/kg showed the inhibitory effect of hepatocellular injury by *P. pellucida* extract. Although unable to reach normal levels or effects equivalent to silymarin control, EPP, specifically at a dose of 400 mg/kg BW, could significantly reduce ALT and AST levels, reaching half to a quarter of the CCL_4 control values. The results of this study strengthened the report that *P. pellucida* extract did not cause hepatotoxic effects, although, in the study, there were no hepatotoxic inducing agents other than distilled water.[20] The AST and ALT values from this study were smaller when compared to the reported AST and ALT values,[20] but were within the normal range of AST and ALT values [21] and the changes shown were consistent with the previously reported hepatoprotective effects.[16] Therefore, the

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

significant decrease ($p < 0.05$) in ALP observed in EPP-treated rats could be a clinical indication of cholestasis inhibition by extract.[11,22]
At 200 and 400 mg/kg doses, EPP also reduced total bilirubin levels, although it could not reach values like the normal group ($p<0.05$), the effect was no different from the silymarin group ($p>0.05$) (Figure 1). Total bilirubin is the sum of conjugated and unconjugated bilirubin in the blood.[13] In hepatotoxic conditions, serum bilirubin levels increase due to inadequate absorption of bilirubin by the liver.[11] This study showed high bilirubin levels in negative control rats compared to normal controls. In contrast, groups treated with EPP extract at 200 and 400 mg/kg doses showed a significant decrease in bilirubin levels ($p < 0.05$). These results were similar to other studies showing that administration of plant extracts decreased bilirubin after hepatotoxic induction with CCl_4.[23] The decrease in the value of these biochemical parameters was attributed to the presence of phytoconstituents in the extract, such as phenolic, flavonoids, terpenes, and alkaloids, that could protect the hepatocyte membrane.[11,19]

Liver Organ Macroscopic
The results of macroscopic observations of the liver are shown in Figure 2. The normal group generally showed normal liver conditions with a reddish-brown color, smooth surface, no spots, and no nodules on the surface. In contrast, the liver organ of the negative control group showed abnormal liver architecture, namely a paler color, a spotted surface, and nodules were found. The formed nodules showed necrosis or cell death in the liver tissue. Meanwhile, the EPP and silymarin groups showed macroscopic images of the liver organ that were similar to the normal group (Figure 2).

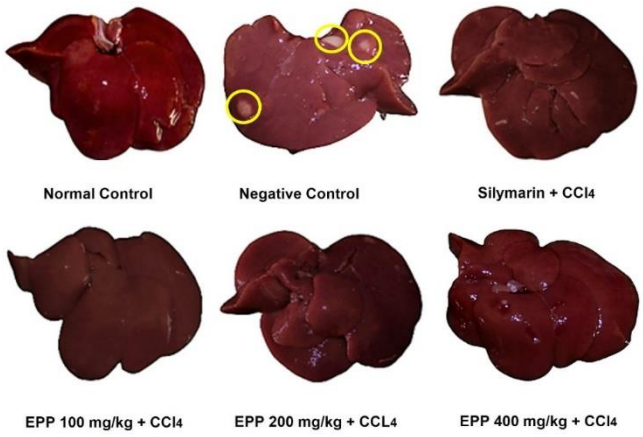

Figure 2: The effect of *P. pellucida* extract on the macroscopic profile of the liver induced by CCl_4 hepatotoxicity. In the negative control group, nodules were found (yellow circles).

The relative weight of the liver (Liver Index) is presented in Table 1. The results of the normality and homogeneity tests of the liver index showed that the data were normally distributed and homogeneous ($p>0.05$). The analysis results with one-way ANOVA showed that the liver index data exhibited significant differences between groups ($p<0.05$). Tukey HSD analysis showed that the negative control group was significantly different from the normal group, positive control, and treatment group ($p<0.05$). The administration of EPP showed a change in the value of the liver organ ratio approaching normal, consistent with the increase in dose. Although it could not reach the equivalent of the normal group and silymarin control ($p<0.05$), the administration of EPP showed a significant decrease and was different from the negative control group ($p<0.05$), as presented in Table 1. The organ index was a more sensitive value than organ weight.[24] The large liver index value in the CCL_4 control group (Table 1) showed an increase in liver mass, which was associated with fat accumulation and liver tissue disintegration.[3] Animals treated with EPP showed a liver organ profile that was similar to normal, and an organ index that decreased with increasing dose.

Table 1: Effect of *P. pellucida* extract on liver index

Groups	Liver Index (%)
Normal control	2.33 ± 0.05^{b}
Negative control	$3.53 \pm 0.14^{a,c}$
Silymarin + CCl_4	2.38 ± 0.07^{b}
EPP 100 mg + CCl_4	$3.09 \pm 0.05^{a,b,c}$
EPP 200 mg + CCl_4	$2.85 \pm 0.07^{a,b,c}$
EPP 400 mg + CCl_4	$2.58 \pm 0.11^{a,b,c}$

Description: Data were presented as mean (n = 5) ± SD. The lower-case letters of the liver index values showed significant differences ($p <0.05$) among (a) the normal group, (b) the negative control group, and (c) the positive control group.

Liver Microscopy
Histopathological examination was conducted to confirm the results of biochemical analysis and macroscopic observations. CCL_4 is a liver toxin that could cause direct damage to hepatocytes, causing necrosis, inflammation to cirrhosis, and carcinoma.[23,25] In this study, the administration of EPP extract significantly improved the structure of liver tissue induced by CCl_4. The higher the extract concentration, the better the liver architecture. Figure 3 shows the results of histological observations on the liver, and the results of the analysis of the level of tissue damage are presented in Table 2.

Table 2: Assessment of the analysis results of the level of liver organ damage

Groups	Steatosis	Inflammation	Necrosis
Normal control	0	0	0
Negative control	3	3	3
Silymarin + CCl_4	0	0	0
EPP 100 mg + CCl_4	2	1	2
EPP 200 mg + CCl_4	1	1	0
EPP 400 mg + CCl_4	0	1	0

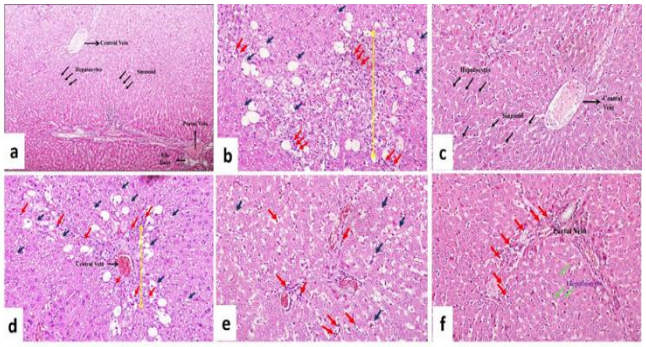

Figure 3: Histopathological image of the liver of animals induced by hepatotoxicity and treated with EPP (H&E; magnification 400 times (a) Normal Control, (b) Negative Control, (c) Positive Control, (d) EPP 100 mg/kg, (e) EPP 200 mg/kg, (f) EPP 400 mg/kg.
Description: Steatosis; Inflammation; Necrosis

The histopathological analysis of the liver showed no morphological changes in the normal group, indicating no steatosis, inflammation, or necrosis. The liver architecture appeared intact, and hepatocytes, sinusoids, central veins, and portal veins were in normal condition (Figure 3a). The negative control group showed damage to the liver

tissue in the form of steatosis, inflammation, and necrosis (Figure 3b). Steatosis formed in the tissue exceeded 60%, and necrosis was more than 50% of the tissue. These results were consistent with the study of,[25] that CCl_4 triggered the formation of steatosis in liver tissue. Steatosis refers to a condition in which more than five percent of hepatocytes experience fat accumulation.[11] Although it was reversible, ~~but~~ when the damage was severe enough and lasted a long time, it became irreversible in the form of necrosis.[16] In the negative control, inflammatory cells were also found to spread throughout the liver tissue (diffuse) with grade 3. This inflammation was characterized by the accumulation and activation of Kupffer cells in the sinusoid area and could be due to the entry of CCl_4 into liver cells, activating Kupffer cells, which then release pro-inflammatory cytokines such as TNF-α and nitric oxide. Untreated inflammation progressed to necrosis.[16]
However, no inflammation and necrosis were found in the positive control group; hepatocyte cells in this group were similar to normal (Figure 3c). Meanwhile, in the EPP group with a dose of 100 mg/kg BW (Figure 3d), steatosis, inflammation, and necrosis were found, ranging from 31 to 60% steatosis, mild inflammation, and less than 10% necrosis. In the EPP group with a dose of 200 mg/kg BW, steatosis and inflammation were found with a mild grade, and no necrosis (Figure 3e). Histopathological observations showed that inflammation and steatosis were significantly reduced in rats treated with 400 mg/kg BW EPP (Figure 3f); this group showed normal hepatocyte cells and were polygonal in shape with extensive granular cytoplasm, no steatosis or necrosis was found, but there was still inflammation with a mild grade. These changes were consistent with the improvement in liver cell structure due to CCl_4, which was produced by date fruit extract.[19]
The protective effect produced by EPP was associated with extract's antioxidant and anti-inflammatory activities. The underlying mechanisms of its efficacy were antioxidant, anti-inflammatory, free radical scavenging, and the ability to block oxidative stress, cytokine production, and stabilize liver cell membranes.[6] EPP had been reported to increase levels of catalase, SOD, and glutathione enzymes and decrease levels of IL-6 and TNFα in mice exposed to aluminium chloride toxicants.[7,26] The main phytoconstituents of *P. pellucida* that produced effects were alkaloids, flavonoids, sterols, triterpenoids, phenols, and quinones.[8,27] Flavonoid compounds, vitexin, isovitexin, isoswertisin, pellucidatin, and caryatin rhamnoside, as well as various lignan compounds such as sesamin, piperomine A-E from *P. pellucida* extract, contributed to antioxidant and anti-inflammatory effects to extract.[28] Vitexin and isovitexin showed anti-inflammatory activity through activation of the MAPK signaling pathway and inhibited the secretion of COX-2, IL-1β, IL-6, MCP-1, and TNF-α.[29] Furthermore, Dillapiole and apiole isolated from *P. pellucida* were anti-inflammatory, and benzodioxol was the group that played a role in the activity.[30] The main terpenes in *P. pellucida*, such as a-terpineol and phytol, could attenuate the expression of IL-6 receptors, and suppress the secretion of TNF-α and IL-1β, which led to reduced leukocyte migration.[9,31] Suppression of inflammatory marker expression attenuated the activation of the JAK-STAT and NF-jB signaling pathways. The antioxidant and anti-inflammatory mechanisms that had been reported could explain the protective mechanism shown by *P. pellucida* extract against liver injury caused by CCL_4.

Conclusion
In conclusion, the results of this study show that the ethanol extract of *P. pellucida* had the potential to protect the liver organs of rats induced by hepatotoxicity with CCl_4. The results suggested that *P. pellucida* extract may be used as a new hepatoprotective herb and had the potential to be used as a functional food.

Conflict of Interest
Authors declare no conflict of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements
The authors are grateful to the Pharmacological Laboratory of the University of Sriwijaya for the support of the experimental facilities.

References

1. Qu J, Huang P, Zhang L, Qiu Y, Qi H, Leng A, Shang D. Hepatoprotective effect of plant polysaccharides from natural resources: A review of the mechanisms and structure-activity relationship. Int. J. Biol. Macromol. 2020; 161:24–34. Doi: 10.1016/j.ijbiomac.2020.05.196.
2. Feng XH, Xu HY, Wang JY, Duan S, Wang YC, Ma CM. In vivo hepatoprotective activity and the underlying mechanism of chebulinic acid from *Terminalia chebula* fruit. Phytomedicine. 2021; 83:1–10. Doi: 10.1016/j.phymed.2021.153479
3. Hamad Shareef S, Abdel Aziz Ibrahim I, Alzahrani AR, Al-Medhtiy MH, Ameen Abdulla M. Hepatoprotective effects of methanolic extract of green tea against Thioacetamide-Induced liver injury in Sprague Dawley rats. Saudi J. Biol. Sci. 2022; 29:564–573. Doi: 10.1016/j.sjbs.2021.09.023
4. World Health Organization. World health statistics 2024: Monitoring health for the SDGs, Sustainable Development Goals. 2024.
5. Ministry of Health of Indonesia. Indonesian Health Survey 2023. Jakarta: 2023. 185-188. p
6. Gonfa YH, Bachheti A, Semwal P, Rai N, Singab AN, Bachheti R kumar. Hepatoprotective activity of medicinal plants, their phytochemistry, and safety concerns: a systematic review. Zeitschrift Für Naturforsch C. 2025; 80:61–73. Doi: 10.1515/znc-2024-0116
7. Ndudi J, Yovwin GD, Igben VO, Abe B, Akoko S, Oritsemuelebi B, Chimezie J, Emeka A & Dagogo G. Chemical characterization, in vivo anti-oxidant, anti-inflammatory and anti- apoptotic properties of *Peperomia pellucida* in modulating vascular tone in Swiss mice exposed to aluminium chloride. Pharmacol. Res. Nat. Prod. 2024; 3:1–11. Doi: 10.1016/j.prenap.2024.100034
8. Alves NSF, Setzer WN, da Silva JKR. The chemistry and biological activities of *Peperomia pellucida* (Piperaceae): A critical review. J. Ethnopharmacol. 2019; 232:90–102. Doi: 10.1016/j.jep.2018.12.021
9. Ho KL, Yong PH, Wang CW, Kuppusamy UR, Ngo CT, Massawe F, Ng ZX. *Peperomia pellucida* (L.) Kunth and eye diseases: A review on phytochemistry, pharmacology and toxicology. J. Integr. Med. 2022; 20(4):292–304. Doi: 10.1016/j.joim.2022.02.002
10. Ahmad I, Ambarwati N, Elya B, Omar H, Mulia K, Yanuar A, Negishi O & Muńim A. A new angiotensin-converting enzyme inhibitor from *Peperomia pellucida* (L.) Kunth. Asian Pac. J. Trop. Biomed. 2019; 9(6):257–262. Doi: 10.4103/2221-1691.260398
11. Amang AP, Kodji E, Mezui C, Baane MP, Siwe GT, Kuissu TM, Emakoua J & Tan PV. Hepatoprotective Effects of Aqueous Extract of *Opilia celtidifolia* (Opiliaceae) Leaves against Ethanol-Induced Liver Damage in Rats. Evidence-based Complement. Altern. Med. 2020; 2020:1–8. Doi: 10.1155/2020/6297475
12. Fitrya F, Amriani A, Puspa Novita R, Fatya Sahara V, Rizki M, Rahma M,Muharni, M & Wahyuni FS. Hepatoprotective effect of the ethyl acetate fraction of Indonesian Artocarpus species against CCl_4 induced liver injury in rat. J. Res. Pharm. 2024; 28(4):952–960. Doi: 10.29228/jrp.778.
13. Sinaga E, Fitrayadi A, Asrori A, Rahayu SE, Suprihatin S, Prasasty VD. Hepatoprotective effect of *Pandanus odoratissimus* seed extracts on paracetamol-induced rats. Pharm. Biol. 2021; 59(1):31–39. Doi: 10.1080/13880209.2020.1865408
14. Rakib A, Ahmed S, Islam MA, Haye A, Uddin SMN, Uddin MMN, Hossain MK, Emran TB. Antipyretic and

hepatoprotective potential of *Tinospora crispa* and investigation of possible lead compounds through in silico approaches. Food Sci. Nutr. 2020; 8(1): 547–556. Doi: 10.1002/fsn3.1339

15. Hashem MM, Salama MM, Mohammed FF, Tohamy AF, El Deeb KS. Metabolic profile and hepatoprotective effect of *Aeschynomene elaphroxylon* (Guill. & Perr.). PLoS One 2019; 14(1):1–24. Doi: 10.1371/journal.pone.0210576
16. Iqbal N, Zubair HM, Almutairi MH, Abbas M, Akhtar MF, Aleya L, Kamel M, Saleem A, Jabeen Q, Noreen S, Baig MMFA & Abdel-Daim MM. Hepatoprotective effect of *Cordia rothii* extract against CCl4-induced oxidative stress via Nrf2–NFκB pathways. Biomed. Pharmacother. 2022; 156:1–9. Doi: 10.1016/j.biopha.2022.113840
17. Oleshchuk O, Ivankiv Y, Falfushynska H, Mudra A, Lisnychuk N. Hepatoprotective effect of melatonin in toxic liver injury in rats. Med. 2019; 55(304):1–11. Doi: 10.3390/medicina55060304
18. Meng X, Tang GY, Liu PH, Liu Q, Li H Bin, Zhao CJ. Antioxidant activity and hepatoprotective effect of 10 medicinal herbs on CCl_4-induced liver injury in mice. World J. Gastroenterol. 2020; 26(37):5629–5645. Doi: 10.3748/wjg.v26.i37.5629
19. Alqahtani NK, Mohamed HA, Moawad ME, Younis NS, Mohamed ME. The Hepatoprotective Effect of Two Date Palm Fruit Cultivars' Extracts: Green Optimization of the Extraction Process. Foods. 2023; 12(6): 1-27. Doi: 10.3390/foods12061229
20. Huang YW, Tuozo AJ, Tan RS. Hepatotoxicity and Mutagenicity assessment during chronic in vivo exposure to aqueous extracts from *Peperomia pellucida*. An. Acad. Bras. Cienc. 2022; 94(2): 1–9. Doi: 10.1590/0001-3765202220191255
21. Delwatta SL, Gunatilake M, Baumans V, Seneviratne MD, Dissanayaka MLB, Batagoda SS, Udagedara AH & Walpola PB. Reference values for selected hematological, biochemical and physiological parameters of Sprague-Dawley rats at the Animal House, Faculty of Medicine, University of Colombo, Sri Lanka. Anim. Model. Exp. Med. 2018; 1(4): 250–254. Doi: 10.1002/ame2.12041
22. Iluz-Freundlich D, Zhang M, Uhanova J. Minuk G Y The relative expression of hepatocellular and cholestatic liver enzymes in adult patients with liver disease. Ann. Hepatol. 2020; 19(2020): 204–208. Doi: 10.1016/j.aohep.2019.08.004
23. Geresu GD, Umer S, Arayaselassie M, Ashebir G, Makonnen E. Hepatoprotective Effects of Crude Stem Bark Extracts and Solvent Fractions of *Cordia africana* against Acetaminophen-Induced Liver Injury in Rats. Can. J. Gastroenterol. Hepatol. 2022; 2022:1–11. Doi: 10.1155/2022/1449286.
24. Fitrya, Lamin S, Ashfa FA, Novita A, Amriani A. The potency of ethyl acetate fraction of cempedak (*Artocarpus champeden*) leaves in attenuating the nephrotoxic effect in gentamicin-piroxicam-induced rat models. J. Ayurveda Integr. Med. 2025; 16(2025): 1–7.Doi: 10.1016/j.jaim.2024.101040
25. Zhang G, Wang X, Chung TY, Ye W, Hodge L, Zhang L, Chng K, Xiao YF & Wang YJ. Carbon tetrachloride (CCl_4) accelerated development of non-alcoholic fatty liver disease (NAFLD)/steatohepatitis (NASH) in MS-NASH mice fed western diet supplemented with fructose (WDF). BMC Gastroenterol. 2020; 20(339):1–13. Doi: 10.1186/s12876-020-01467-w.
26. Beltran-Benjamin KS, Co EL, Gaspi SAD, Matibag JLR, Su GLS. Enzyme Activity and Histopathology of Rat Liver Treated with Crude Methanolic Extract of *Pepperomia pellucida* (L). J. Biol. Sci. 2013; 13(4):183–195. Doi: 10.3923/jbs.2013.183.195.
27. Ng ZX, Than MJY, Yong PH. *Peperomia pellucida* (L.) Kunth herbal tea: Effect of fermentation and drying methods on the consumer acceptance, antioxidant and anti-inflammatory activities. Food Chem. 2021; 344 (2021):1-9. Doi: 10.1016/j.foodchem.2020.128738.
28. Gomes PWP, Barretto H, Reis JDE, Muribeca A, Veloso A, Albuquerque C, Teixeira A, Braamcamp W, Pamplona S, Silva C & Silva M. Chemical Composition of Leaves, Stem, and Roots of *Peperomia pellucida* (L.) Kunth. Molecules. 2022; 27(6):1–12. Doi: 10.3390/molecules27061847
29. He M, Min JW, Kong WL, He XH, Li JX, Peng BW. A review on the pharmacological effects of vitexin and isovitexin. Fitoterapia. 2016;115:74–85. Doi: 10.1016/j.fitote.2016.09.011.
30. Ho K, Tan GG, Yong PH, Wang CW, Lim SH, Kuppusamy UR, Ngo Ct, Massawe F, Ng ZX. Extraction of phytochemicals with health benefit from *Peperomia pellucida* (L.) Kunth through liquid-liquid partitioning. J. Appl. Res. Med. Aromat. Plants. 2022; 30(2022): 1–10 . Doi: 10.1016/j.jarmap.2022.100392
31. Silva RO, Sousa FBM, Damasceno SRB, Carvalho NS, Silva VG, Oliveira FRMA, Sousa DP, Aragão KS, Barbosa AL R, Freitas RM & Medeiros JVR. Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. Fundam. Clin. Pharmacol. 2014; 28(4):455–464. Doi: 10.1111/fcp.12049.