

Ameliorative Potentials of *Persea americana* Mill (Avocado) Seed Extract on Acetaminophen-induced Hepatic Insufficiency in Rats

ABSTRACT

Aim: The ability of ethylacetate extract of *Persea americana* Mill (avocado) seed to ameliorate the hepatotoxic effect of acetaminophen in rats was assessed.

Methodology: Thirty experimental rats were placed in 6 groups of 5 animals per group. Two animal groups received distilled water and the drug vehicle, dimethyl sulphoxide (DMSO). Four other groups received acetaminophen (750 mg/kg bw/day) in DMSO for 3 days with 3 of the 4 groups receiving, after 3 days of acetaminophen administration and for 11 days, *Persea americana* seed extracts (150-, 350 mg/kg bw) and silymarin (200 mg/kg bw). Animals were sacrificed and both serum and liver tissue analysed for markers of liver integrity (AST, ALT, ALP), liver function (total protein, albumin, total cholesterol, triglyceride), antioxidant status (SOD, Catalase) and liver architecture.

Results: The result showed induction of hepatotoxicity by acetaminophen with increased AST, ALT, ALP and decreased SOD and CAT activities. Hepatotoxicity was also observed as increase in total and conjugated bilirubin and decrease in total cholesterol and triglyceride. Administration of *Persea americana* seed ethylacetate extract reversed the status of the parameters towards normal (except for AST, total protein and albumin). Also the liver architecture which hitherto revealed inflammation, fatty changes and hepatocyte necrosis also reversed to mild inflammation to normal histology. The action of the extract was dose dependent and its effectiveness comparable to silymarin (200 mg/kg bw).

Conclusion: The ethylacetate extract of *Persia americana* showed ameliorative effect against acetaminophen-induced hepatotoxicity at higher dose, with potentials to reverse disruptions in liver integrity and function, and restore balance in endogenous antioxidant system.

Keywords: *Persea americana* Mill, Acetaminophen, Hepatotoxicity, Antioxidant, Silymarin.

1. INTRODUCTION

Persea americana Mill. (family Lauraceae), commonly known as avocado is a tropical fruit native to Mexico, Central and South America but is now grown worldwide for food and medicinal uses [1,2,3]. The fruit pulp contain avocado oil also rich in bioactive components such as chlorophyll, carotenoids, phenolics and phytosterol which is mainly applied in cosmetic industry [4].

[5] reported presence of flavonoids, sterols, phenolics, triterpenes and fatty acids in ethanolic extract of leaves and fruits of *P. americana* and attributed their antioxidant activities to the polyphenols and flavonoids [4]. *Persea americana* seed was reported as source of bioactive phytochemicals with antihypercholesterolemia, antihypertensive, anti-inflammatory and antidiabetic potentials but yet underutilized and a source of worry to avocado processors [6]. Antioxidants in medicinal plants can function as free radical scavengers, reducing agents, metal chelators, hydrogen donors or involved in quenching singlet oxygen. Hence their actions put the free radicals under check and protect the body against oxidative stress [7]. The LD50 values of *P. americana* in rats were, at various times reported to be below 5000 mg/kg body weight and 1200.75 mg/kg body weight by [8] and [9] respectively.

Paracetamol, also known as *N*-acetyl-*p*-aminophenol or acetaminophen (APAP) is an over-the-counter medication which at therapeutic doses is indicated for treatment of pain, fever and inflammation [10]. Metabolism of APAP by liver cytochrome P450 (Cyt-P450) leads to the generation of a reactive metabolite, *N*-acetyl parabenzoquinoneimine (NAPQI). NAPQI is usually conjugated with glutathione (GSH) for elimination to the outside as increase in its cellular concentration can lead to liver injury due to binding with cellular proteins [11]. Such increase usually arise when, in excessive dosing of APAP, glutathione conjugation process is overwhelmed due to excessive generation of NAPQI and subsequent depletion of GSH. This results in paracetamol hepatotoxicity and liver damage [12].

Some serum indicators of liver damage include aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, total bilirubin, conjugated bilirubin, total cholesterol and triglycerides [13,14,15]. In the absence of glutathione (GSH), a non-enzymatic endogenous antioxidant, the enzymatic antioxidants such as superoxide dismutase (SOD) and catalase (CAT) play vital roles in protecting the liver against paracetamol toxicity [16,17].

Silymarin is a natural product found in *Silybum marianum* fruit and contain among other phytochemicals, flavonoids and polyphenols [18]. Its hepatoprotective, antioxidant and anti-inflammatory potentials were reported by [19] and its role in the in the protection against liver fibrosis due to acetaminophen toxicity reported by [20]. It usually acts as free radical scavenger and modulator of enzymes responsible for cellular damage, fibrosis and cirrhosis and improve glycemic parameters in diabetic and alcohol cirrhosis patients [21]. Silymarin has been implicated in both lipid and carbohydrate metabolism and also ameliorates insulin resistance [22]. It has been reported to prevent against paracetamol-induced hepatotoxicity in animal models [23,24,25].

This study intends to evaluate antioxidant potentials of *Persia americana* seed extract and the effect of the extract on paracetamol-induced liver toxicity in rats.

2. MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Sample Procurement and Identification

Mature avocado fruits were purchased in sango market, Mile 1 Port Harcourt River state, Nigeria. It was identified and deposited in the herbarium of the Plant Science and Biotechnology Department, University of Port Harcourt with voucher number, UPH/C/102.

2.1.2 Sample Preparation

The mature avocado fruits were allowed to ripe, after which the fleshy parts were removed. The seeds were chopped into thin slices and air dried until constant weight. The dried sample was then pulverized and stored in a container. Weighed pulverized plant sample (600 g) was defatted in *n*-hexane and extracted with ethylacetate using Soxhlet extraction methods.

2.1.3 Experimental Animals

Thirty (30) adult wistar rats of both sexes weighing 150 – 180g were purchased from animal house Department of Biochemistry, University of Port Harcourt, Port Harcourt. They were acclimatized for 2weeks, in the animal house, kept in laboratory cages and allowed free access to standard rat feed and water.

2.2 METHODS

2.2.1 Experimental Design

The animals were divided into 6 groups of 5 animals per group. Groups 1, 2 and 3 were given distilled water, dimethyl sulphoxide and paracetamol (750 mg/kg bw). Group 4 and 5 recieved *Persea americana* seed extract (150 mg/kg bw and 350 mg/kg bw respectively), while group 6 is the

standard group which received silymarin (200 mg/kg bw). Groups 3,4,5 and 6 received paracetamol for 3 days and after that the *Persea americana* extract and silymarin were given for 11 days.

2.2.2 Sample Collection and Preparation

At the end of the experiment, all animals were sacrificed under anesthesia and blood collected via cardiac puncture into a heparin sample bottle. Serum from centrifuged whole blood was used for biochemical and antioxidant assays. Portions of the liver were also harvested into sample bottles containing 10% of formalin solution for histological examination.

2.2.3 Biochemical Analysis

The serum activities aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and concentrations of total protein, albumin, conjugated and unconjugated bilirubin were assayed in the serum using Randox commercial kits (RANDOX, UK). Triglyceride concentration was determined using Triglyceride kit (Agappe Diagnostics, Switzerland). Analytical procedures followed were as contained in the kit manuals. Absorbance readings were taken using a Spectrophotometer (Surgispec SM -23D, Surgified medical, England). AST activity was ascertained by monitoring the concentration of oxaloacetate hydrazone (measured at 540 nm wavelength) formed with 2,4-dinitrophenyl hydrazine. ALT activity was measured by monitoring the concentration of pyruvate hydrazone (at 540 nm) formed from 2, 4-dinitrophenyl hydrazine. Alkaline phosphatase (ALP) yields inorganic phosphate and p-nitrophenol from hydrolyses of a colourless substrate (p-nitrophenylphosphate). Under alkaline condition, the p-nitrophenol released and measured spectrophotometrically at 405nm, is proportional to the activity of ALP.

Total protein was measured on the basis of interaction between cupric ions, in an alkaline medium, and peptide bonds in protein resulting in the formation of a coloured complex measured at 546 nm wavelength. The measurement of serum albumin was based on its quantitative binding to the indicator 3,3',5,5'-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The concentration of albumin in the sample is directly proportional to the albumin-BCG-complex which absorbs maximally at 578 nm. Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. Total bilirubin then reacts with diazotized sulfanilic acid in the presence of activator (TAB) to form azobilirubin. Triglyceride measurement was by enzymatic method involving Lipoprotein Lipase, Glycerol Kinase, Glycerol-3-phosphate Oxidase and Peroxidase as specified in the kit manual.

The activity of SOD was determined following [26] as reported by [27]. A xanthine/xanthine oxidase system generates superoxide radical (O_2^-) which oxidizes epinephrine to adrenochrome. Adrenochrome yield is responsive to increase in pH and proportional to epinephrine concentration. The inhibition of autooxidation of epinephrine (pH 10.2 @ 30°C) by SOD leading to changes in the level of adrenochrome constitutes the basis of this test.

Catalase activity was determined as described by [28]. Catalase in the sample preparation splits its substrate, hydrogen peroxide which absorbs spectrophotometrically at 240nm. One unit of catalase activity equals protein concentration that converts 1 $\mu\text{mol H}_2\text{O}_2/\text{min}$ to water and oxygen.

2.2.4 Liver Histopathological Assay

Liver from each group of experimental animals were fixed with 10% formalin. This was followed by dehydration in isopropyl alcohol and embedding in paraffin wax. Embedded tissues were later trimmed out and sectioned on a microtome. Sections were adsorbed on slides, and stained with hematoxylin and eosin, and examined under light microscope.

2.2.5 Statistical Analysis

The analysis was carried out using SPSS Statistics 17.0 (SPSS Inc., Chicago, IL). Data were presented as mean \pm SEM, and analysed using one-way analysis of variance (ANOVA) followed by Bonferroni *post hoc* test. Significance was set at $p = .05$.

3. RESULTS AND DISCUSSION

3.1 BIOCHEMICAL ASSAYS

In table 1, the activities of liver integrity marker enzymes and relative liver weight of rats administered ethylacetate extract of *Persea americana* seed is shown. The administration of the extract vehicle, Dimethylsulfoxide (DMSO) at 0.003ml/ kg body weight (bw) did not cause significant changes in the enzyme activities and relative liver weight. DMSO at very low doses has been reported to serve as vehicle for administration of acetaminophen without significant biochemical effect on the animals [29,30]. Significant changes were observed for all the parameters except relative liver weight following administration of acetaminophen (750 mg/kg bw). Acetaminophen at 750mg/kg bw induced liver toxicity in rats [31] resulting in the increase in serum liver enzymes activities. The reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) arising from the activities of the cytochrome P450 (CYP) enzyme on acetaminophen is responsible for the liver damage. NAPQI, initiates oxidative stress in the liver by binding to protein sulfhydryl groups, leading to cell necrosis and lipid peroxidation [32].

Two dose levels of 150 and 350 mg/kg bw were carefully selected in a buildup to an effective dose as different literatures revealed varied values as median lethal dose (LD₅₀) of the extracts [8,9,33]. Administration of the ethylacetate extract of *P. americana* caused dose dependent significant changes in the activities of the liver enzyme activities except for AST which varied insignificantly for all doses of extract and standard drug, silymarin (200mg/kg bw) compared to acetaminophen treated group 3. The extract at 350 mg/kg bw (group 5) caused significant reductions in the activities of ALT and ALP, and also increased significantly the relative liver weight of this group compared to group 3. These changes were insignificant when compared to the result for the silymarin treated group 6. Silymarin has been reported to ameliorate acetaminophen toxicity by reducing expression and activity of CYP2E1 and stemming production of its toxic metabolites [25].

Flavonoids and phenolics, among other phytochemicals have also been reported for *P. americana* seed. [34] and it was also noted to possess antioxidant and anti-inflammatory properties [35]. These may have contributed to its amelioration of acetaminophen toxicity.

Table 1. Activities of liver integrity marker enzymes (AST, ALT, ALP) and relative liver weight of Rats administered ethylacetate extract of *Persea americana* seed

GROUP	TREATMENT	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Relative Liver Weight (%)
Group 1	Distilled water	83.93 \pm 1.77 ^a	25.91 \pm 1.36 ^{ab}	23.70 \pm 1.47 ^a	2.40 \pm 0.03 ^a
Group 2	DMSO only	96.50 \pm 2.50 ^{ab}	31.25 \pm 0.75 ^{ab}	32.50 \pm 1.71 ^a	2.87 \pm 0.05 ^a

Group 3	750mg/kgbw Acetaminophen only	123.33 3.33 ^c	± 37.67 ± 1.45 ^c	58.33 ± 4.91 ^d	2.41 ± 0.10 ^a
Group 4	750mg/kgbw Acetaminophen+ Extract (150mg/kg bw)	122.50 2.50 ^c	± 31.75 ± 1.18 ^{ab}	38.00 ± 2.65 ^a	2.83 ± 0.09 ^a
Group 5	750mg/kgbw Acetaminophen+ Extract (350mg/kg bw)	114.67 6.06 ^{bc}	± 31.33 ± 0.67 ^{ab}	37.25 ± 3.86 ^a	3.27 ± 0.05 ^b
Group 6	750mg/kgbw Acetaminophen+ Silymarin(200mg/kg bw)	111.33 3.67 ^{bc}	± 31.00 ± 1.60 ^{ab}	33.67 ± 4.48 ^a	3.00 ± 0.21 ^b

Data represent mean ± standard error mean SEM (n=5). Values with different superscripts down the column are statistically significant at $p = .05$.

Table 2 shows levels of the liver function markers (total protein, albumin and bilirubin) of rats administered ethylacetate extract of *Persea americana* seed. Administration of acetaminophen (750 mg/kg bw) caused insignificant changes in the levels of total protein and albumin, but significant increase in the levels of total and conjugated bilirubin compared to the control (group 1). Insignificant changes in the levels of serum total protein and albumin after acetaminophen administration were reported by [36] and [37]. It may result from the duration of exposure being too short that protein metabolism was not impacted or a waiting period after last drug administration being enough to allow recovery of protein metabolic mechanisms. Increases in total and conjugated bilirubin after acetaminophen administration were reported by [38] and [39].

Biotransformation of xenobiotics is among the biological functions of the liver and uptake of unconjugated (indirect) bilirubin and its subsequent conjugation in the liver is one of such. Conjugated (direct) bilirubin, now water soluble, is either excreted to the bile or returned to the bloodstream for excretion through the kidney [40]. The increased serum level of bilirubin in the acetaminophen treated group is an indication of drug induced necrosis which affected the binding, conjugation and excretory capacity of the liver cells [41]. The various doses of *P. americana* seed ethylacetate extract may have reversed the effect of acetaminophen leading to significant reduction in the levels of both total and conjugated bilirubin. The effectiveness of this reversal is same (statistically) for all doses of the extract and the standard drug, silymarin.

Table 2. Serum levels of liver function markers (total protein, albumin and bilirubin) of Rats administered ethylacetate extract of *Persea americana* seed

GROUP	TREATMENT	TOTAL PROTEIN (g/l)	ALBUMIN (g/l)	TOTAL BILIRUBIN (µmol/l)	CONJUGATE BILIRUBIN (µmol/l)
1	Distilled water only	80.33 ± 2.40 ^{ab}	44.60 ± 0.75 ^a	13.83 ± 0.49 ^{ab}	8.10 ± 0.40 ^{ab}
2	DMSO only	83.20 ± 0.80 ^b	46.40 ± 2.29 ^a	9.93 ± 0.69 ^a	7.70 ± 0.40 ^{ab}
3	750mg/kg bw Acetaminophen Only	80.75 ± 1.03 ^{ab}	46.75 ± 3.33 ^a	19.40 ± 1.42 ^c	12.60 ± 0.40 ^c

4	750mg/kg bw Acetaminophen + Extract (150mg/kg bw)	76.40 ± 0.40 ^a	43.50 ± 2.53 ^a	15.10 ± 0.30 _{bc}	10.65 ± 0.65 ^{bc}
5	750mg/kg bw Acetaminophen + Extract (350mg/kg bw)	82.75 ± 2.13 ^b	42.60 ± 0.68 ^a	13.60 ± 1.64 _{ab}	8.20 ± 0.90 ^{ab}
6	750mg/kg bw Acetaminophen + Silymarin (200mg/kg bw)	79.75 ± 1.08 ^{ab}	43.00 ± 0.71 ^a	11.10 ± 0.64 _{ab}	7.30 ± 0.96 ^{ab}

Data represent mean ± standard error mean SEM (n=5). Values with different superscripts down the column are statistically significant at p = .05.

The activities of the antioxidant enzymes; Superoxide dismutase (SOD) and Catalase and the serum lipid levels of total cholesterol and triacylglycerol (TAG) were also monitored in all the animal groups (table 3). In tissue damage resulting from acetaminophen toxicity, these serum parameters are usually decreased [42,43,44].

The liver is central to lipid metabolism especially cholesterol and lipoproteins and also involved in regeneration of reduced glutathione (GSH) required to regulate lipid peroxidation processes [42]. Thus, the observed significant decrease in the activities of these enzymes and lipid levels may have resulted from induction of hepatic damage by acetaminophen. These decreases were reversed by the administration of varied doses of *P. americana* seed ethylacetate extract to a level comparable to the standard drug.

Table 3. Activities of liver antioxidant enzymes (SOD, catalase) and serum lipid status (total cholesterol, TAG) of Rats administered ethylacetate extract of *Persea americana* seed

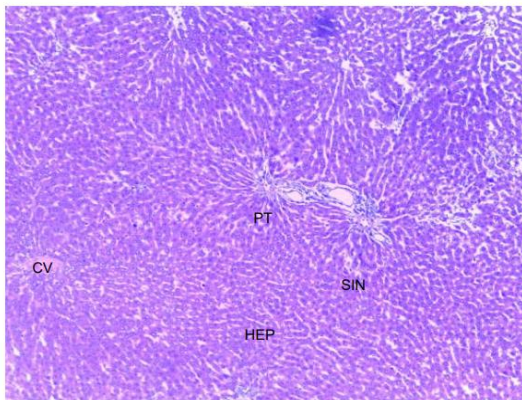
GROUP	TREATMENT	SOD (U/mL)	CATALASE (U/mL)	T.CHOL (mmol/l)	TAG (mmol/l)
1	Distilled water only	0.31 ± 0.02 ^{ab}	2.80 ± 0.27 ^{ab}	3.40 ± 0.07 ^{ab}	2.17 ± 0.04 ^{ab}
2	DMSO only	0.40 ± 0.02 ^b	4.00 ± 0.57 ^a	3.38 ± 0.09 _{ab}	2.07 ± 0.03 _{ab}
3	750mg/kg bw Acetaminophen Only	0.20 ± 0.02 ^c	2.20 ± 0.60 ^b	2.91 ± 0.12 ^c	1.69 ± 0.09 ^c
4	750mg/kg bw Acetaminophen + Extract (150mg/kg bw)	0.26 ± 0.02 ^a	3.56 ± 0.20 ^a	3.19 ± 0.03 _{bc}	1.92 ± 0.04 ^{bc}
5	750mg/kg bw Acetaminophen + Extract (350mg/kg bw)	0.30 ± 0.02 ^b	3.53 ± 0.37 ^a	3.55 ± 0.13 _{ab}	2.27 ± 0.08 _{ab}
6	750mg/kg bw Acetaminophen + Silymarin (200mg/kg bw)	0.32 ± 0.01 ^{ab}	3.37 ± 0.18 ^a	3.17 ± 0.08 _{ab}	2.33 ± 0.14 _{ab}

Data represent mean ± standard error mean SEM (n=5). Values with different superscripts down the column are statistically significant at p = .05.

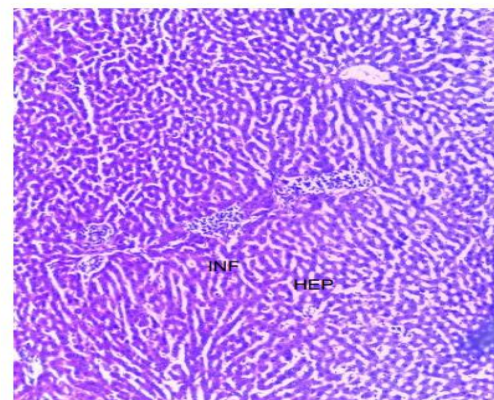
3.1 LIVER HISTOPATHOLOGY ASSAY

Histopathological observations of liver sections of the two experimental control groups show normal liver cells with moderate inflammation and cell infiltrates in the DMSO control. The group induced acetaminophen hepatotoxicity revealed inflammation, fatty changes and hepatocyte necrosis. Mitochondrial dysfunction has been implicated in acetaminophen toxicity leading to hepatocyte necrosis. This is followed by recruitment of pro-inflammatory cytokines or chemokines, Tumor necrosis factor-alpha (TNF- α) and innate immune cells, mainly neutrophils and nonocytes [45]. Also acetaminophen is known to inhibit β -oxidation of fatty acid, disrupt lipid metabolism and cause triglyceride accumulation in liver and serum. This is preparatory to onset of steatosis, a major cause of liver damage [46].

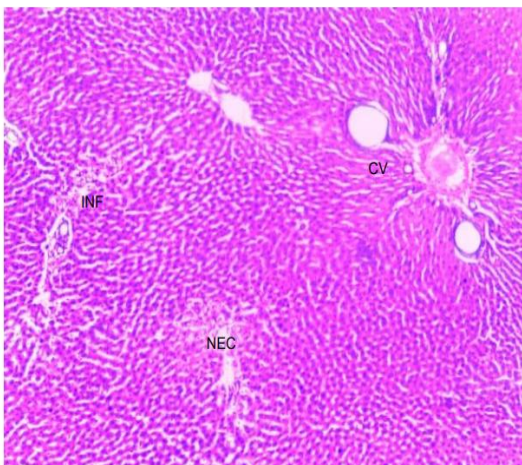
The lower dose (150 mg/kg bw) extract treated group presents low moderation of the acetaminophen toxicity with diminishing fatty changes and hepatic necrosis. Both the extract- treated (350 mg/kg bw) and the silymarin-treated groups revealed normal cellular architecture with mild inflammation changes in the extract group (Fig.1). Most liver protecting natural products are known to possess anti-inflammatory, antioxidant, immunomodulatory and antiviral effects [47].



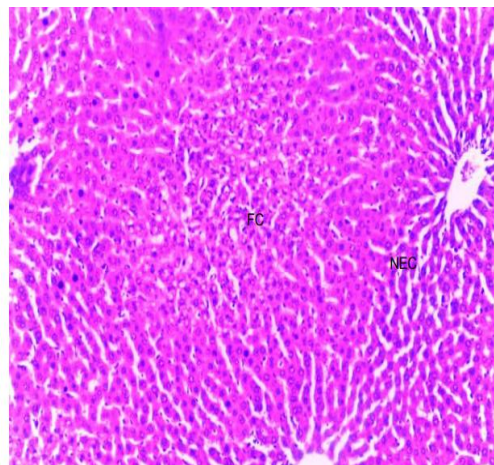
A



B



C



D

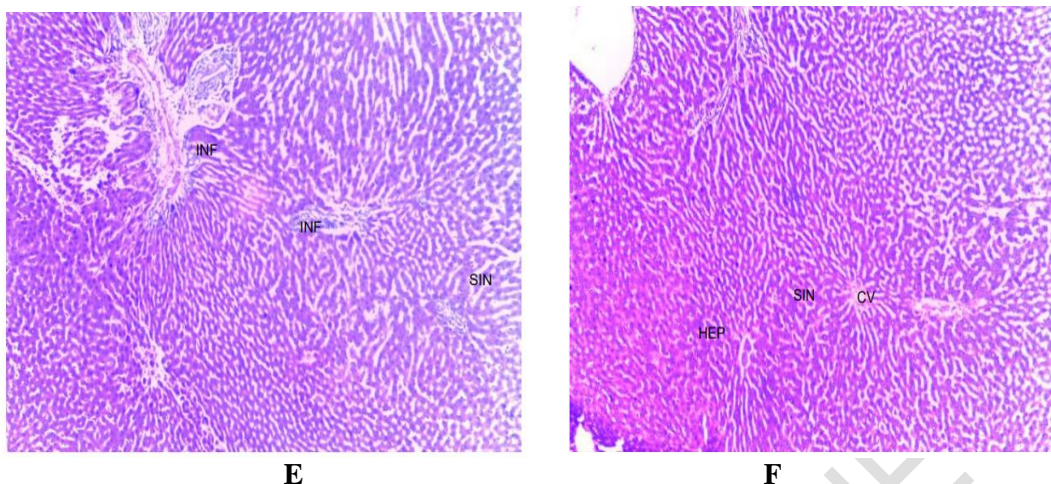


Fig.1. Microscopic view of H & E stained liver sections (x 100 magnification) of experimental rats exposed to acetaminophen toxicity and treated with doses of *P. americana* seed ethylacetate extract and reference drug, silymarin. (A) Showing normal liver of control animals. (B) Normal liver section of the DMSO control rats with moderate inflammation and cell infiltrates. (C) Liver section of acetaminophen (750 mg/kg bw)- treated group showing inflammation, fatty changes and hepatocyte necrosis. (D) Section of liver tissue of group that received lower dose (150 mg/kg bw) *P. americana* seed extract showing diminishing fatty changes and hepatocyte necrosis. (E) Liver section of group that received higher dose (350 mg/kg bw) *P. americana* seed extract showing normal cells with mild inflammation changes. (F) Shows normal liver architecture of the group that received acetaminophen followed by reference drug, silymarin (200 mg/kg bw). CV: Central vein; PT: Portal tract; HEP: Hepatocyte; SIN: Sinusoids; INF: Inflammation; NEC: Necrosis; FC: Fatty changes.

4. CONCLUSION

In conclusion, the ethylacetate extract of *Persia americana* showed ameliorative effect against acetaminophen-induced hepatotoxicity at higher dose. This is as a result of increased concentration of relevant phytochemicals and may indicate possibility of achieving effective dose at higher extract concentrations. It may, therefore, have potentials to reverse disruptions in liver integrity and function, and restore balance in endogenous antioxidant system. Investigations aimed at ascertaining the effective dose of the extract and its other biological activities and mechanisms of actions are underway.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

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