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Hepatoprotective activity of ethanolic extract of *Plectranthus amboinicus* (lour.) spreng leaf in DMBA induced rats

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ABSTRACT

The hepatoprotective activity of ethanolic of *Plectranthus amboinicus* Lour Spreng leaf extract (PEE) on blood biochemical profiles, non-specific immune system, liver histology were evaluated in rats induced DMBA. Twenty five female rats were divided into five groups, each with 5 rats. The negative control group (NC) received only food and water. The positive control group (PC) administered orally DMBA 20 mg/kg body weight (bw) once every four days for 32 days. The treatment groups received the PEE with three different doses of 175 (T1), 350 (T2), 700 (T3) mg/kg bw, respectively for 27 days after DMBA induction. At the end of the treatment, blood samples were collected to investigate the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, total protein, albumin and globulin as well as the hematological parameter, such as neutrophils, monocyte, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) were monitored. The results showed an increased level in ALT, AST, ALP, and bilirubin in the PC group. However, the T3 group (PEE 700 mg/kg) showed a significant decrease value ($p < 0.05$) in ALT, ALP, and bilirubin compared to the PC group. Our finding revealed that all PEE treatments had a significant increase ($p < 0.05$) in the total protein, albumin and globulin compared to the PC group. The neutrophils (18.60 ± 4.64) and monocytes (61.40 ± 4.99) are lowest in the T2 groups as well as the value of MCH, RDW and MCV were significantly alleviated compared to all other groups. Histopathological observation demonstrated that the administration of PEE improved hepatocyte architecture and reduced the number of necrosis and hydrophilic degeneration. In conclusion, PEE has hepatoprotective activity by improving liver function, enhancing the non-specific immune system and recovering histopathological hepatocytes in rats exposed to DMBA.

1. Introduction

The liver is a vulnerable organ to respond to the exposure of toxic compounds by detoxifying enzymes (H. Liu et al., 2017). The toxic compound damages hepatocytes and induces proliferation of liver cancer in excessive and prolonged exposure. The early development of liver cancer is remarkable with chronic fibrosis and cirrhosis in liver injury. It is the sixth most common malignancy and the second leading cause of cancer death worldwide (Kang and Ahn, 2017).

DMBA is a toxic substance that can cause several diseases in the body. Research explains that DMBA given to rats can cause damage to their livers (Kumar et al., 2014) by increasing levels of ALT, AST and ALP significantly compared to those in the control group. Administration of DMBA to rats increased levels of urea, uric acid, creatinine, LDH, ALT and AST, but decreased levels of total protein, albumin and globulin (Ozdemir et al., 2006). Furthermore, Bio-indicator of high dose of hazard compound in animals included level of liver enzyme (Sun et al., 2018), hematological profile (Lumban Gaol-Adriana et al., 2023), serum biochemical parameter (Mekonnen and Wondmeneh, 2022), and histopathological alteration (Shah and Parveen, 2022).

P. amboinicus is an ethnomedicinal from the Lamiaceae family found in Indonesia and traditionally used, especially among the Bataknese lactating mother to enhance breast milk production (Arumugam et al., 2016). The previous study revealed that consumption of *P. amboinicus* leaves could induce lactation with no side effects, non-toxic and high nutrient content such as iron and carotene (Silitonga 1993 and Pillai, 2011). In addition, this medicinal edible species has many phytochemical which was analyzed by NMR metabolomic data, for instance

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apigenin, luteolin, quercetin and eriodictyol, potentially as antioxidant, antihyperglycemic and anti-inflammatory (Yuliana et al., 2018). Our previous study reported the level apigenin of PEE by HPLC method is about 0.0236 ng/μl (Silitonga et al., 2014) tend to be contributed in hepatoprotective activity against CCl_4-induced mice (Yue et al., 2020). Apigenin relieved hepatic fibrosis by inhibiting hepatic stellate cell activation (Martic, 2017) and autophagy via TGF-Line 1/Smad3 and p38/PPARα (Ji Jie et al., 2021). Hereafter, apigenin-containing dietary supplements decreased the replication of Hepatitis C Virus (HCV) in chronically infected patients (Shibata et al., 2013).

Moreover, our previous studies have revealed the potential of PEE in elevating immunostimulant activity (Silitonga and Silitonga, 2017), preventing hematological profile against Rhodamine B (Silitonga et al., 2018), and reducing cholesterol level (Silitonga et al., 2022), however the mechanism of the hepato-protection of PEE against DMBA has not been explored. This study aimed to evaluate the hepatoprotective properties of PEE. Observation parameters include ALT, AST, total bilirubin, direct bilirubin, and ALP assay. We also assess the level of albumin, globulin, total protein, hematological and histopathological examination of hepatocytes in rats exposed to DMBA.

2. Materials and methods

2.1. Animals

Twenty-five female rats (*Rattus norvegicus*) aged 12 weeks, weighing between 150 and 200 g, were obtained from the Pharmacy Laboratory, University of North Sumatra, Indonesia. The rats were then acclimatized at a temperature of 24 ± 27 °C for 7 day at the Biology Laboratory of the State University of Medan, Indonesia. After that, the animals were treated with DMBA 20 mg/kg and PEE with three doses of 175, 350, 700 mg/kg bw, respectively; fed, and drank freely ad libitum water for 58 days (NRC National Research Council, 2011). The method has obtained ethical clearance from Animal Research Ethics Committees/AREC No 0453/KEPH-FMIPA/2019.

2.2. Plant collection and preparation of PEE

P. amboinicus L. Spreng leaves were acquired from Sipahutar district, North Tapanuli and Dairi district, Sidikalang, North Sumatra- Indonesia. The preparation of PEE based on previous study (Sinaga et al., 2021). The fresh leaves of *P. amboinicus* (3000 g) were washed, placed on parchment paper, and dried in a closed cabinet equipped with a 40-W light bulb. Drying was done until the leaves became brown and the texture was crispy (±7 days). The dried leaves were mashed using a blender until they became powder as simplicia. The simplicia (1000 g) was put into a stainless pan and then 96% ethanol was added in a ratio of 1: 10 (1000 g of simplicia:10 L of ethanol) and then covered with aluminum foil. This immersion was carried out for five days and stirred every two days. After the maceration process, the marinade was filtered for three times using Wattman filter paper to obtain PEE. The extract was concentrated using a rotary evaporator to remove or evaporate the ethanol, so that a concentrated pure extract was obtained. These extracts were stored at 4 °C for further analysis.

2.3. Chemical material and kits

7,12-Dimethylbenz[a]anthracene (DMBA) was purchased from Sigma-Aldrich. Ethanol was acquired from Merck. Measurement of AST and ALT used a biochemistry analyzer (Dyasis). Bilirubin analysis was performed using the MAK126 Sigma-Aldrich Bilirubin Assay Kit. Total protein test used Total Protein Kit (DiaLab) and Albumin, while Globulin used Globulin (Plant) Microplate Assay Kit and Diasys® kit was used for ALP test. The liver histology preparations used the H&E method, while the hematology test used the Hematology Analyzer kit (Abdelghffar et al., 2022).

2.4. Experimental design

Twenty five rats were divided into five treatment groups at completely randomized design. The five treatment groups are as follows: Negative Control (NC), given only feed and water. Positive Control (PC), given orally DMBA 20 mg/kg bw 15 times (4 days) for 59 days. Treatment 1(T1), received orally DMBA 20 mg/kg bw once every 4 days for 32 days and after that given PEE 175 mg/kg bw daily for 27 days starting on day 33 to day 59. Treatment 2 (T2), administered orally DMBA 20 mg/kg bw once every 4 days for 32 days and after that given orally PEE 350 mg/kg bw daily for 27 days starting on day 33 to day 59. Treatment 3 (T3), received orally DMBA 20 mg/kg bw once every 4 days for 32 days, after that was given orally PEE 700 mg/kg bw daily for 27 days starting on day 33 to day 59.

On the 60th day, the rats were weighed, neck dislocation was performed, and the blood samples were collected in two groups of tubes, the one tube has anticoagulant ethylenediamine tetra acetic acid (EDTA) and the other tube without anticoagulant (Brígido et al., 2021). Eppendorf tubes coated with EDTA are for hematological assay and the other tube is for serum analysis. Blood and liver samples were collected 24 h. Then, the animals were carefully dissected and the livers of rats were collected for further histopathological examination.

2.5. Parameter measurement

2.5.1. Liver function test

Liver function tests observed in this study were AST, ALT, ALP, total bilirubin and direct bilirubin. The serum of blood in tubes without anticoagulants were centrifuged at 3000 rpm (Jain et al., 2019). AST and ALT levels were determined with the enzymatic kinetic method conducted at the Regional Health Laboratory of North Sumatra Province. Measurement of ALP activity was based on the enzymatic method using the Diasys® reagent kit and analyzed with a spectrophotometer. ALP contained (R1) 2-Amino-2-methyl-1-propanol pH 10.4 1.1 mmol/L, magnesium acetate 2 mmol/L, zinc sulfate 0.5 mmol/L, HEDTA 2.5 mmol/L, (R2) p-nitrophenyl-phosphate 80 mmol/L. The sample solution contained a mixture of R1 and R2 with a ratio of 4: 1. Serum for 10 μl ALP measurement was added with 500 μl mono reagent that was then vortexed and incubated at room temperature for 1 min. Then, the sample was read for absorbance with a UV–Vis spectrophotometer at a wavelength of 405 nm at three times every a minute. The measurement of total and direct bilirubin was used by the automated Cobas C111 chemical analyzer, and the procedure followed with the manufacturer's instructions of bilirubin assay. The threshold of normal values for Total Bilirubin was 8.0–17.0 mol/L and the level of direct Bilirubin amounted to 8 mol/L (C.N.C. et al., 2021).

2.5.2. Measurement of total protein, albumin and globulin levels

The total serum protein concentration was determined with the biuret method. First of all, alkaline copper reacted with protein peptide bonds to form the characteristic pink to purple biuret complex. Sodium potassium tartrate prevented precipitation of copper hydroxide, and potassium iodide prevented automatic reduction of copper. The intensity of the color was directly proportional to the protein concentration. The absorbance was measured at 546 nm. The concentration of total serum protein was expressed as g/dL.

Serum albumin concentration was measured using the bromocresol green method explained by (Basil, 1997). The buffer solution containing albumin reacted with the anionic green dye bromocresol and produced a green color. Measurements were made at the absorbance of 628 nm (Abu-Serie and Habashy, 2020). The intensity of the green color was proportional to the concentration of albumin present in the sample and was expressed as g/dL.

Serum globulin levels were calculated by subtracting the albumin value from the corresponding total protein value. The serum globulin concentration was expressed as g/dL. Albumin and globulin ratio, The

A/G ratio, was calculated based on albumin serum and globulin measurements.

2.5.3. Evaluation of hematological values

The blood sample that had been mixed with 200x dilution reagent for hemolyzing process was used to measure the number of leukocytes. Then the sample was further diluted 200x (so 40,000x) to measure erythrocytes and platelets. Erythrocyte differentiation included Hemoglobin, the total amount of hemoglobin in the blood; Hematocrit, the percentage of the number of red blood cells in the blood; MCV (mean corpuscular volume), the average size of red blood cells; MCH (mean corpuscular hemoglobin), the average amount of hemoglobin in red blood cells; MCHC (mean corpuscular hemoglobin concentration), the density of the hemoglobin molecule in red blood cells; and RDW (red cell distribution width), the variation in the size of red blood cells. Leukocyte differentiation consisted of Basophils, Eosinophils, Neutrophils, Monocytes. Measurement of hematological values used a Hematology Analyzer, based on standard procedure (Ejeh et al., 2023).

2.5.4. Histopathological liver assay

The histopathological assay was measured at the Pathology Laboratory of the Medan Veterinary Center, North Sumatra - Indonesia. The liver specimens of rats were collected, fixed in 10% formalin, dehydrated, cleared, infiltrated, and embedded in paraffin, sectioned (4–5 μm), examined with hematoxylin-eosin (H&E) method (Villanueva-Toledo et al., 2020). Then, it was observed by a microscope and assessed with the Manja Roenigk scoring method (Ilyas et al., 2019; Ielciu et al., 2021). The assessment of histopathological parameters consist of normal cell count, hydropic degeneration, necrosis cells and parenchymatous degeneration cells.

2.6. Statistical data

The data are expressed as the means ± SE. The results were presented using one-way analysis of variance (ANOVA) followed by the Tukey's test with a difference value $p < 0.05$ is considered as statistically significant.

3. Results

3.1. The effect of EEP on liver function assay

3.1.1. ALT and AST

We demonstrated the level of ALT and AST to observe impaired liver function. The results of the measurement of ALT and AST can be seen in Fig. 1. Our study presented there was an increase in ALT levels in DMBA (PC) group. However, the level of ALT decreased significantly ($p < 0.05$) in all the PEE treated groups compared to the PC treatment. In Fig. 1a, the data showed the higher the PEE dose, the higher the decrease in ALT levels.

In addition, the measurement of level of AST presented an increase significantly in DMBA (PC) treatment group rats which indicated the direction of hepatic injury. Meanwhile, there was a significant decrease in AST ($p < 0.05$) in all PEE treated groups compared to the DMBA-only treatment group (PC). The levels of AST in T1 and T2 were significantly higher than the control group (NC), but the T3 was not significantly different ($p > 0.05$) from the control group (NC).

3.2. Bilirubin direct, total bilirubin and alkalin phosphatase

Our finding showed that the levels of direct bilirubin and total bilirubin increased significantly in the DMBA treatment group (Fig. 2). However, the administration of PEE in the T1 and T3 treatment groups significantly reduced both in the value of direct and total bilirubin ($p < 0.05$) is similar with the value of the control treatment in Fig. 2.

The current study revealed the level of ALP significantly increased in DMBA (PC) treatment group rats. This indicated the presence of tissue damage due to DMBA exposure. Meanwhile, the level of ALP decreased significantly in the group of rats exposed to DMBA and PEE (T1, T2 and T3), compared to ALP in the PC group (Fig. 2c).

3.3. Effect of P. amboinicus ethanol extract on total protein, albumin and globulin

We analyzed the levels of total protein, albumin and globulin decreased in the DMBA (PC) treatment group. The data exhibited that an increased significantly the total protein in all PEE treated groups as well as control group (Fig. 3c). Besides, the highest level of albumin increase was found in the T3 treatment. Furthermore, the level of globulin

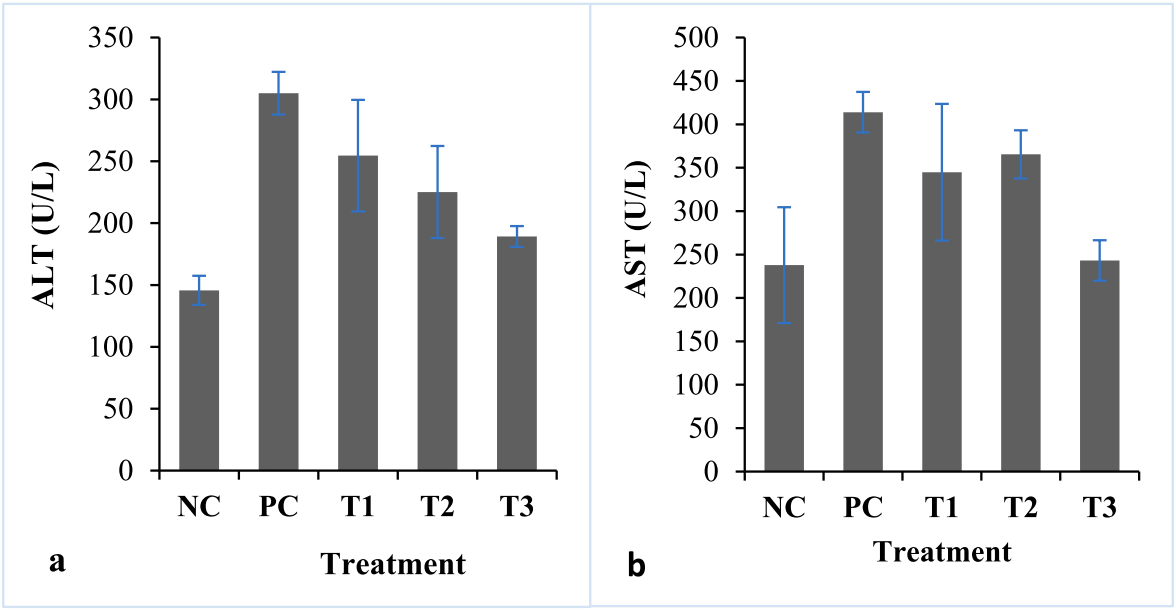

Fig. 1. Comparison of levels of ALT (a) and SGOT (b) in different treatment groups.

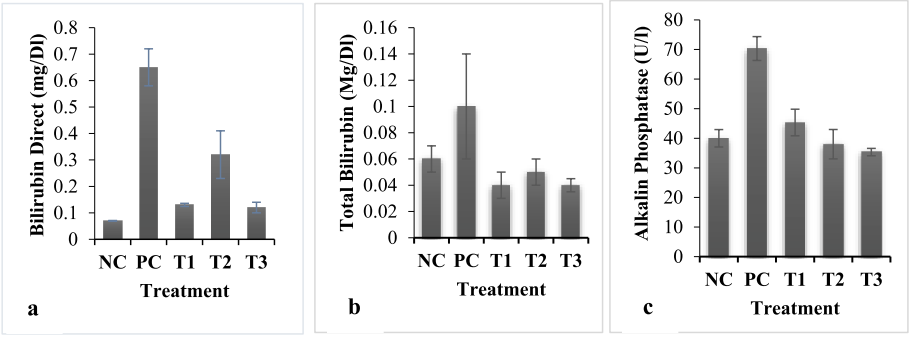

Fig. 2. Comparison of concentrations of Direct Bilirubin (a) Total Bilirubin (b) and Alkalin Phosphatase (ALP) in different treatment groups.

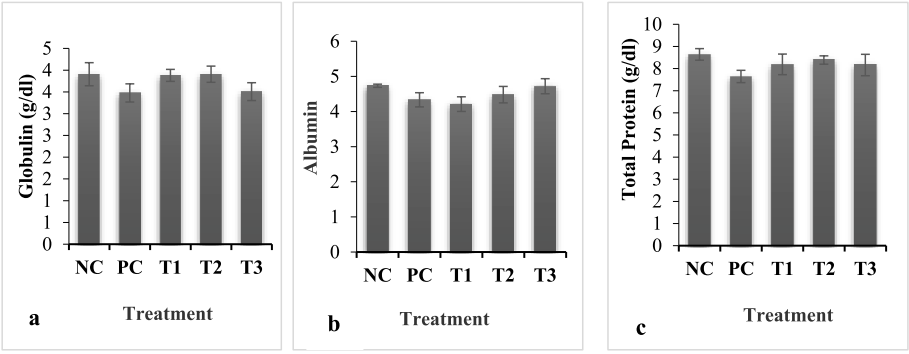

Fig. 3. Globulin (a) Albumin (b) and Total Protein (c) levels in different treatment groups.

elevated significantly ($p < 0.05$) in the T1 and T2 treatment groups, while the T3 group was not significant ($p > 0.05$) compared to the PC group.

3.4. Hematology profile

The administration of PEE in this study (T1 and T3) increased the platelet count significantly compared to the control (NC) and the positive control (PC) groups (Table 1), and the highest platelet levels were found in the T3 group. However, there was a significant decrease in platelets in the T2 group.

The experiment data stated that both basophils and eosinophils decreased in number due to exposure to DMBA in rats. In T1, T2 and T3 treatment groups, the number of basophils increased compared to PC and this increase did not reach the number of basophils in the control treatment. Giving PEE increased the number of eosinophils in the T1, T2 and T3 treatment groups. In T2 and T3 treatment groups, there was a significant increase compared to PC. The same thing also occurred with basophils. It increased significantly in the T1, T2 and T3 treatment groups although it did not reach the normal value as the value of basophils in the control treatment. The highest increase in eosinophils occurred in the T1 group.

Neutrophils and Monocytes are non-specific immune systems in the body. In this study, neutrophils and monocytes increased in DMBA treatment. Neutrophils decreased again in the T1, T2 and T3 treatment groups. Monocytes decreased in the T1, T2 and T3 treatment groups until they reached the same level of monocytes as in the control treatment. The highest decrease in neutrophils occurred in the T1 group, but neither the T1, T2 nor the T3 reached the same eosinophil value as in the control group. The number of monocytes in the DMBA (PC) treatment group increased significantly compared to the control (NC) group. However, there was a decrease after giving PEE in the T1, T2 and T3 treatment groups.

Our study presented that the level of MCH, MCV and RDW increased significantly in the PC group. In the treatment groups of T1, T2 and T3, the three parameters decreased significantly to reach the control value (NC). There was no difference in MCHC and MVP in all treatment groups.

Table 1
Effect of *Plectranthus amboinicus* L. Spreng on the hematological profile of DMBA induced rats.

Groups	Platelets	Basophils	Eosinophils	Neutrophils	Monocytes
NC	697.33 ± 218.02^{b}	3.00 ± 1.9^{c}	5.57 ± 1.08^{c}	13.00 ± 5.74^{a}	62.00 ± 2.94^{a}
PC	636.75 ± 218.55^{b}	0.60 ± 0.44^{a}	2.33 ± 0.41^{a}	29.20 ± 5.84^{c}	72.00 ± 5.94^{c}
T1	775.50 ± 104.55^{c}	1.00 ± 0.00^{b}	5.25 ± 1.16^{c}	21.00 ± 2.64^{b}	53.20 ± 8.55^{b}
T2	462.75 ± 96.87^{a}	0.80 ± 0.36^{a}	3.50 ± 1.48^{b}	18.60 ± 4.64^{b}	61.40 ± 4.99^{a}
T3	932.60 ± 219.55^{d}	1.00 ± 0.00^{b}	3.00 ± 2,45^{b}	19.40 ± 4.64^{b}	66.20 ± 5.60^{a}

3.5. Effect of P. amboinicus L. Spreng on DMBA-induced liver histology

In our study, the histopathological measurement in the liver can be seen in Fig. 4a–e. The control group (Fig. 4a) was seen as the normal hepatic central vein architecture. However, the histopathological of the liver in the DMBA (PC) group (Fig. 4b) showed that the effect of administration of DMBA altered the hepatocyte architecture with hydropic degeneration, parenchymatous degeneration and necrosis. In Table 3, the number of normal cells was decreased and the number of necrotic cells increased. The hepatocytes indicated pyknosis is blackened and larger in cell membranes and the nucleus fragmented is unclear.

In T1-treated groups (Fig. 4c), the number of normal cells was significantly higher than the PC group. Furthermore, the number of hydrophilic degeneration cells was significantly lower than the NC, T2

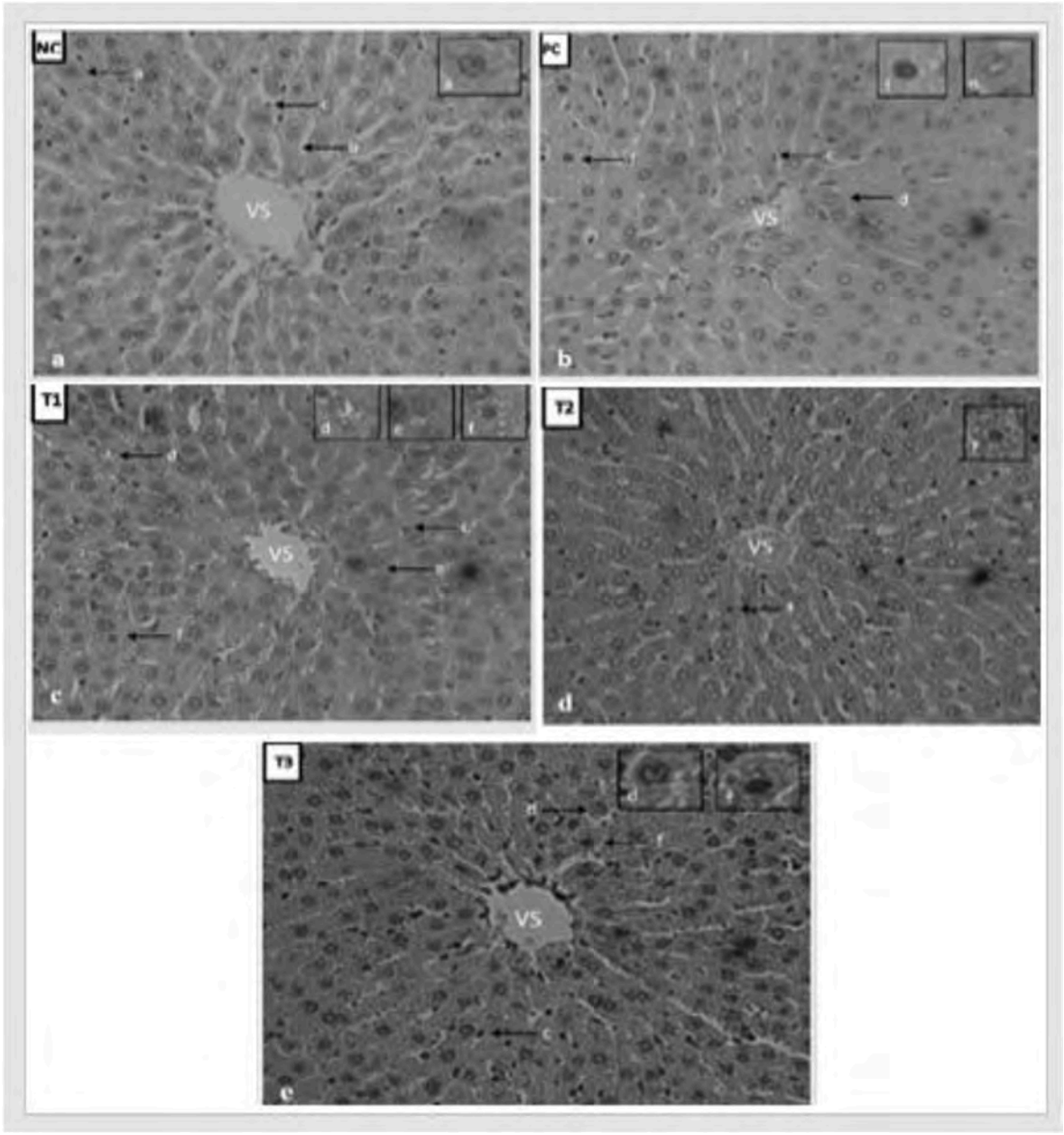

Fig. 4. Effect of *Plectranthus amboinicus* administration on the histological aspects of the liver: (a) negative control, namely the group that did not receive either PEE or DMBA; (b) the group receiving DMBA (20 mg/kg b. w.) showed inflammatory infiltrates and hepatocyte necrosis; the group that received PEE therapy at a dose of: 175 mg/kg b. w. (c); 350 mg/kg b. w.; (d); and 700 mg/kg b. w. (e). a. normal hepatocytes; b. Sinusoids; c. kuffer cells. Hematoxylin & Eosin staining; Bars, 100 m.

and T3 treatment groups. In addition, the number of necrosis was significantly lower than the PC group, while it was still higher than the control group, T2 and T3 treatment groups.

Histological observations of Fig. 4d showed that the number of normal cells increased significantly ($p < 0.05$) in the T2 group compared to the PC group. The number of hydrophilic degeneration cells was significantly higher than the CN, PC and T1 treatment groups. The number of necrosis cells decreased significantly compared to the PC group, but it was still higher than the CN and T3 groups. The number of parenchymatous degeneration was significantly lower in the T1 group than the CN group, while it was not significantly different compared to PC, T1 and T3 groups.

Our study showed that the T3 group has significantly higher number of the normal cells and hydrophilic degeneration cells compared to all other groups in Fig. 4e. The number of necrosis cells was significantly lower than that of PC, T1 and T2 groups, however it was higher than the CN group.

In Table 3, the number of normal hepatocytes decreased significantly in the PC treatment group. This indicated that DMBA caused necrosis of liver cells as observed in the histological image of Fig. 4b. The DMBA (PC) treatment group had a significantly higher number of necrotic cells ($p < 0.05$) compared to all groups. Hepatocyte damage assessment scores can be seen in Table 3.

4. Discussion

The liver is an important key role in the human body with various functions especially to respond to several serious diseases. Liver disease caused by drugs, toxins, or heavy alcohol consumption. In addition, contemporary medicines are less effective to protect liver injury, therefore development of less toxic ethnomedicine treatment is needed to maintain liver activity.

Our finding claimed that the PEE has significant hepatoprotective activity in reducing the level of AST compared to the PC group displayed in Fig. 1b. AST is a mitochondrial enzyme which is released from the heart, liver, skeletal muscles, and kidneys. The high doses of toxicity compounds elevated the level of AST due to tissue damage resulting in acute necrosis (Pawandeep et al., 2019). However, the administration of PEE significantly reduced AST levels which indicate that the extract had significant efficacy as the hepatoprotectant.

Hereinafter, the ALT is an important enzyme responsible for the reversible transfer of the amino group from L-alanine to pyruvate and glutamate (Yadav et al., 2022). The results showed that DMBA could damage liver function by increasing ALT activity to more than 400 U/L, while the normal value of ALT in rats was 54–298 U/L (Upadhyay et al., 2019). The level of ALT in PC groups was higher compared to its value (Sehitoglu et al., 2018) in rats induced apomorphine and paracetamol. We confirmed that the levels of ALT decreased significantly in the T1, T2 and T3 rats due in consequence of several phytochemicals of PEE, such as quercetin, apigenin, and luteolin, tend to cover the liver damage in order to normalize the level of ALT and AST. Quercetin of PEE is an effective compound in preventing acute liver injury exposed by difenoconazole which has properties to be anti-inflammatory agents, antioxidants, and antiaggregant and hepatoprotective (Bahadir et al., 2017). In addition, apigenin of PEE (8.4 mg/g) could be an important compound that has hepatoprotective properties (El-hawary et al., 2012). Apigenin reduces liver injury (Jimmi, 2021). and kidney toxicity by attenuating oxidative stress and markers of tissue injury, histopathological changes, apoptosis, and inflammation in rats exposure methotrexate (Sahindokuyucu-Kocasari et al., 2021). Apigenin also recovers liver function impaired in induced paracetamol and CCl_4 by improving the level of liver enzyme into control values (Martic, 2017; Yue et al., 2020). This is in line with the previous observation that the level of AST and ALT an increase significantly in mice induced CCl_4, however the administration of apigenin could stimulate ischemic injury for relieving the level of ALT and AST (Tsiaousidou et al., 2016; Ji et al., 2021).

Bilirubin has two types as the product of heme catabolism, such as indirect bilirubin (IDB) and direct bilirubin (DB). IDB is converted into DB in liver cells and excreted into bile acids. Bilirubin, is known as hematoidin, forms the yellow color as breakdown product of normal heme catabolism. Heme is found in hemoglobin, which tend to be the main component of red blood cells which is excreted in the bile and urine and the elevation of its levels could be an indicator of certain diseases (Odiegwu et al., 2021). The liver injury could be provoked by increasing the level of total or indirect bilirubin (Guerra Ruiz et al., 2021). Likewise, the dose of DMBA has caused liver damage by increasing bilirubin, however the administration of PEE normalized the number of bilirubin which indicate the potential of PEE as hepatoprotectant.

ALP is an important blood serum which the normal serum ranges at 20–140 U/L to detect liver diseases which is found in sinusoids and bile duct membranes (Shi et al., 2021). There is a rise in the level of ALP in the PC group as shown in Fig. 3, while the PEE as hepatoprotectant significantly degraded the level of ALP in rats administered DMBA. The elevation of ALP values is caused by impaired hepatic excretion in liver parenchyma or ductal cells (Pawandeep et al., 2019).

Furthermore, the total protein in blood is helpful for clinical biochemistry and is one of the most reliable methods for identification of blood proteins that is considered as an abnormality indicator (Franca et al., 2011). Our finding demonstrated that the DMBA caused abnormalities in rats by decreased levels of total protein, albumin and globulin, while *P. amboinicus* leaves that have many bioactive compounds, could enhance the levels of total protein, albumin and globulin in rats induced DMBA. This is in line with Ekam and Udosen (2012) reported that paracetamol at dose 171.41 mg/kg administered orally in rats could damage the liver as shown in the reduction of the levels of total protein, albumin and globulin. However, the extract of *Vernonia amygdalina* as hepatoprotectant at dose 200 mg/kg significant increase ($P < 0.05$) in total protein (g/dl) in rats received paracetamol. The previous study (Mujahid et al., 2017) revealed that the reduction of total protein and albumin in rats induced the antitubercular drugs Isoniazid and rifampicin, while the administration of *Erythrina indica* leaf extract 200 mg/kg body weight increased the total protein and albumin levels in serum significantly (1.90 ± 0.16 to 2.48 ± 0.99) for total protein and (1.39 ± 0.09 to 2.3 ± 0.14) for albumin. The bioactive phytochemical of the *Erythrina indica* leaf, for instance apigenin, genkwanin, iso-vitexin, swertisin, and saponarin, contributed to the improvement of level of total protein and albumin.

P. amboinicus contains apigenin (Sugiharto et al., 2020; Silitonga et al., 2014) as hepatoprotective activity (Ji et al., 2021). According to (Saddick et al., 2023), CCl_4 significantly increased the activity of ALT and AST enzymes in blood serum, as well as levels of MDA and 8-OHdG in liver tissue, and decreased GSH levels. Pre-treatment with apigenin was not only able to suppress the increase in ALT, AST, MDA and 8-OHdG, and inhibit the reduction of GSH in vivo, but also reduced hepatocyte damage in vitro. Apigenin has strong antioxidant activity against reactive oxygen species (ROS) in vitro depending on concentration as ROS scavengers. These results obtained in vivo and in vitro indicated that apigenin had a protective effect against chemical-induced liver oxidative injury.

The liver plays an important role in normal erythropoiesis and the synthesis of clotting factors and inhibitors, and it maintains homeostasis in the human body. The liver also functions as a storage of some minerals for example iron, vitamin B12 and folic acid which is required to promote hematopoiesis (Jha, 2019). Therefore, the alteration of hematological profile could be an indicator of abnormalities and/or injury in the liver. Besides, the liver is involved in various hematological disorders due to its unique portal circulation and synthetic functions for example clotting factors, and thrombopoietin, as well as its function in the immune system. In addition, liver disorders such as cirrhosis can lead to hematological abnormalities and primary hematological diseases which affect the changes in the hematological profile such as erythrocytes, leukocytes, platelets and disorders of coagulation (Satué et al., 2023). We discovered liver function disorders occurred in rats exposed to DMBA by changing the level of blood parameters. Table 1 provides the data that platelets value is lower in PC groups in rats administered DMBA. Our result is similar to the previous observation that explained that 48% of patients with liver disorders had thrombocytopenia, which is a decreased platelet count (Luis Calleja et al., 2023). On the other hand, the PEE could restore liver function and play as hepatoprotective agent by raising the production of platelets in the T1 and T3 groups. Our finding is in line with the previous observation (Asiimwe et al., 2014) that the level of thrombocytes (platelets) enhanced due to the immunostimulant of *P. amboinicus*. The bioactive compounds include saponins, flavonoids, and alkaloids tend to enhance the immune system through stimulation of cell division and lymphocyte transformation (Sinaga et al., 2020).

Other indicators of alteration hematologically related to liver disorders caused by DMBA induction are the elevation of the number of neutrophils and monocytes and the reduction of the number of eosinophils and basophils performed in Table 1. The experiment data are in line with Brantley (2019) that the administration of DMBA 20 mg/kg bw to rats increased the level of monocyte from 2.33 ± 0.66 in the control group to 3.66 ± 0.33. The enhancement of the number of neutrophils is usually due to the presence of bacteria or infection as well as the existence of autocrine, paracrine, and immune system modulating functions involved in liver diseases, including viral hepatitis, nonalcoholic steatohepatitis, alcoholic liver disease, liver fibrosis, cirrhosis, liver failure, and liver cancer (Liu et al., 2017; Tang et al., 2021). Neutrophils are the largest number of circulating leukocytes which participate in various processes of immune reactions (Rosales, 2018) and inflammation (Hassouna et al., 2022). For example, the worsening of liver occurs in the development of alcoholic liver disease (ALD) lead to produce the high number of neutrophils and migrate from the blood circulation to

the tissues injury that regulated by chemokines, cytokines, and adhesion molecules (Yang et al., 2022). Meanwhile, the highest number neutrophils is found in patients acute cancer by releasing its homolog, that is neutrophil-released neutrophil elastase (ELANE), destroyed cancer cell types and activated cytotoxic T lymphocytes to weaken the tumor development (Cui et al., 2023). We verified that the number of neutrophils and monocytes is higher in PC group compared to NC group in rats induced DMBA, while *P. amboinicus* extract recovers liver injury and reduces cell necrosis. According to Brantley (2011), the administration of DMBA, which is a carcinogen compound, induced mammary cancer in mice and increased both the number of neutrophils and monocytes than the control group.

Furthermore, the number of eosinophils could be an indicator in liver disorder due to destruction of pathogens by inducing basophil to secrete antibodies and prevent blood clots. Eosinophils, promote the immune system and inflammatory responses and accumulate in blood and tissues by expression of 12/15 lipoxygenase in infectious and acute diseases, tend to be a regulator of immune system and homeostatic function (Zhao et al., 2021). Our experiment data showed that the level of eosinophil is lower in the PC group, however PEE increased ($p < 0.05$) the level of eosinophil at 5.25 ± 1.16 in T1 group in order to improve the destruction of the immune system by DMBA compound. In addition, the number of monocytes is the highest in the PC group as shown in Table 1 which indicated the disruption in the liver due to DMBA exposure. We claimed that the number of monocytes is reduced in all of PEE treatment compared to PC groups in rats administered DMBA. The elevation of monocytes value caused by hepatic impairment in order to maintain tissue integrity (Triantafyllou et al., 2018). The previous study described a rise in the level of monocytes in patients with chronic liver disease due to immunological imbalance (Cardoso et al., 2021).

Apigenin is a flavonoid compound that possess antioxidant and anti-inflammatory properties in treating asthma, insomnia, Parkinson's disease, neuralgia, and herpes zoster and restoring the immune system in suppression of inflammation (Li et al., 2018). Apigenin is also found abundance in *P. amboinicus* (Silitonga et al., 2014; Jimmy, 2021) purposed to maintains immune cells. In this study, the hematological profile (Table 1) showed that the bioactive of PEE plays a role in normalized the altered immune system related to liver in rats exposure to DMBA.

Hereafter, We examined the value of MCV was a significant increase in the PC group. Our observation is similar with the previous study revealed that the mean MCV was significantly higher in patients with liver disease compared to normal controls according to the *t*-test ($p < 0.001$), even the MCV increased in reduction of the level of albumin (Das et al., 2011). Our study suggested that DMBA increased the size or volume of MCV caused the disruption in liver function, whereas the administration of PEE potentially reduced MCV levels in the T2 and T3 treatment groups which is similar with the value of MCV in the control group (CN). The ability of PEE to normalize the level of MCV is due to several phytochemicals of PEE such as vitamins (vitamin C, vitamin B1, vitamin B12, beta carotene) (Lans and van Asseldonk, 2020) Fe, and the bioactive compound such apigenin and quercetin (Hullatti et al., 2011).

MCH (mean corpuscular hemoglobin) is an indicator of erythrocyte to confirm the type of anemia associated with cirrhosis of liver, for instance, the highest of MCH levels are usually a sign of macrocytic anemia (Sheng et al., 2022). The experimental data showed that an increase in the level of MCH due to DMBA exposure. The higher dose of toxicity compound caused a risen of the level of MCH in liver disease such as cirrhosis by excessive alcohol drinking (J. Liu et al., 2022) and chronic liver disease (Rappai et al., 2019). The administration of PEE reduced the level of MCH as displayed in Table 2 due to Vitamin B12 in PEE to regulate the level of erythrocytes to maintain hemoglobin homeostatis in the rats exposured to DMBA.

The mean corpuscular hemoglobin concentration or MCHC is a calculation of the concentration or average level of hemoglobin in one red blood cell. In this study, MCHC decreased with DMBA administration in line with previous studies (Silitonga et al., 2018) that

Table 2
Values of physical form of red blood cells (MCH, MCHC, MCV, RDW) and MVP in different treatment groups.

Treatment	MCH (pg)	MCHC (g/dl)	MCV (fl	MVP (fl)	RDW (%)
NC	19.56 ± 1.27[a]	31.08 ± 0.49[a]	60.20 ± 1.64[a]	8.32 ± 0.19[a]	15.84 ± 1.16[a]
PC	23.98 ± 2.60[b]	32.14 ± 1.26[a]	63.12 ± 3.18[b]	8.38 ± 0.31[a]	16.06 ± 1.43[b]
T1	19.82 ± 0.78[a]	32.54 ± 0.33[a]	63.70 ± 3.35[b]	8.02 ± 0.19[a]	15.60 ± 1.37[a]
T2	19.62 ± 0.83[a]	32.52 ± 1.32[a]	60.96 ± 0.72[a]	8.66 ± 0.25[a]	14.50 ± 1.11[a]
T3	19.80 ± 0.44[a]	33.22 ± 0.33[b]	60.40 ± 1.69[a]	8.32 ± 0.24[a]	15.56 ± 1.53[a]

Table 3
Modified hepatocyte damage assessment score for manja roegnik cretaceous.

Treatment	Normal cells	Hydrophilic cells Degeneration	Necrosis cells	Parenchymatic cells Degeneration
CN	13.92 ± 1.75[b]	2.42 ± 1.758[a]	1.94 ± 0.712[a]	2.50 ± 0.58[b]
PC	7.89 ± 2.136[a]	2.22 ± 2.136[a]	9.84 ± 2.699[b]	1.72 ± 0.65[a]
T1	13.96 ± 1.74[b]	1.05 ± 1.740[b]	4.32 ± 0.807[c]	1.00 ± 0.00[a]
T2	14.16 ± 1.21[b]	4.18 ± 1.211 [ab]	3.97 ± 1.920[c]	1.70 ± 0.30[a]
T3	16.40 ± 0.86[b]	6.40 ± 0.86 [ab]	2.23 ± 0.584[a]	1.40 ± 0.54[a]

Rhodamine-B decreased levels of MCHC and hemoglobin significantly compared to the control groups. This decrease in MCHC and Hb indicated anemia. However, the PEE as a preventive agent normalizes the level of MCHC due to the presence of the bioactive compound and iron to regenerate the Hb value (Silitonga et al., 2018).

We explored that the PEE could suppress the highest level of RDW in rats exposed. The increase of the level of RDW is associated with impaired liver function. RDW is a method to calculate the number of anisocytosis (variation in cell size) and poikilocytosis (variation in cell shape) of red blood cells on peripheral blood examination. RDW indicates the coefficient of variation of the red blood cell volume distribution which is a potential prognostic index for liver disease (Hu et al., 2013). RDW was positively correlated with serum bilirubin and creatinine levels, prothrombin time, and negatively correlated with platelet count and serum albumin concentration. The elevation of the level of RDW could be caused by several factors such as inflammatory cytokines can suppress erythrocyte maturation faster, larger and accelerate reticulocytes to enter the peripheral circulation (Ren et al., 2023).

Mean platelet volume (MPV) is the average size of thrombocytes/platelets. The increase of level of MPV associated with the elevation of the number of platelets produced in the body. Elevated MVP usually occurs in hepatocellular carcinoma (HCC) (Omar and Elbehisy, 2018) and MPV differs significantly between patients with different severity of liver fibrosis (Dehghani et al., 2020). In contrast, the reduction of level of MPV could indicate the decreased platelet count (thrombocytopenia) (Delwatta et al., 2018). Our observation revealed that the level of MVP was no difference between all treatment groups in rats exposed DMBA.

Our experiment data demonstrated that liver damage caused by DMBA displayed the lack of normal cells and the number of cells undergoing necrosis (Table 3) in the liver tissue. This is in line with the previous study (Abdelghffar et al., 2022) which stated that the liver sections of DMBA-treated rats showed a loss of hepatic cord regularity due to hepatocellular swelling. Blood sinusoids were no longer visible except in some pericentral areas where some hepatocytes showed vacuolated cytoplasm. In addition, the alteration of necrotic was observed among the periportal hepatocytes showed that liver lesions

reduce the number of hepatocytes, interfere the uptake of indirect bilirubin from the plasma and reduce the mechanism function of direct bilirubin through the bile ducts (Guerra Ruiz et al., 2021). This study is in line with the research conducted by Mujahid (Mujahid et al., 2017) that the number of necrotic cells increased with the treatment of Isoniazid and rifampicin (3.39 ± 0.98), while the administration of *Erythrina indica* leaf extract 200 mg/kg decreased to 1.56 ± 0.95. Furthermore, apigenin is a flavonoid compound contributing to the mechanism of reduction necrosis as hepatoprotective agent. Similarly, we found that the hepatoprotective activity *of P. amboinicus* leaves on liver histology is associated with the bioactive compounds of PEE, for instance apigenin. The decrease in the number of necrosis cells in the T1, T2 and T3 treatment groups (Fig. 4c and d, and Table 3) indicated a hepatoprotective effect of PEE on the impaired liver due to DMBA induction successfully inhibiting liver cell necrosis. Furthermore, we also stated the value of bilirubin is higher in the PC group (Fig. 2). The bilirubin level indicates the severity of the necrosis (Jiang et al., 2022). The hepatoprotective effect of PEE significantly decreases the bilirubin levels in the T1, T2 and T3 exposed DMBA.

The administration of ethanol extract of *P. amboinicus* leaves reduced the level of liver damage in rats treated with DMBA. Apigenin reduced liver injury by ameliorating inflammation and oxidative stress through suppression of the non-canonical NF–B pathway (Xu et al., 2023). This indicates the potential of apigenin for the treatment of liver damage. The previous research (Warkad et al., 2021) also explained that oxidative stress caused lesions in liver cancer cells, while apigenin reduce a cell growth as anticancer and improve Kupffer cells and lymphocyte cell aggregation around the central vein in mice treated metformin. Apigenin could repair liver injury by reducing the production of excessive amounts of oxidative stress in apoptosis (Tsiaousidou et al., 2016). Apoptosis is a physiological process that serves as an important mechanism of tissue homeostasis by eliminating abnormal cells. Moreover, Apigenin is a bioactive compound of PEE contributed to protect against ischemia/reperfusion injury in the liver. Our finding is similar to the previous study which claimed that Apigenin induces apoptosis of Hep G2 cells which examined by flow cytometric assay-staining with propidium iodide in the proliferation of human liver cancer cell (Qanash et al., 2022). This indicated that apigenin had a selective growth inhibitory effect on hepatoma cells. Apigenin at dose 25 mg/kg bw protected the liver against oxidative stress and DNA damage caused by carcinogens in rats induced N-nitroso-diethylamine and methotrexate (Goudarzi et al., 2021).

Saponarin (apigenin-6-C-glucosyl-7-O-glucoside) was isolated from *Gypsophila trichotoma* (Simeonova et al., 2013) showed that this compound was able to repair the damage caused CCl_4-induced by decreasing MDA levels, increasing CSH levels and preventing oxidative damage in order to maintain antioxidant activity. In addition, saponarin tends to prevent histopathological changes, namely lymphocytic infiltration, steatosis and centrilobular necrosis in rats administered CCl_4 (Simeonova et al., 2014). In addition, the saponarin could scavenge free radicals by preventing the inflammatory response (Zeng et al., 2018) and protect the liver injury in rats induced with paracetamol. Moreover, *P. amboinicus* leaves have bioactive compounds, for instance apigenin, tend to be the hepatoprotective agent. Epidemiological studies have shown that a diet rich in flavonoids is associated with a reduced risk of certain cancers, particularly breast, gastrointestinal, skin, prostate, and certain hematological malignancies (Shukla and Gupta, 2010).

5. Conclusion

DMBA causes impaired liver function as indicated by the elevation of levels of ALT, AST, ALP, total, and direct bilirubin. *P. amboinicus* acts as a hepatoprotective to suppress the levels of ALT, AST, ALP, total, and direct bilirubin in the T1, T2, and T3 groups. *P. amboinicus* served as hepatoprotective by increasing total protein, albumin, and globulin significantly compared to the DMBA group. PEE increased platelets, basophils, and eosinophils significantly compared to the control groups. Neutrophils and monocytes increased significantly in the T1 and T2 groups compared to all other groups. *P. amboinicus* decreased MCH, RDW, and MCV significantly and had no effect on MCHC and MVP. Based on histological observations, *P. amboinicus* acted as hepatoprotective by increasing normal hepatocytes, hepatocytes undergoing hydrophilic degeneration, and decreasing the number of hepatocytes undergoing necrosis and hydrophilic degeneration. In conclusion, *P. amboinicus* had hepatoprotective activity by enhancing liver function, increasing the non-specific immune system and improving the liver histology of exposed mice.

CRediT authorship contribution statement

Melva Silitonga: Conceptualization, Formal analysis, Investigation, Project administration, Writing–original draft. **Erlintan Sinaga:** Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – review & editing. **Meida Nugrahalia:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Pasar M. Silitonga:** Conceptualization, Investigation, Writing–review & editing.

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Ethical Statement

Authorships and affiliatio

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On behalf of all the authors, I declare that:

1) This material is the authors' own original work, which has not been previously published elsewhere.
2) The paper is not currently being considered for publication elsewhere.
3) The paper reflects the authors' own research and analysis in a truthful and complete manner.
4) The paper properly credits the meaningful contributions of co-authors and co-researchers.
5) The results are appropriately placed in the context of prior and existing research.
6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

The violation of the Ethical Statement rules may result in severe consequences.

I agree with the above statements and declare that this submission

follows the policies of Toxicon as outlined in the Guide for Authors and in the Ethical Statement.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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