Research Article

The Antioxidant and Hepatoprotective Activities of the Ethyl Acetate Leaf Extract of *Ficus pseudopalma* Blanco

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ABSTRACT

The antioxidative and hepatoprotective properties of defatted ethyl acetate extract fractionated from the crude methanolic leaf extract of *Ficus pseudopalma* Blanco, an endemic Philippine medicinal plant, were investigated. The extract possessed high total flavonoid (21.80 ± 0.16 mg quercetin equivalent/g) and total phenolic (12.93 ± 0.57 mg gallic acid equivalent/g) content as well as strong 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity ($IC_{50} = 0.71 \pm 0.01$ µg/mL). Assessment of the *in vitro* antioxidant activity of the total phenolic fraction (TPF) from the extract revealed a concentration-dependent DPPH (IC_{50} IC50= 2.73 ± 0.04 µg/mL) and nitric oxide ($IC_{50}= 4.96 \pm 0.05$ µg/mL) scavenging activity as well as strong Fe^{+3} reducing ability ($RC_{50}= 11.74 \pm 0.19$ µg/mL). An *in vivo* hepatoprotective study using Sprague-Dawley rats revealed that the extract possessed significant ($p<0.05$) hepatoprotective activity against paracetamol (500 mg/kg)-induced oxidative damage. Extract treatment markedly attenuated increase in serum transaminases, alkaline phosphatase (ALP), and liver malondialdehyde (MDA) levels while preventing marked decrease in albumin (ALB), reduced glutathione (GSH) and catalase (CAT) levels. The extract also ameliorated histopathological changes to liver tissue in a dose-dependent manner. The extract at a dose of 400 mg/kg was comparably similar ($p>0.05$) to the hepatoprotective effect of N-acetylcysteine (NAC) at 100 mg/kg. High performance liquid chromatography (HPLC) revealed the presence of lupeol and quercetin that were likely responsible for the antioxidant and hepatoprotective activities exerted by the extract.

Keywords: *Ficus pseudopalma*, ethyl acetate extract, antioxidant, hepatoprotective, lupeol, quercetin

INTRODUCTION

Drug-induced liver injury accounts for most cases of acute liver failure and transplantation in developed Western countries[1-2]. In particular, paracetamol (acetaminophen) in overdose is known to be the principal cause of acute liver failure (ALF) in the United States, United Kingdom and most of Europe[3]. Drug-induced liver injury in the case of paracetamol poisoning is an example of liver disease associated with oxidative stress. When taken in overdose, it is converted to a free radical, N-acetyl-p-benzoquinoneimine (NAPQI), causing glutathione depletion, more free radical formation and eventually, cell death[4]. As a precursor of glutathione, N-acetylcysteine (NAC) is clinically used as the antidote for paracetamol poisoning[3].

In the search for better drugs, researchers have turned to plants for their bioactive constituents such as antioxidants that may have possible therapeutic effects against such oxidative stress-related diseases[5]. An endemic species of antioxidant-rich *Ficus* found in the Philippines – *Ficus pseudopalma* or the Philippine fig – locally known as *Lubi-lubi* has long been used for either medicinal or nutritional purposes[6-7]. Studies using the crude ethanolic leaf extract from the plant have shown its strong antioxidant and potent scavenging activities[8-9]. The plant has also been studied for its hypoglycemic[10-12], anti-urolithiatic[13] and antibacterial properties[14], as well as its cytotoxic and apoptotic activity against certain cancer cell lines[15-17]. A previous study using the crude ethanolic leaf extract has shown that the plant may have hepatoprotective potential[18]. Recent studies have revealed that the ethyl acetate fraction from the crude leaf extract possesses potential bioactive properties[13,16], and have detected the presence of lupeol, a pharmacologically-active triterpene[19], in the ethyl acetate fraction. This study was performed to assess the antioxidant as well as the hepatoprotective properties of the ethyl acetate extract partitioned from the crude alcoholic leaf extract of *F. pseudopalma*, using rats as *in vivo* models for paracetamol-induced oxidative liver damage. The study also aimed to identify what compound/s present in the said extract may be responsible for such activities.

MATERIALS AND METHODS

Standards, Reagents and Chemicals
Analytical-grade reagents and chemicals like paracetamol and NAC were purchased from Sigma-Aldrich through Belman Laboratories, Inc. Standards such as quercetin and lupeol were also from Sigma-Aldrich. Standard kits used for biochemical assays such as aspartate aminotransferase

(AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and albumin (ALB) were from Human Diagnostics (Human Gmbh) through Biocare Health Resources, Philippines.

Plant Preparation and Extraction

Ficus pseudopalma Blanco leaves were collected and authenticated by the Botany Division of the National Museum of the Philippines. Leaves were allowed to air-dry under shade for three weeks until crumbly, ground using Wiley Mill then percolated with methanol for 48 hours. This procedure was repeated five to six times. The crude methanolic extract produced was further fractionated following a modified Kupchan's partition scheme[20] using solvents of increasing polarity: petroleum ether<chloroform<ethyl acetate<n-butanol<water.

Determination of total phenolic content (TPC)

Total phenolic content was determined based on the procedure of Singleton *et al* [21]. A volume of 0.5 mL of diluted sample was reacted with 0.20 M Folin-Ciocalteau's reagent for four min, and then added with saturated sodium carbonate solution (75g/L). The tubes were swirled and incubated at room temperature for two hrs then absorbance readings were taken at 760 nm. TPC was expressed as milligrams of gallic acid equivalent (mg GAE)/g extract.

Determination of total flavonoid content (TFC)

Total flavonoid content was determined using aluminum chloride colorimetric method according to Woisky and Salatino[22]. A calibration curve was first made from several dilutions of quercetin. Similar dilutions of the sample were prepared and separately mixed with 95% ethanol, 10% aluminum chloride, 1M sodium acetate and distilled water. After 30 min of incubation at room temperature, absorbance of the reaction mixture was read at 415 nm. The TFC of the different extracts was expressed as quercetin equivalent (mg QE)/g extract.

DPPH Radical Scavenging Activity

The scavenging activity of the extracts against DPPH radical was measured according to the method of Batool[23]. Different concentrations of the extracts were prepared in 95% methanol. Freshly prepared DPPH solution (0.125 mM) in methanol was added to 0.5 mL solutions of crude extract and fractions. The reaction mixture was allowed to incubate for 15 min at room temperature and absorbance was read at 517 nm. Ascorbic acid was used as standard.

Preparation of Total Phenolic Fraction and In vitro Antioxidant Assays

The extract with the highest TPC and TFC content and the lowest DPPH IC_{50} value was further separated via silica gel column chromatography. The extract was permeated on Silica Gel 60 mm (Merck) for column chromatography with solvent systems of increasing polarities as eluents: hexane hexane:chloroform (1:1), chloroform, chloroform:ethyl acetate (70/30), ethyl acetate, ethyl acetate:n-butanol (70/30) and n-butanol, respectively. Five milliliters of column fraction were collected and each fraction was tested for TPC and TFC. The column fractions with significantly higher TPC and TFC compared with other fractions were pooled together into the total phenolic fraction (TPF) and used for *in vitro* antioxidant assays: DPPH and nitric oxide scavenging assays and ferric-

reducing antioxidant power (FRAP) assay.

Nitric Oxide Scavenging Assay

Nitric oxide (NO●) was measured via the the Griess-Illosvoy reaction[24]. The reaction mixture containing sodium nitroprusside (10 mM) in phosphate buffer solution (pH 7.4) and various concentrations of the test compounds (0-70 µg/mL) was incubated at 25°C for 150 min. Every 30 min, 0.5 mL of the incubated sample was removed and 0.5 mL of Griess reagent was added. The pink chromophore resulting from the diazotization of nitrite ions was measured at 540 nm against a blank sample. Ascorbic acid was used as the standard while sodium nitroprusside in PBS was used as blank.

Ferric-reducing Antioxidant Power (FRAP) Assay

The procedure was based on the method of Saha *et al* [25]. An aliquot of 0.5 mL of different dilutions of the fraction in methanol was mixed with phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide then incubated for 20 min at 50°C. Trichloroacetic acid (1.25 mL, 10%) was added to the mixture and then shaken vigorously for 10 min. An aliquot from the upper layer of this solution was taken and added with distilled water and $FeCl_3$ (0.25 mL, 0.1%) and the absorbance was read at 700 nm. Ascorbic acid was used as a standard and phosphate buffer (pH 6.6) as blank. The antioxidant power of the fraction was expressed in reducing concentration (RC_{50} in µg/mL), necessary to reduce 50% of Fe^{+3} ions in solution to Fe^{+2}.

Animal handling and treatment

Adult male albino Sprague-Dawley rats weighing between 180 – 250g were obtained from the Food and Drug Administration (FDA) Philippines, and kept in standard laboratory conditions under natural light-dark cycle. Rats were maintained on standard pellet diet and water *ad libitum*. The test animals were subjected to three (3) to seven (7) days acclimatization period. Food was withdrawn 12 hrs before starting the experiments but water was given *ad libitum*. Approval from the Institutional Animal Care and Use Committee (IACUC) of the University of Santo Tomas, Philippines was sought prior to performing the animal experiment.

Toxicity Test (Limit Test)

Since leaves of the plant are eaten regularly as a vegetable dish, only a limit test was carried out using five male Sprague-Dawley rats following OECD 425 Guidelines[26]. The safety profile of *F. pseudopalma* leaves is further supported by a previous acute oral toxicity study in which the crude ethanolic leaf extract was found to be practically non-toxic at the limit dose of 2000 mg/kg[27]. For the limit test on the ethyl acetate extract, one rat was given 2000 mg/kg of extract via oral gavage. The rat survived hence the rest of the animals were given the same dose of extract and observed within a 14-day period. After this study period, all the rats were sacrificed via cervical dislocation. A control rat, given rodent diet and water only, was used as basis for comparison with the limit dose-treated rats.

Hepatoprotective assay procedure

The procedure was based on the method of Sabir *et al* [28] with modifications. Thirty-six (36) healthy and properly identified young adult male Sprague-Dawley rats were randomly divided into six (6) groups of six (6) rats each:

Group I (Normal Control) – received the vehicle (2% Tween 80 in distilled water, 1 mL/kg *per orem*).

Group II (Toxicant) – given 500 mg/kg paracetamol only for seven days.

Group III (Low-dose extract) – administered paracetamol (500 mg/kg) and a low dose (200 mg/kg) of extract.

Group IV (High-dose extract) – received the same dose of paracetamol and a higher dose of extract (400 mg/kg).

Group V (Standard/NAC) – administered a similar dose of paracetamol and given NAC (100 mg/kg).

Group VI (Extract-only) – given extract alone (400 mg/kg) to determine any toxic effects toward the liver

Plant extracts and NAC were administered four (4) to six (6) hours after paracetamol administration. All treatments and toxicant were given orally (per orem) by means of a gavage tube for seven (7) days. Blood (serum) was collected via tail clipping before (Day 0) and after (Day 8) the administration of all test substances, for assessment of biochemical parameters of hepatoxicity and liver function: ALT, AST, ALP and ALB. The weight of each rat was recorded on the 8th day. Following blood collection on the 8th day, all animals were sacrificed via cervical dislocation. The liver from each rat was collected and weighed. A major portion of the liver from the sacrificed animal was preserved in 10% buffered formalin for histopathological analysis while the remaining portion was used to prepare the liver homogenate for glutathione and catalase assays and for the measurement of lipid peroxidation via thiobarbituric acid-reactive substances (TBARS) assay. Slides from liver samples were taken to a histopathologist for reading and interpretation.

Reduced glutathione (GSH) Assay

The procedure was based on the method of Saeed *et al*[29] with modifications. An aliquot of 0.5 mL supernatant from the liver homogenate was precipitated with sulphosalicylic acid (0.5 mL, 4% w/v). The mixture was kept at a temperature of at least 4°C for one hour then centrifuged for 15 min at 1200 x g. A 10 µL aliquot from the supernatant of the centrifuged mixture was taken and added with 270 µL of phosphate buffer (50 mM, pH 7) and 20 µL of 5,5′-Dithiobis-2-nitrobenzoic acid or DTNB (4.5 mM). A blank was used and the absorbance read at 412 nm with the concentration of GSH (µmol/g sample) computed from the molar absorption coefficient (ε) of TNB (14,150 $M^{-1}cm^{-1}$ at 412 nm).

Catalase (CAT) Activity Assay

Catalase activity was determined by the method of Bogdanska *et al* [30]. An aliquot of supernatant (10 µL) was pipetted into a microplate and reaction was started by the addition of 100 µL freshly prepared H_2O_2 (19 mM) in phosphate buffer, and 195 uL of phosphate buffer (50 mM, pH 7.4) The rate of H_2O_2 decomposition was measured at 240 nm per minute and CAT activity in U/l was calculated using the ε of H_2O_2 (43.6 $M^{-1}cm^{-1}$) at 240 nm.

TBARS Assay

The degree of lipid peroxidation was assessed through the method of Manna *et al*[31]. A volume of 60 µL thiobarbituric acid (1% w/v) and 75µL trichloroacetic acid (10% w/v) were added to 15 µL of liver homogenate. The reaction mixture was heated in a boiling water bath for 15 min then centrifuged at 10,000 x g for five min. The pink chromogen (TBARS) produced from the reaction was measured at 532 nm and computed using ε of malonaldehyde (MDA) which is 1.56 x 105 $M^{-1}cm^{-1}$.

Statistical analysis

All assays were performed in triplicate. Results were expressed as mean ± standard error (SEM). Independent t-test and one-way analysis of variance (ANOVA) were used to compare two or more groups of data. Post hoc analysis using Tukey and LSD were used with ANOVA and *p* values less than 0.05 were considered significant. SPSS software version 19 was used for statistical analysis.

High Performance Liquid Chromatography (HPLC)

In brief, HPLC analysis was conducted using Agilent Series II-HPLC machine equipped with quaternary pump and autosampler at 30°C. For the identification of quercetin, the mobile phase used was methanol:acetonitrile:water (60:20:20 v/v/v). A quantity of 5 mg of sample was accurately weighed and dissolved in 10 mL of mobile phase to make 0.5 mg/ml of solution. A volume of 10 µL sample solution was injected at a flow rate of 1.1 mL/min for a total run time of 14 min and detection at 262 nm[32]. For the identification of lupeol, methanol:acetonitrile (30:70 v/v) was used as mobile phase. About 10 µL of sample solution (0.5 mg/mL) prepared in methanol was injected at a flow rate of 1 mL/min for a total run time of 10 min and detection at 210 nm[33]. Quantification of detected antioxidant compounds was carried out using a calibration curve from various concentrations (25 to 150 ppm) of standards lupeol and quercetin.

RESULTS AND DISCUSSION

Extraction Yield

Different fractionated extracts were obtained through a partitioning scheme using solvents of increasing polarity. Petroleum ether yielded the highest amount of extract (1.74%) followed by water (1.40%), chloroform (1.20%), n-butanol (1.02%) and ethyl acetate (0.70%). The percentage yield of each extract was computed per kg of plant sample.

Total Phenolic and Flavonoid Contents, and DPPH-Scavenging Activity of Extracts

Among all the fractionated extracts, the ethyl acetate extract had the highest TPC (12.93 ± 0.57 mg GAE/g) and TFC (21.80 ± 0. 16 mg QE/g) as well as the strongest DPPH radical scavenging activity (IC_{50} = 0.71 ± 0.01 mg/mL) as shown on Figure 1. The TFC and DPPH IC_{50} of the ethyl acetate extract differed significantly ($p<0.05$) with the rest of the extracts.

Preparation of Total Phenolic Fraction

The ethyl acetate extract was further subjected to column chromatography producing 12 different fractions each labeled according to their order of elution and tested for TPC and TFC. Among the column fractions, fractions six (19.16 ± 0.14 mg GAE/g), seven (18.08 ± 0.15 mg GAE/g) and eight (14.97 ± 0.25 mg GAE/g) had significantly higher ($p<0.05$) TPC than the rest of the fractions while fractions one (21.77 ± 0.51 mg QE/g), two (10.31 ± 0.02 mg QE/g), three (8.67 ± 0.24 mg QE/g) and four (15.16 ±

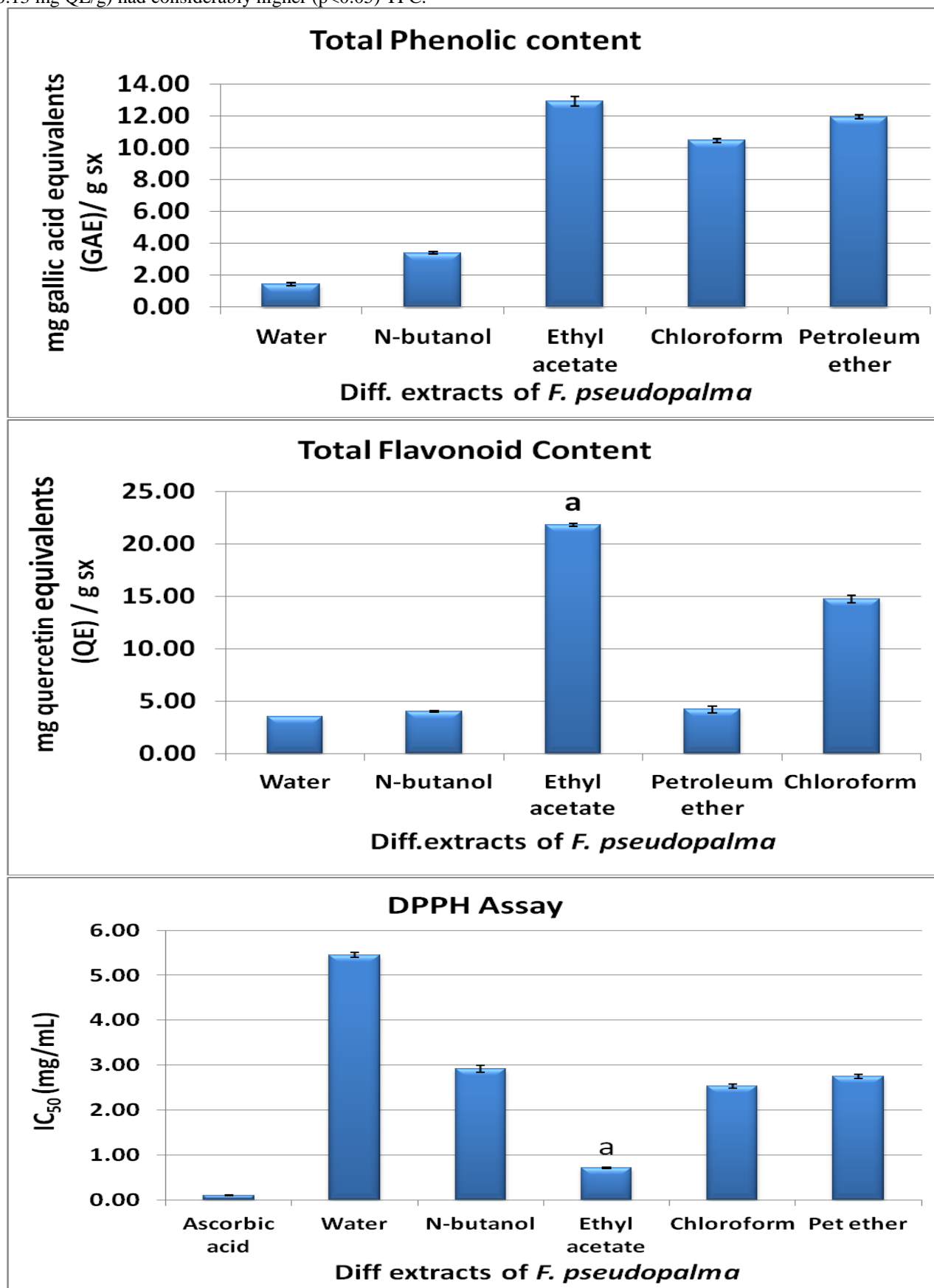0.13 mg QE/g) had considerably higher (p<0.05) TFC.

Figure 1. Total phenolic content (A), total flavonoid content (B) and DPPH-scavenging activity (C) of the fractionated extracts. Values (n=3) represent mean±SEM, [a] p<0.05 compared with the rest of the extracts.
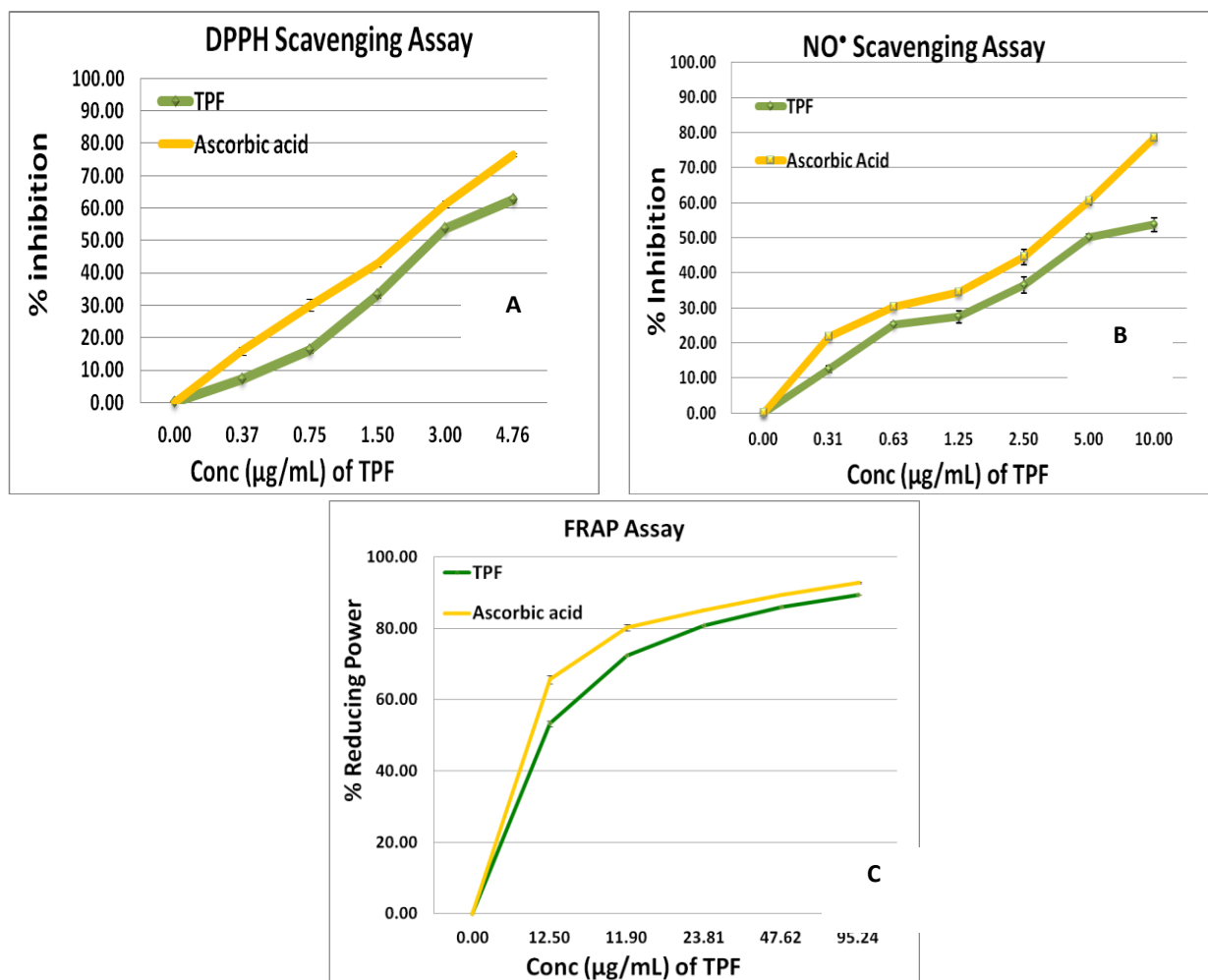
Figure 2. *In vitro* antioxidant activity of the TPF from the ethyl acetate extract of *F. pseudopalma* showing dose–dependent scavenging activity against (A) DPPH radical; (B) NO●; and (C) Reducing ability towards Fe^{+3} ions, compared with ascorbic acid.

These fractions were pooled together to obtain the total phenolic fraction (TPF) used for the in vitro antioxidant assays.

In vitro Antioxidant Activity

Data from previous research have shown that the crude ethanolic leaf extract from *F. pseudopalma* possessed strong antioxidant and free radical/reactive species–scavenging activity[8-9]. To determine if the ethyl acetate extract possessed the same properties, the TPF from the said extract was subjected to the following *in vitro* antioxidant activity assays.

DPPH Scavenging activity

One of the most widely used methods for determining the free radical scavenging activity of antioxidants such as polyphenolic compounds is by using 2,2-Diphenyl-1-picrylhydrazyl (DPPH), a stable free radical[34]. The total phenolic fraction exhibited concentration-dependent scavenging activity against the DPPH radical. As shown on Figure 2A, the scavenging activity of the TPF (IC_{50} value = 2.73 ± 0.04 μg/mL) was almost comparable to ascorbic acid. This shows that the TPF has a potent free radical scavenging activity.
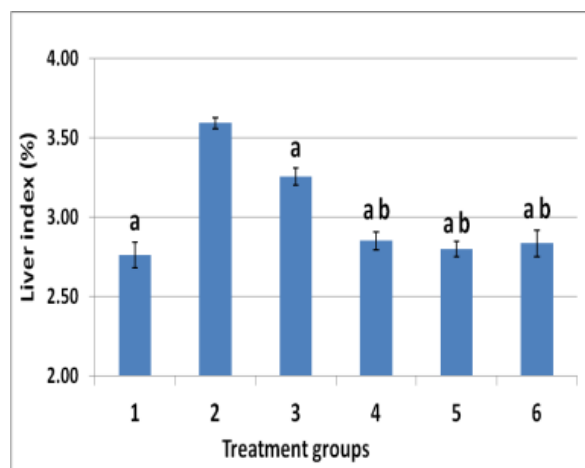
Nitric Oxide (NO●) Scavenging Activity

Figure 3. Effect of extract treatment on liver index. Results (n=6) represent mean $\pm$ SEM data from six rats, [a] $p<0.05$ compared with toxicant group and [b]$p>0.05$ compared with normal control group. (1 = Normal control, 2 = Toxicant, 3 = Low-dose extract, 4 = High-dose extract, 5 = NAC, 6 = Extract only)

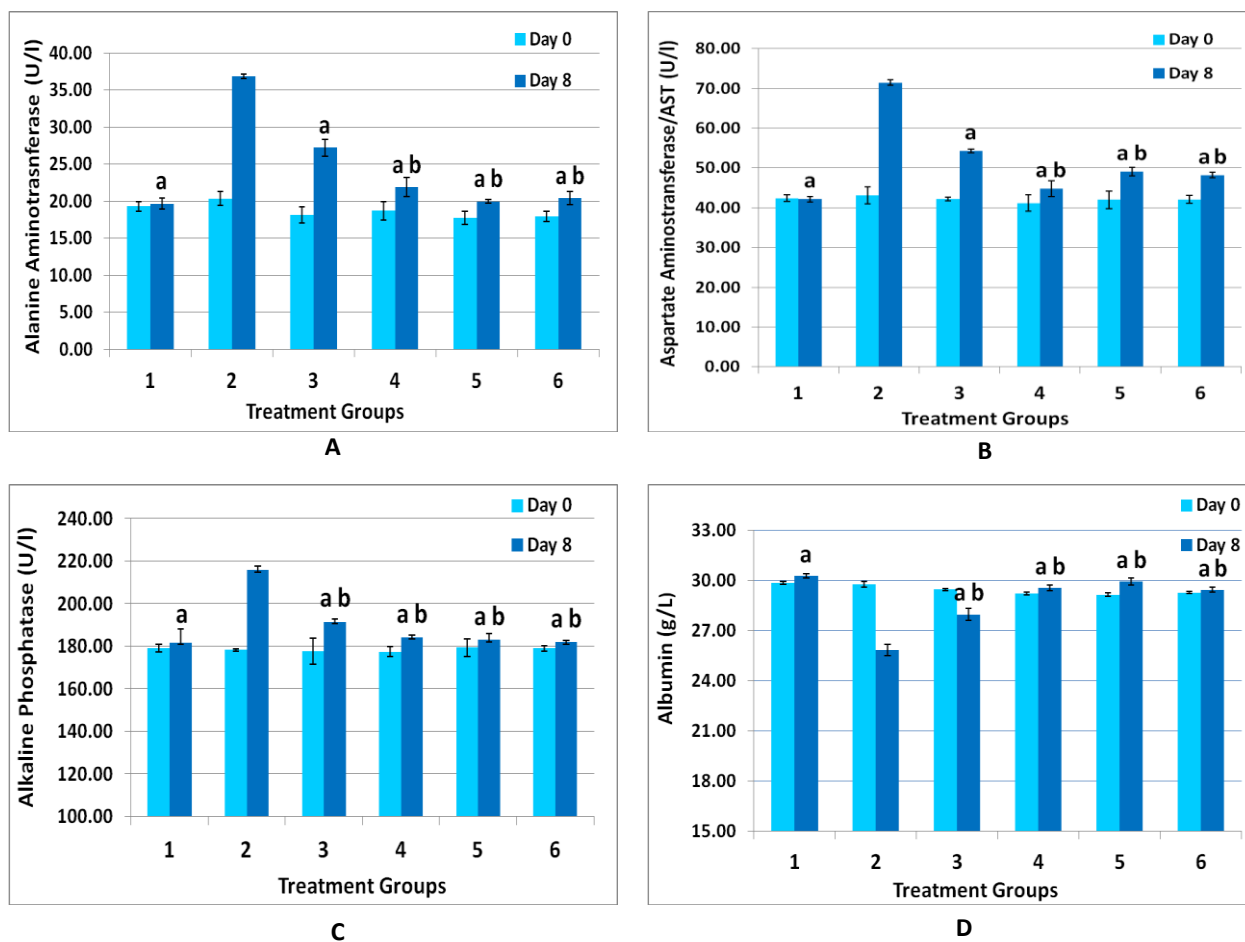Nitric oxide generated from an aqueous solution of sodium

Figure 4. Effect of extract administration on certain biochemical parameters of liver function and damage: ALT (A), AST (B), ALP (C) and ALB (D). Results (n=3) represent mean ± SEM of three trials, [a] p<0.05 compared with toxicant group and [b] p>0.05 compared with normal control group. (1 = Normal control, 2 = Toxicant, 3 = Low-dose extract, 4 = High-dose extract, 5 = NAC, 6 = Extract only)

nitroprusside at physiological pH can react with oxygen producing nitrite ions that can be quantified using Griess reagent[24]. When an antioxidant-containing sample is added to the reaction mixture, it competes for the oxygen present leading to decreased production of nitrite ions[24].The TPF demonstrated a strong concentration-dependent scavenging activity against NO● as shown on Figure 2B. The TPF exerted a strong NO● scavenging activity at a potent dose (IC_{50} = 4.96 ± 0.05 µg/mL). NO● is generally unreactive than most ROS but in the event of paracetamol-induced oxidative damage, it can react with superoxide to form another more oxidatively active radical, peroxynitrite, that can promote lipid oxidation[35].

Ferric-Reducing Antioxidant Power (FRAP) assay
The reduction of Fe^{+3} to Fe^{+2} ions is frequently used to indicate electron-donating ability, an essential mechanism of polyphenolic antioxidant action[25]. A dose-dependent increase in reducing power is observed with the TPF exhibiting an RC_{50} value of 11.74 ± 0.19 µg/mL. Data is shown on Figure 2C.

Toxicity Test (Limit Test)
No overt signs of clinical toxicity and death were noted in any of the rats during the 14-day period following the

administration of 2000 mg/kg of ethyl acetate extract. All rats gained weight continuously for 14 days after administration of limit dose. This means that the extract is practically non-toxic at the dose given.

In vivo Hepatoprotective Assay
Liver Index
The liver index (%) is the ratio of liver weight relative to the body weight and an indication of paracetamol toxicity[36]. Based on Figure 3, the toxicant group had the highest liver index among all groups. The significant increase in liver weight (relative to body weight) in the event of paracetamol poisoning is thought to be due to the accumulation and subsequent congestion of red blood cells (RBCs) in the sinusoids owing to decreased intra-hepatic and portal vein pressure[36]. Data from the high dose extract-treated group showed no significant difference (p>0.05) with the normal control group. It could be said that the extract was able to dose-dependently mitigate the transient increase in liver weight due to paracetamol toxicity.

Biochemical Parameters of Liver Damage and Function
In the event of oxidative damage, drastic increase in serum ALT and AST are observed due to the lysis of hepatocytes[37]. Meanwhile substantial elevations in ALP
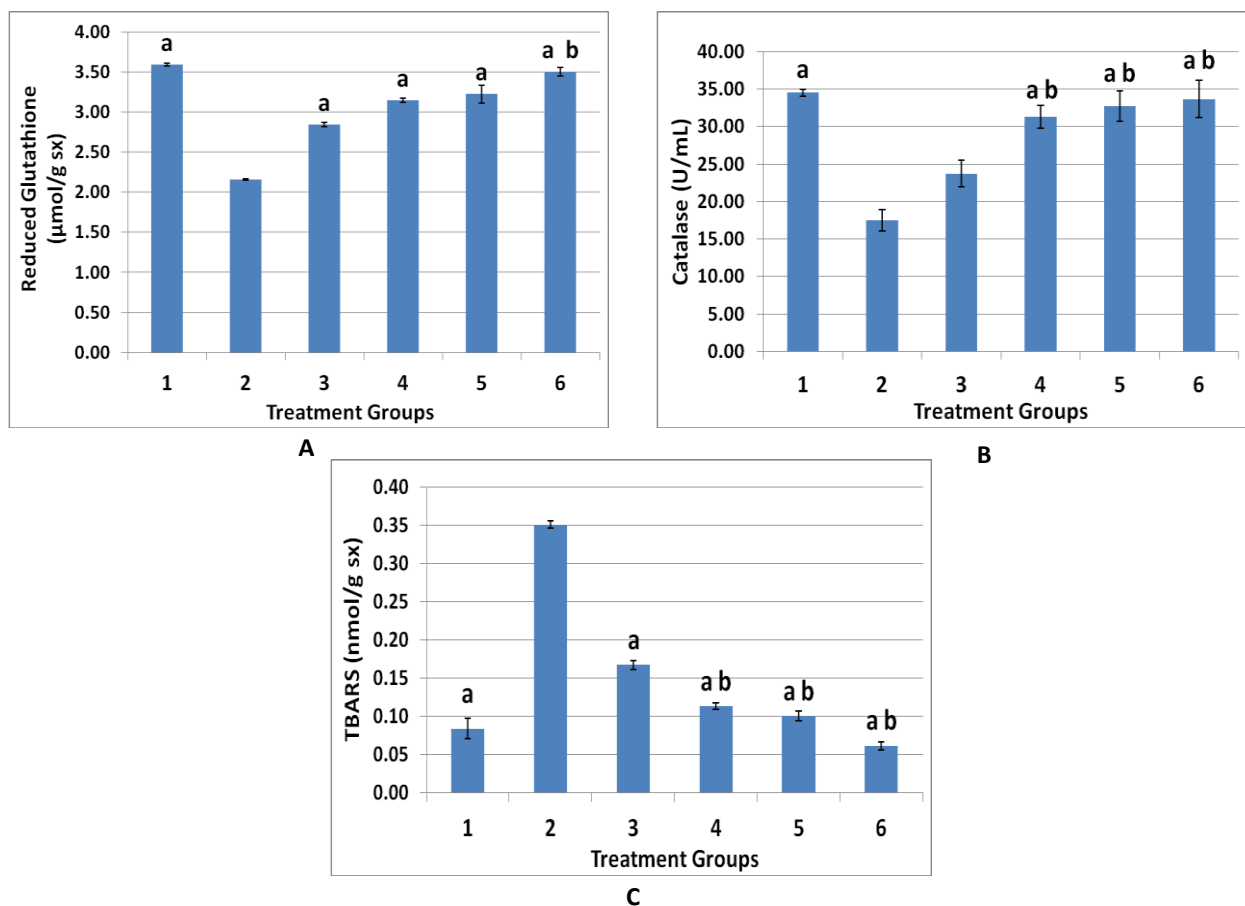
Figure 5. Effect of extract administration on GSH (A), CAT (B), and MDA (C) levels. Results (n=3) represent mean ± SEM of three trials, ^{a}p<0.05 compared with toxicant group and ^{b}p>0.05 compared with normal control group. (1 = Normal control, 2 = Toxicant, 3 = Low-dose extract, 4 = High-dose extract, 5 = NAC, 6 = Extract only)

indicate hepatobiliary obstruction[38]. Serum ALT, AST and ALP levels were significantly elevated (p<0.05) in the toxicant group based on Figure 4. On the other hand, enzyme levels were brought back to normal with extract administration (p>0.05) indicating the prevention of extensive damage to hepatocytes. The measurement of albumin is also important in acetaminophen toxicity; damage to hepatocytes results in the loss of synthetic function of the liver resulting in decreased ALB levels[39].The extract therefore, may have prevented further damage to the liver, essentially helping maintain normal liver function.

Estimation of Liver Antioxidant Capacity and Malondialdehyde (MDA) levels
To assess the liver's own antioxidant defense system against oxidative damage due to paracetamol toxicity, reduced glutathione (GSH) and enzyme catalase (CAT) levels were assayed from the liver homogenate. GSH directly scavenges NAPQI as well as reactive oxygen/nitrogen species while CAT is a powerful H_2O_2–destroying enzyme with a very high activity in the liver[40]. The level of MDA was also measured to determine the degree of lipid peroxidation in the liver, MDA being the final major product in the lipid peroxidation pathway[35]. Drastic decrease in CAT and GSH levels and a three-fold increase in MDA levels were observed in the toxicant

group as shown on Figure 5. Meanwhile, extract administration prevented significant decrease in CAT and GSH levels while ameliorating marked increase in lipid peroxidation (MDA). Both CAT and MDA were restored to normal levels (p<0.05) with high-dose extract; however GSH was only partially restored to levels similar to the NAC group. The prevention of marked decrease in CAT and GSH levels indicate that the extract was able to work with the liver's own antioxidant defense. By scavenging important reactive species in paracetamol-induced oxidative damage, these endogenous compounds were protected by the extract from significant depletion in the liver. Since lipid peroxidation ensues from the ROS and RNS attack on lipids[35], their detoxification by the extract halts this deleterious process that can lead to further hepatocyte damage and eventual death[37].

Histopathological Analysis
Histopathological changes following paracetamol-induced oxidative toxicity are often revealed through microscopic analysis of hepatic tissue samples[37]. Microphotographs of liver samples taken from each of the treatment groups are shown on Figure 6. Figure 6A presents a representative liver tissue sample from the control group showing normal hepatic architecture with well-preserved hepatocytes possessing well-defined cytoplasm, and no remarkable changes around the central vein. A contrasting image of
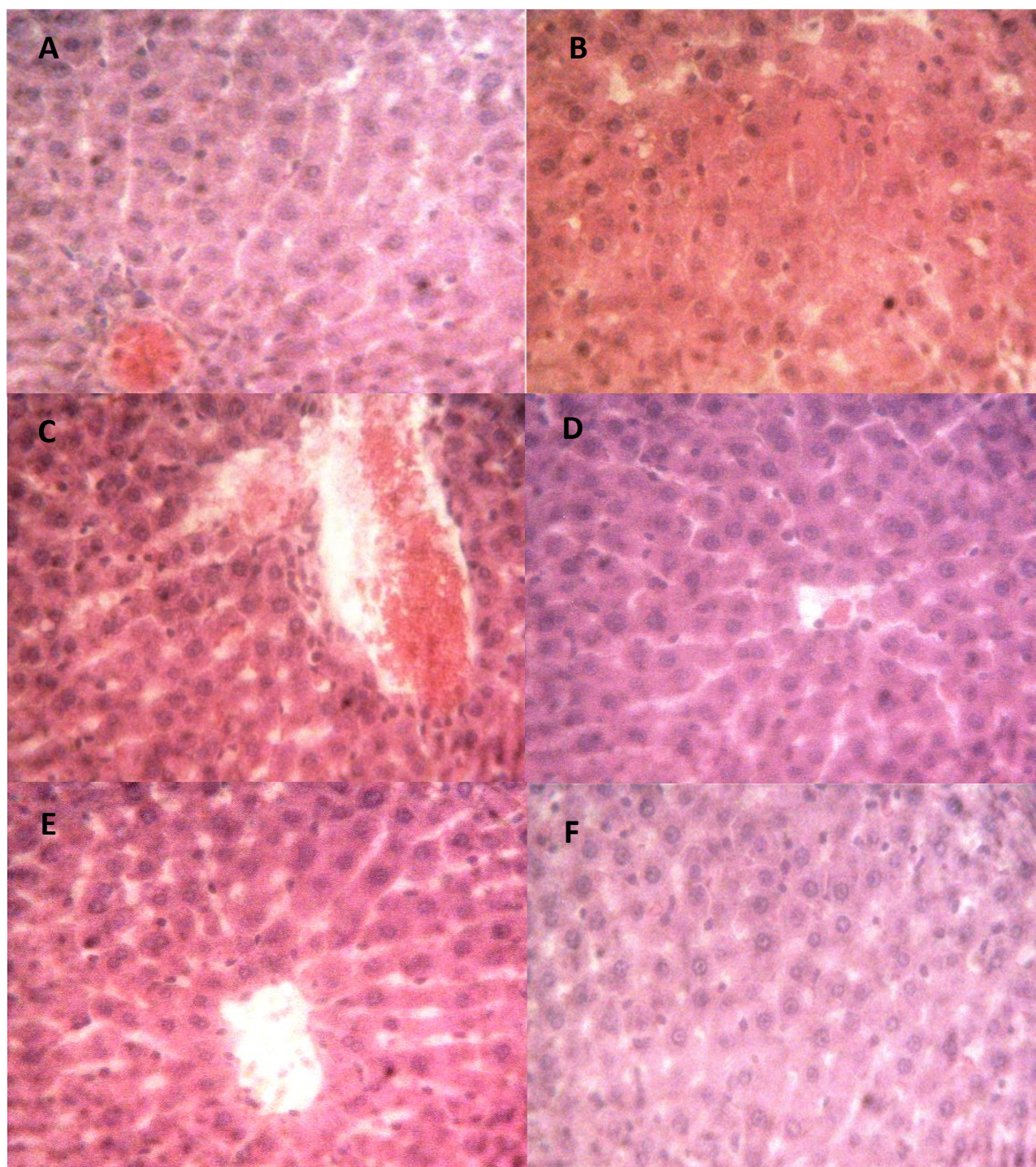
Figure 6. Light microphotographs (400x) of hematoxylin and eosin-stained sections of representative hepatic tissue taken from the diff. treatment groups: Normal control (A), Toxicant (B), Low-dose extract (C), High-dose extract (D), Standard/NAC (E) and Extract-only (F)

hepatic architecture is observed from a liver sample taken from the toxicant group (B). A certain degree of necrosis is noticeable in the tissue sample. According to Larson[41] a classic feature of paracetamol toxicity is hepatocyte necrosis around the centrilobular region. Sinusoidal congestion with red blood cells, hepatocyte swelling, fatty changes, and the presence of inflammatory infiltrates like neutrophils and lymphocytes were also observed in other tissue samples from the group. These changes to hepatic

morphology were improved by extract administration in a dose-dependent manner. A liver sample from the low dose-extract group (C) shows a dilated and congested central vein but with thriving hepatocytes. Sinusoidal congestion, fatty changes and cellular swelling were also noted. Hepatic architecture was better in both the high-dose extract (D) and NAC-treated groups (E) showing the presence of prominent-nucleated hepatocytes with intact cytoplasm around the central vein, minimal sinusoidal
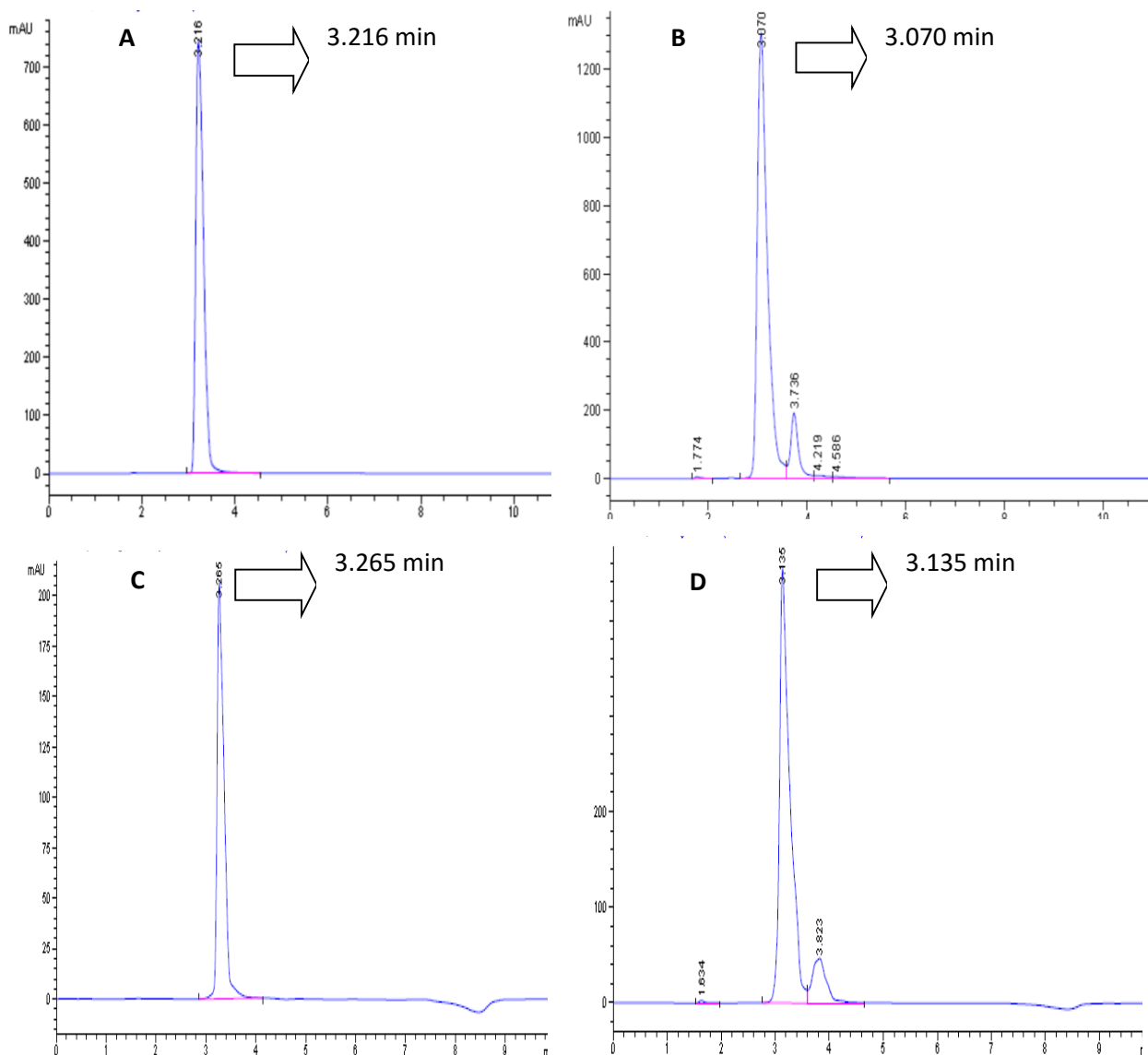
Figure 7. HPLC Chromatogram of quercetin (A) and diluted sample (B) at 262 nm showing peak retention time at 3.216 mins and 3.070 mins, respectively. HPLC Chromatogram of lupeol (C) and diluted sample (D) at 210 nm showing peak retention time at 3.265 and 3.135 mins, respectively.

congestion, and regenerative changes as evidenced by bi-nucleated hepatocytes. Administration of extract alone (F) did not produce any remarkable changes to hepatic tissue, showing well-preserved hepatocytes.

High Performance Liquid Chromatography

HPLC analysis was performed on the total phenolic fraction from the ethyl acetate extract. Quercetin, a flavonoid, and lupeol, a triterpenoid with hepatoprotective properties[42] were detected in the sample. Good linearity was achieved for both standards in the range of 25-150 ppm with an R^2 coefficient of 0.9916 and 0.9826 for lupeol and quercetin, respectively. The concentration of lupeol in the sample was found to be 471.311 ppm while that of quercetin was 211.31 ppm. Figure 7 presents the chromatograms of quercetin and lupeol, as well as of diluted sample.

In recent studies, lupeol has been previously described as present in the crude dichloromethane[19] and ethanolic leaf extracts[9] as well as in the ethyl acetate fractions thereof [13,16]. Similarly, quercetin has also been detected in the crude ethanolic leaf extract of *F. pseudopalma*[16]. In a hepatoprotective study against paracetamol-induced oxidative damage on rat hepatocytes, Kumari and Kakkar [42] noted that lupeol counteracted ROS generation, maintained oxidative balance and afforded protection against damage to critical cellular components such as DNA and mitochondria. Quercetin on the other hand, considered to be one of the most potent free radical scavengers among flavonoids[43], is able to scavenge important ROS and RNS involved in paracetamol-induced oxidative damage[44]. These two bioactive compounds present in the ethyl acetate extract may have been responsible for the strong *in vitro* antioxidant and radical/reactive species scavenging activity, and in effect the dose-dependent ameliorative action of the extract against paracetamol-induced oxidative liver damage.

CONCLUSION

The ethyl acetate extract partitioned from the crude methanolic leaf extract of *Ficus pseudopalma* Blanco possessed high total phenolic and flavonoid contents and exhibited strong, dose-dependent *in vitro* antioxidant activity. Consequently, it also exerted a dose-dependent hepatoprotective activity *in vivo* against paracetamol (500 mg/kg)-induced oxidative damage. The extract at a dose of 400 mg/kg was comparably similar to the hepatoprotective effect of the standard antidote, N-acetylcysteine at 100 mg/kg. Compounds quercetin and lupeol were likely responsible for the observed antioxidant and hepatoprotective activities of the extract.

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