DOI: 10.2478/cipms-2023-0033 Curr. Issues Pharm. Med. Sci., Vol. 36, No. 4, Pages 189-193

Current Issues in Pharmacy and Medical Sciences
Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: http://www.curipms.umlub.pl/

Hepatoprotective effect of Indonesian propolis from *Apis mellifera* in carbon tetrachloride (CCl_4) induced liver injury in mice

Dian Ayu Juwita[1], Almahdy Ahmadin[1], Rahmad Abdillah[1*], Fitri Rachmaini[1], Sherly Veronica[2]

[1] Departement of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Andalas, Indonesia
[2] Undergraduate Pharmacy Study Program Faculty of Pharmacy, Universitas Andalas, Indonesia

ARTICLE INFO

Received 09 May 2022
Accepted 25 October 2023

Keywords:
hepatoprotective,
propolis,
alanine aminotransferase,
aspartate aminotransferase,
alkaline phosphatase,
liver histology.

ABSTRACT

Propolis has been reported to have a broad spectrum of biological activities. The objective of this study was to investigate the hepatoprotective effect of propolis on liver injury induced by carbon tetrachloride (CCl_4). Twenty-five adult mice were randomly divided into five groups of five. The normal group received distilled water; positive control group was given CCl_4 at 2.8 mL/kg BW orally, other groups were given the same dose of CCl_4, followed by oral propolis at 25, 50, and 100 mg/kg BW, respectively, for 14 days. On the 15th day, the mice were sacrificed for the measurements of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, and albumin, as well as examination of liver histology. The data were analyzed using a one-way ANOVA and, subsequently, by Duncan's Multiple Range Test. The results demonstrated that propolis had a hepatoprotective effect as shown by significant improvement of the biochemical parameters ($p < 0.05$), which was confirmed by the liver histological analytical results. The most potent hepatoprotective effect was found after the dose of 100 mg/kg BW.

INTRODUCTION

Liver diseases are common worldwide, including in Indonesia. Free radicals and reactive oxygen species (ROS) play a crucial role in developing liver diseases [1], and the liver is the first organ exposed to the damaging effects of any newly formed toxic substance. Therefore, protective armaments for the liver is a particular concern.

Bio-activation of xenobiotics such as pharmaceuticals or poisonous foods in the liver might contribute to reactive metabolic species reacting with cellular macromolecules, leading to protein malfunction, oxidative stress, lipid peroxidation, and DNA damage [2]. Despite significant improvements in pharmacological development, effective and safe hepatoprotective medicines remain in high demand [3]. Alternative therapies, such as the use of natural medicines derived from medicinal plants and their formulations, have sparked the interest of pharmaceutical companies.

Many researchers are turning to natural products because of the limited drugs used as hepatoprotectives [2]. Traditional medicine recommends a variety of herbal treatments to treat liver diseases. The majority of these treatments work as free radical scavengers, while some operate as antioxidant modulators. Thus, natural products may serve as therapeutics to cope with liver damage [1]. Among the natural products providing a wide range of antioxidants, propolis has been the subject of considerable investigation [4].

Propolis is a viscous, dark-colored substance collected by honeybees from living plants. They blend it with wax and utilise it to build and remodel their nests, mostly to seal fractures in the honeycomb and to defend against dangerous microorganisms. Propolis has been used since ancient times as folk medicine [5,6]. Because of its valuable characteristics, propolis is extensively used in complementary and alternative medicine in food and beverages to improve health, and to prevent diseases such as inflammation, heart disease, diabetes and cancer [7]. It has been claimed that propolis contains remarkable biological activities, among others, antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, anticancer properties, immunoregulatory, and bone strengthening effects owing to the aromatic acids, phenolic compounds and flavonoids found in it [8-11].

In addition, as a hepatoprotective, propolis also has the most potent antioxidant activity against oxidants and free radicals when compared to the results of investigations

* **Corresponding author**
e-mail: rahmadabdillah@phar.unand.ac.id

involving other bee products [12,13]. Its extract has been found to contain phenolic acids and caffeic acid phenethyl ester (CAPE), that could act as hepatoprotectors [14,15]. It has been suggested that the biological activities of propolis may be due to the presence of a large number of terpenoid and flavonoids [16]. The terpenoid content in propolis has antibacterial activity, anti-inflammatory activity and immunomodulatory effect. Moreover, propolis has demonstrated activity in decreasing nuclear factor-KB (NF-KB) stimulation in the inflammatory cascade. Research suggests that the content of flavonoids can reduce the destructive effects of free radicals by inhibiting lipid peroxide through activation of peroxidase to haemoglobin, an endogenous antioxidant [17]. Propolis has also been found to capture hydroxyl and superoxide radicals and neutralize free radicals, hence can maintain the structural integrity of cells/tissues and membrane lipids against undesirable reactions [18]. This current study aims to determine the hepatoprotective effect of propolis on liver damage caused by CCl_4, with ALT, AST, ALP, Albumin and Total Protein as parameters.

MATERIALS AND METHODS

Materials

Carbon tetrachloride (Merck, Germany), sesame oil (PT. ABC, Indonesia), formaldehyde (Sigma Aldrich, Singapore), sodium carboxymethylcellulose 0.5% (PT. Brataco Indonesia), monosodium and disodium phosphate (PT. Brataco Indonesia), alcohol 96% (Sigma Aldrich, Singapore), sodium chloride 0,9% for injection (Otsuka, Indonesia), aqua injection (Otsuka, Indonesia). Propolis from *Apis mellifera* subsp. *mellifera* was purchased from local bee farmers in Solok, West Sumatra, Indonesia.

Extraction of propolis

The Propolis (1 kg) was cut into small pieces and extracted with 96% ethanol (1:10, w/v) at room temperature. The main constituents of propolis were previously reported by Trusheva, et al. [19].

Animals and experimental design

All experiments were carried out according to the National Institute of Health Guidelines for the care and use of laboratory animals and the European Council Directive of 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), and approved by The Research Ethics Committee, Faculty of Medicine, Universitas Andalas (No. 177/KEP/FK/2020). Twenty-five BALB/c mice aged 2-3 months weighing 20-30 grams were used in this research. All animals were acclimatized to laboratory condition for seven days under 12 hours light-dark cycle, (25±2)°C and (55±5)% of relative humidity. The animal were fed with standard laboratory diet and water *ad libitum* for the adaptation of laboratory conditions.

All animals were divided into five groups. The normal control group received distilled water. The positive control group was orally treated with CCl_4 at 2.8 mL/kg BW dissolved in sesame oil. Other groups were given CCl_4 as hepatotoxicity inductor orally and administered with propolis at dose 25, 50 and 100 mg/kg BW dissolved in sesame for 14 days as the range of propolis dose used by Lee, *et al.* [20]. At the end of the study, the rats were anesthetized with pentobarbital, and blood was drawn using cardiac puncture to collect data on biochemical markers. Blood was placed in a microtube that had been given heparin. Following the collection of a blood sample from the heart, cervical dislocation was done, and the liver was collected through abdominal incision.

Measurement of Biochemical Markers

Serum was separated from blood samples by centrifugation. The alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, and total protein levels were determined using reagent kits from DiaSys® Diagnostic.

Histology examination of the liver

The liver was fixed in a 10% buffered formalin solution for 24 hours before sectioning. The liver tissue was successively dehydrated in 70%, 80%, 90%, 96% alcohol, xylol, and liquid paraffin. Tissue vacuuming and embedding were the next steps. The liver tissue was sectioned at a thickness of 4-6 mm and stained with hematoxylin and eosin. Histological observations of the liver included observations of central veins, hepatocytes, the arrangement of hepatocyte cells and the presence of abnormal liver abnormalities (observed in the form of parenchymal degeneration, hydropic degeneration and necrosis of liver hepatocytes). Liver tissue was analyzed using a 40 times magnification microscope with Optilab®, in five different fields of view. ImageJ software (NIH-Bethesda, MD, USA) was employed to calculate the stained-area and staining intensity. Each biological replicate had its own set of photographs taken. As a representative of that duplicate, the average stained-area and staining intensity of the 5 fields were chosen [21,22]. Histopathological features of hepatic injury were evaluated by applying a semi-quantitative histopathology score adapted from the recently accepted AASLD criteria [23,24]

Data analysis

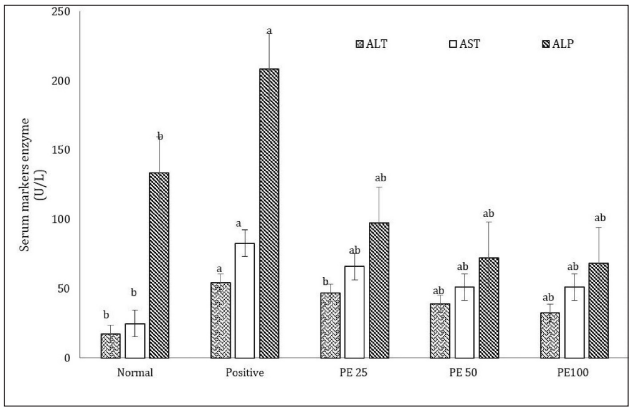
All values are expressed as means ± standard deviation (SDs). A one-way analysis of variance (ANOVA) test was introduced to analyze the data, followed by Duncan's Multiple Range Test (DMRT). This data was processed by means of the statistical software, SPSS version 22.

RESULTS

Effect of Propolis on Serum Biochemical Markers Levels

This study found that the addition of CCl_4 significantly increases the activities of ALT, AST, and ALP when compared to the normal group. In contrast, propolis supplementation resulted in significantly lower ALT, AST and ALP levels ($p < 0.05$) in the animal group. Propolis dose 100 mg/kg BW gave the best activity at ALT and ALP levels (32.34±5.04 U/L); (68.44±7.44 U/L), compare to the normal control group (17.14±8.83 U/L); (133.55±12.37 U/L). Moreover, propolis dose 50 and 100 mg/kg BW gave the best activity on AST level, which gives an equal result

(51.06±6.01 U/L), as compared to the normal control group (24.90±8.44 U/L) – as seen in Figure 1. The treatment of propolis extract (PE) at 50 mg/kg BW and 100 mg/kg BW recovered the impaired liver functions induced via CCl_4 damage.



Data are the mean ± SE of five mice, a=significant difference compared to normal group (p<0.05); b=significant difference compared to positive group (p<0.05)

Figure 1. The effect of propolis on serum marker level in CCl4-induced mice

Effect of Propolis on Albumin and Total Protein Level

Table 1 presents the results of albumin and total protein measurements. The levels of albumin (2.86 g/L) and total protein (4.81 g/L) were significantly lower in the CCl_4 group compared to the normal control group ($p < 0.05$), indicating liver injury. Propolis supplementation significantly improved these condition ($p < 0.05$), bringing them back to normal in a dose-dependent manner. PE 100 provided better results, with comparable levels to the control group.

Tabel 1. The effect of propolis extract (PE) on albumin and total protein level in CCl^4-induced mice

Group	Albumin (g/L)	Total Protein (g/L)
Normal control	4.48±0.84	6.77±0.72
Positive control	2.86±1.09^{a}	4.81±2.31^{a}
PE 25	3.31±0.71^{ab}	6.94±2.17^{ab}
PE 50	3.58±0.63^{ab}	6.46±1.07^{ab}
PE 100	4.43±0.61^{b}	7.22±1.45^{b}

Data are the mean ± SE of five mice, a=significant difference compared to normal group (p<0.05); b=significant difference compared to positive group (p<0.05)

Histology Analysis

Histology results in the CCl_4 control group showed degeneration of parenchyma, hydropic degenerative and necrosis. In the hepatocyte cell degeneration, we can see the granular in the cytoplasm, as well as an enlarged cell size because of a swelling cell. Figure 2 illustrates the liver histopathology in a qualitative manner, providing the liver description of this study. In the positive control group, the mean histopathology score increased during CCl_4 induction to as high as 3.74±0.12. This is significantly different compared to normal controls ($p < 0.05$), as seen in Table 2. The histopathology score of PE 100 demonstrated the greatest hepatoprotective effect (2.92±0.13), and is statistically different to other propolis doses.

Tabel 2. The effect of propolis on liver histopathology score in CCl_4-induced mice

Group	Liver histopathology score
Normal control	1.01±0.02
Postive control	3.74±0.12^{a}
PE 25	3.40±0.36^{a}
PE 50	3.04±0.14^{a}
PE 100	2.92±0.13^{ab}

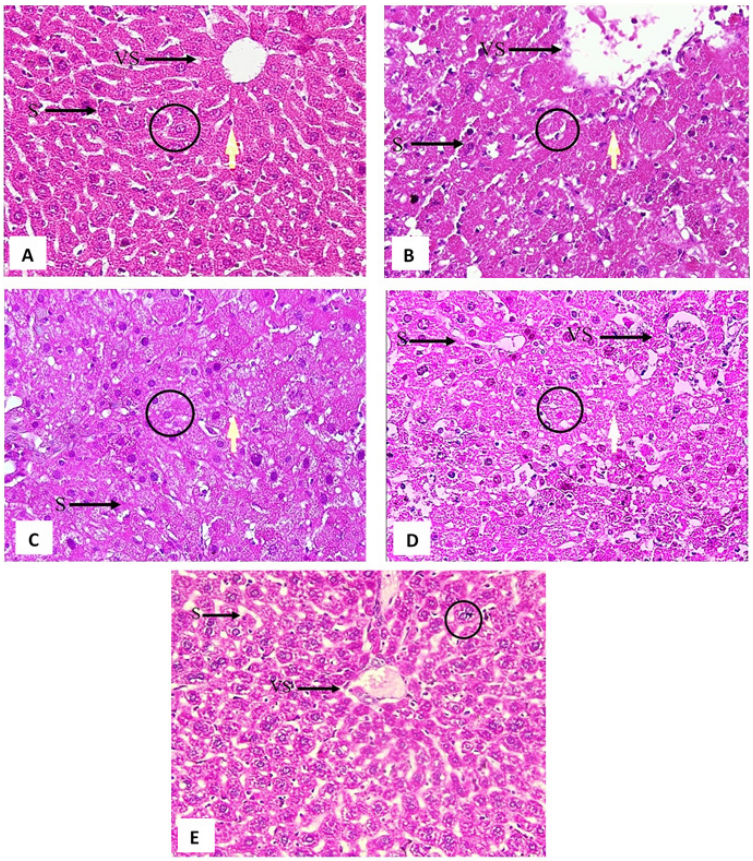
Data are the mean ± SE of five mice, a=significant difference compared to normal group (p<0.05); b=significant difference compared to positive group (p<0.05)

DISCUSSION

This study investigated the effects of CCL4 administration on liver cells and the effect of propolis administration on preventing hepatocyte damage. Hepatic function marker such as the enzymes ALT, AST and ALP characterize hepatocyte damage. When liver cells are injured, these enzymes are released from cells into the circulation and generate significantly elevated serum levels[19]. Our findings showed that CCl_4 administration causes severe acute liver injury in mice. This finding validates the previous study's finding that CCl_4 is a preferred chemical agent for inducing hepatic cell damage in animals.[20,25] Hepatic damage induction with CCl_4 is potentially reversible, and it precisely models actual liver cirrhosis by producing free radicals and inducing pro-inflammatory and pro-fibrotic cytokines. In the liver, CCl_4 is metabolized to $CCl_{3\cdot}$. Once exposed to O_2, it transforms from $CCl_{3\cdot}$ to $CCl_3O_{2\cdot}$, $CCl_{3\cdot}$ and $CCl_3O_{2\cdot}$ – which are highly reactive lipid and protein binders. All of the structural changes generated by free radicals will result in a decrease in protein production. Damage to the cytoplasmic membrane lipids will eventually result in a rise in hepatic serum indicators such as ALT, AST and ALP [26,27].

CCl_4 exposure caused severe liver damage in the current study, as seen by higher serum AST, ALT, and ALP levels. These cytoplasmic marker are released into the blood following cellular injury [28]. This research revealed the effects of a combination of hepatic damage, hepatocellular (predominantly first ALT elevation) and cholestatic (initial ALP rise). AST acts as a catalyzer in converting alanine to pyruvate and glutamate. AST thus could be a better biomarker of liver damage caused by viral hepatitis, myocardial infarction or liver injury. In these conditions, there was less of an elevation in ALT level. Meanwhile, serum ALP is associated with hepatic cell activity. An elevation in serum ALP is caused by increased synthesis due to increasing biliary pressure [27].

These present study findings demonstrated a decline in the levels of serum marker enzymes following PE administration. In this condition, the free radicals produced during CCl_4 biotransformation must be either eliminated or suppressed to reduce liver injury. Mice with CCl_4 – induced liver damage treated with PE at 25, 50 and 100 mg/kg BW were able to reduce AST, ALT and ALP. In this study, the ALT and AST values in the test group were still higher than in the normal group. However, these were much lower than that in the positive control group. This happens because the hepatocyte cell repairing process is still going on and takes a long time. These conditions indicate the hepatoprotective effect



The arrow pointed to S: Sinusoids, and VS: Central vein

Figure 2. Histology of liver mice cells in CCl_4-induced mice in normal group (A), CCl_4 control group (B), Normal group (C), Propolis 25 group (D) Propolis 50 (E) Propolis 100 group. H & E was used to stain 6-mm slices of liver at a magnification of 40×. The circle represented normal cells with intact nuclei and regular sinusoids

of propolis. This finding was backed by Nakamura *et al.* [29] The optimal effect in reducing ALT, AST and ALP shown in dose PE100, as seen in Table 1.

Caffeic Acid Phenethyl Ester (CAPE) is a natural chemical substance in propolis that acts as an antioxidant [30,31]. Changes in ALP, ALT and AST enzyme in the animal group given propolis is associated with the propolis compounds that act as antioxidants. Antioxidants are substances that neutralise a free radical [14]. Sharma and Shukla supported this finding (2011), by reporting that the effect of antioxidant flavonoids can improve the process of regeneration [20,29]. CAPE has 4-6 times antioxidant activity more potent against oxidants and free radicals than vitamin C and N-acetyl-cysteine (NAC) [13]. Propolis can catch radical hydroxyl and superoxide, then neutralize free radicals, hence protecting cells by maintaining their structural integrity, and protecting lipid membranes against undesirable reactions [15].

The largest plasma protein in the bloodstream is albumin, solely made in the liver. It participates in the scavenging of oxygen free radicals. It is also crucial in distributing various compounds in the circulation linked to albumin, including medicines, lipids, hormones and toxins. As the results of our study show a rise in total protein content, this outcome might be regarded as amelioration in the degree of cellular dysfunction in liver disorders [32]. The presence of a high albumin level in the blood indicates cellular leakage and a lack of functional integrity of the cell membrane in the liver. Protein synthesis stimulation has been proposed as a contributing hepatoprotective mechanism that accelerates liver enzyme deformation and production [33].

Although ALT, AST and ALP are considered sensitive enough for indicating the presence of liver cell damage, these measurements lack specificity, so a histology study is necessary to identify microscopical liver damage [34,35]. Histology results in the normal control group (Figure 1) revealed intact central venous and nucleus cells, with no inflammation cells, and that some cells had undergone necrosis. However, this is not included in the incidence of pathology, because necrosis may also occur [29,36].

According to Trefts *et al*., parenchymal degeneration occurs due to failure of oxidation, leading to disturbed protein transport produced by the ribosome, which results in an accumulation of water in the cell that causes swelling. Moreover this effect induces the] appearance of granules in the cytoplasm due to protein sediment [23,37]. In the group dose of 25, 50 and 100 mg/kg BW (Figure 2), there is a decrease in liver damage histology. This was characterized by fewer necrosis cells, normal sinusoid and less damage in the central vein compared to the positive control group. PE100 administration demonstrated the highest decrease in histology score. This came about because of the dose-dependent activity thus, protection of liver damage will also be higher than other doses. Overall, it was concluded that propolis, because of its antioxidant and anti-inflammatory activities, works as a potent hepatoprotective agent and protects the liver from severe CCl4-induced damage.

CONCLUSION

This study shows that propolis has hepatoprotective effect by significantly affecting ALT, AST and ALP levels ($p < 0.05$) and significantly restoring the albumin and total protein level to normal ($p<0.05$). These outcomes were confirmed by the liver histology score in the carbon tetrachloride (CCl_4) – induced mice. Propolis at the dose of 100 mg/kg BW had the highest hepatoprotective effect.

ORCID iDs

Dian Ayu Juwita https://orcid.org/0000-0003-2078-2049
Almahdy Ahmadin https://orcid.org/0000-0003-4311-6233
Rahmad Abdillah https://orcid.org/0000-0001-9072-2522
Fitri Rachmaini https://orcid.org/0000-0003-1865-8540

REFERENCES

1. Vitaglione P, Morisco F, Caporaso N, Fogliano V. Dietary antioxidant compounds and liver health. *Crit Rev Food Sci Nutr.* 2004;44(7-8):575-86.

2. Tasduq SA, Kaisar P, Gupta DK, Kapahi BK, Jyotsna S, Maheshwari HS, et al. Protective effect of a 50% hydroalcoholic fruit extract of Emblica officinalis against anti-tuberculosis drugs induced liver toxicity. *Phyther Res.* 2005;19(3):193-7.
3. Alshehri M, Wahab Amjad M, Mudawi ME. Drugs-inducing hepatotoxicity. *Asian J Pharm Res Heal Care.* 2020;12(3):148-56.
4. Nna VU, Bakar ABA, Mohamed M. Malaysian propolis, metformin and their combination, exert hepatoprotective effect in streptozotocin-induced diabetic rats. *Life Sci.* 2018;211:40-50.
5. Kuropatnicki AK, Szliszka E, Krol W. Historical aspects of propolis research in modern times. *Evid Based Complement Altern Med.* 2013;2013:964149.
6. Mahmoud A, Almas K, Dahlan A. The effect of propolis on dentinal hypersensitivity and level of satisfaction among patients from a university hospital Riyadh, Saudi Arabia. *Indian J Dent Res.* 1999;10(4):130-7.
7. Kapare H, Lohidasan S, Sinnathambi A, Mahadik K. Standardization, anti-carcinogenic potential and biosafety of Indian propolis. *J Ayurveda Integr Med.* 2019;10(2):81-7.
8. Ansorge S, Reinhold D, Lendeckel U. Propolis and some of its constituents down-regulate DNA synthesis and inflammatory cytokine production but induce TGF-β1 production of human immune cells. *Z Naturforsch C J Biosci.* 2003;58(7-8):580-9.
9. Juwita DA, Almahdy A, Abdillah R, Syahputri F. The bone strengthening effects of propolis in ovariectomized female white rats as models for postmenopause. *Curr Issues Pharm Med Sci.* 2021;0(0):119-22.
10. Lu LC, Chen YW, Chou CC. Antibacterial activity of propolis against Staphylococcus aureus. *Int J Food Microbiol.* 2005;102(2):213-20.
11. Higashi KO, de Castro SL. Propolis extracts are effective against Trypanosoma cruzi and have an impact on its interaction with host cells. *J Ethnopharmacol.* 1994;43(2):149-55.
12. Pasupuleti VR, Sammugam L, Ramesh N, Gan SH. Honey, propolis, and royal jelly: a comprehensive review of their biological actions and health benefits. *Oxid Med Cell Longev.* 2017;2017.
13. Nakajima Y, Shimazawa M, Mishima S, Hideaki H. Propolis and its main constituents against oxygen-glucose deprivation stress, with a gene-expression analysis. *Phyther Res.* 2009;23:1431-8.
14. Bankova V. Recent trends and important developments in propolis research. *Evid Based Complement Altern Med.* 2005;2(1):29-32.
15. Šuran J, Cepanec I, Mašek T, Radi B. Propolis extract and its bioactive compounds – from traditional to modern extraction technologies. *Molecules.* 2021;26:1-21.
16. Wagh VD. Propolis: A wonder bees product and its pharmacological potentials. *Adv Pharmacol Sci.* 2013;2013.
17. Mot AC, Damian G, Sarbu C, Silaghi-Dumitrescu R. Redox reactivity in propolis: Direct detection of free radicals in basic medium and interaction with hemoglobin. *Redox Rep.* 2009;14(6):267-74.
18. Bendich A. Physiological role of antioxidants in the immune system. *J Dairy Sci.* 1993;76(9):2789-94.
19. Trusheva B, Popova M, Koendhori EB, Tsvetkova I, Naydenski C, Bankova V. Indonesian propolis: Chemical composition, biological activity and botanical origin. *Nat Prod Res.* 2011;25(6):606-13.
20. Lee MS, Kim YH, Park WS, Ahn WG, Park OK, Kwon SH, et al. Novel antidepressant-like activity of propolis extract mediated by enhanced glucocorticoid receptor function in the hippocampus. *Evid Based Complement Altern Med.* 2013;2013: 217853. :28-35. 114-123.
21. Shao SY, Qi XZ, Sun H, Li S. Hepatoprotective lignans and triterpenoids from the roots of Kadsura longipedunculata. *Fitoterapia.* 2020;142(2):104487.
22. Delgado-Montemayor C, Cordero-Pérez P, Salazar-Aranda R, Waksman-Minsky N. Models of hepatoprotective activity assessment. *Med Univ.* 2015;17(69):222-8.
23. Trefts E, Gannon M, Wasserman DH. The liver. *Curr Biol.* 2017; 27(32):11147-51.
24. Farghali H, Canová NK, Zakhari S. Hepatoprotective properties of extensively studied medicinal plant active constituents: Possible common mechanisms. *Pharm Biol.* 2015;53(6):781-91.
25. Sharma N, Shukla S. Hepatoprotective potential of aqueous extract of Butea monosperma against CCl 4 induced damage in rats. *Exp Toxicol Pathol.* 2011;63(7-8):671-6.
26. Koh C, Zhao X, Samala N, Sakiani S, Liang TJ, Talwalkar JA. AASLD clinical practice guidelines: A critical review of scientific evidence and evolving recommendations. *Hepatology.* 2013;58(6):2142-52.
27. Ahmed A, Al Tamimi DM, Isab AA, Alkhawajah AMM, Shawarby MA. Histological changes in kidney and liver of rats due to gold (III) compound [Au(en)Cl2]Cl. *PLoS One.* 2012;7(12):e51889.
28. Kasdallah-Grissa A, Mornagui B, Aouani E, Hammami M, El May M, Gharbi N, et al. Resveratrol, a red wine polyphenol, attenuates ethanol-induced oxidative stress in rat liver. *Life Sci.* 2007;80(11):1033-9.
29. Nakamura T, Ohta Y, Ohashi K, Ikeno K, Watanabe R, Tokunaga K, et al. Protective effect of Brazilian propolis against liver damage with cholestasis in rats treated with α -naphthylisothiocyanate. *Evid Based Complement Altern Med.* 2013;2013.
30. Al-Sayed E, Martiskainen O, Seif El-Din SH, Sabra ANA, Hammam OA, El-Lakkany NM, et al. Hepatoprotective and antioxidant effect of bauhinia hookeri extract against carbon tetrachloride-induced hepatotoxicity in mice and characterization of its bioactive compounds by HPLC-PDA-ESI-MS/MS. *Biomed Res Int.* 2014;2014.
31. Šuran J, Cepanec I, Mašek T, Radi B. Propolis extract and its bioactive compounds. *Molecules.* 2021;(26):1-21.
32. Hadisoewignyo L, Soeliono I, Hartono SB, Hestianah EP, Mahanani SR. Hepatoprotective effects of curcumin-mesoporous silica nanoparticles on CCl4-induced hepatotoxicity wistar rats. *Indones J Pharm.* 2019;30(2):114-21.
33. Yuliandra Y, Armenia AA, Arief R, Jannah MH, Arifin H. Reversible hepatotoxicity of cassytha filiformis extract: Experimental study on liver function and propofol-induced sleep in mice. *Pharmacogn J.* 2019;11(1):69-74.
34. Kim Y, You Y, Yoon HG, Lee YH, Kim K, Lee J, et al. Hepatoprotective effects of fermented Curcuma longa L. on carbon tetrachloride-induced oxidative stress in rats. *Food Chem.* 2014;151:148-53.
35. Armenia, Permatasari D, Sinamar LP, Estera K, Ahmadin A. The impact of sub acute administration of purified gambier (Uncaria gambir roxb.) to the liver and kidney functions and its reversibility on rats. *Pharmacogn J.* 2021;13(1):44-51.
36. Wahyuni FS, Arisanty D, Hayaty NF, Juwita DA, A A. Sub-acute toxicity study of the ethyl acetate fraction of asam kandis rinds (garcinia cowa roxb.) on the liver and renal function in mice. *Pharmacogn J.* 2017;9(3):345-9.
37. Badriyya E, Latifah W, Aldi Y. Sub-acute toxicity study of pegagan embun (hydrocotyle sibthorpioides lam.) extract on the sgpt and sgot level of wistar white male rats. *Int J Appl Pharm.* 2023;5-9.