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Protective Effect of Methanol Stem Bark Extract of *Cocos nucifera* on Paracetamol-induced Hepatotoxicity in Adult Wistar Rats

Damilola Alex Omoboyowa[1*], Nwogo Ajuka Obasi[2], Glory Otuchristian[1], Garba Jeremiah Danladi[1] and Martin Umoh Okon[1]

[1]Biochemistry Research Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Ebonyi State, Nigeria.
[2]Department of Medical Biochemistry, Federal University, Ndufu-Alike, Ikwo, Ebonyi State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors DAO, MUO and GJD designed the study, wrote the protocol and supervised the work. Authors DAO and MUO carried out all laboratories work. Author DAO performed the statistical analysis. Authors DAO and GO managed the analyses of the study. Authors DAO, GO and NAO wrote the first draft of the manuscript. Authors DAO and NAO managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Cocos nucifera* is a plant used widely in the African system of medicine for its diverse medicinal and nutritional properties. Its extracts have a vast pharmacological activity and are used as raw materials for medicine and other commodities. Each part of this plant has its own therapeutic importance and uses which include: anticancer, reproductive, anti-inflammatory, anti-malaria, antioxidant and others. The present study investigated the effect of methanolic extract of *Cocos nucifera* stem back on liver marker enzymes and liver histology of paracetamol exposed hepatotoxic rats using standad protocols.
Methodology: A total of twenty (20) male wistar rats distributed normally into five groups (n=4)

**Corresponding author: Email: damlexb@yahoo.com;*

were used for the study. Group I rats served as normal control and were not exposed to paracetamol, while groups II, III, IV and V rats were exposed to 750 mg/kg body weight of paracetamol served as intoxicated test groups. Groups II, III, IV and V were treated with 0.5 ml distilled water, 25 mg/kg body weight of silymarin, 200 mg/kg and 400 mg/kg body weight of *C. nucifera* stem bark extract respectively for seven days. At the end of the experimental period, all animals were sacrificed using cervical dislocation method; blood was obtained for assay for the following hepatic marker enzymes Alanine amino transaminase (ALT), Aspartate amino transaminase (AST), Alkaline phosphatase (ALP) and total bilirubin respectively. Liver tissue was removed, fixed in 10% formol saline and processed for histopathological studies using Heamatoxylin and Eosin (H and E) staining technique.

Results: The results indicated the presence of the phytochemical content of the extract in the order: tannins > soluble carbohydrates > flavonoids > alkaloids > saponnins > steroids. Result of the acute toxicity test showed that the extract is safe at a dosage of up to 5000 mg/kg body weight. The results showed that induction of paracetamol caused significant ($P<0.05$) increase in the marker enzymes and a multiple, mild to moderate periportal infiltration of mononuclear leucocytes in hepatocytes. It was observed that treatment with the extract caused dose-dependent significant ($P<0.05$) decrease in plasma aspartate amino transaminase (AST), alanine amino transaminase (ALT), Alkaline phosphatase (ALP) and bilirubin concentrations and increased protection in the damaged hepatocytes.

Conclusion: The hepato-protective activities of this extract might be attributed to the bioactive compounds present and as such implicates the extract as potent tool for ethnomedical practice.

Keywords: Hepatotoxicity; Phytochemical; enzymes; paracetamol; ethno-medical.

1. INTRODUCTION

Cocos nucifera commonly known as coconut palm is a member of the family *Arecaceae* (palm family). It is found throughout the tropic and sub-tropic area, it is known for its great versatility [1]. Coconut is a drupe, which means that it has a hard shell in its fibrous outer layer. When the coconut shell is cracked open, it reveals a fleshy white meat and a liquid which is known as coconut water. The seed provides oil for frying, cooking and for production of margarines, the coconut water contains sugar, dietary fibre, proteins, antioxidants, vitamins and minerals, palm wine can be gotten from the flower Clusters through which alcohol can be distilled [2]. Medicinally, the hexane fraction of coconut peel contains anticancer compounds [2]. Young coconut juice has estrogen-like characteristics, it can be used as intravenous hydration fluid, and the tea from the husk fibre is used to treat severe inflammatory disorders [2].

The liver is the second largest and the most complex organ in the human system, it is responsible for over 500 different functions in the body [3]. This gland plays a major role in metabolism and has a number of functions in the body, including glycogen storage decomposition of the red blood cells, plasma protein synthesis, hormone production and detoxification. The liver as highly specialized tissues regulates wide varieties of high volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions [4].

Paracetamol (acetaminophen) produces sever hepatotoxicity when consumed in higher dose in experimental rats [5]. It is safe in recommended doses but deliberate or accidental overdose is very common because of its wide availability and this often leads to liver damage [6]. Paracetamol overdose has been reported to cause acute hepatic necrosis which is now one of the most common causes of liver failure amongst the populace [7]. Paracetamol is mainly metabolized in the liver to excretable glucuronide and sulphate conjugates [8]. The hepatotoxicity of paracetamol is activated by hepatic cytochrome P_{450} [6] to a highly reactive metabolic N-acetyl-P-benzoquinone imine (NAPQI) [9]. NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid [10]. When the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or sulphur –SH group of protein and alters the homeostasis of calcium after depleting GSH [10].

In Nigeria, there are oral reports among herbal medicinal practitioners linking *Cocos nucifera* with the treatment of stomach pain. Therefore, the present study was undertaken to investigate

the medicinal properties of the plant relating to liver toxicity and its effect on the liver histology of rats intoxicated with paracetamol and treated with the plant.

2. MATERIALS AND METHODS

The chemicals used for this study were of analytical grade in addition to the RANDOX, UK commercial assay kits which were used for the determination of ALT, AST, ALP and bilirubin. The reference drug (sylimarin) and paracetamol were purchased from registered city pharmacist

2.1 Plant Material

Coconut palm (*Cocos nucifera*) bark used for this study was collected from Ogbu Edda, Afikpo south Local Government Area, Ebonyi State, Nigeria. It was authenticated at the botany unit of the Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi state, Nigeria where a voucher specimen with voucher number V/No. 2002016 has been deposited. The bark of the plant was harvested with a cutlass, air dried at room temperature for three (3) weeks, ground with a mechanical blender to a coarse form, extracted with methanol and fix-dried.

2.2 Extraction Procedure

One hundred and sixty eight grams (168 g) of dried ground sample of *Cocos nucifera* bark were macerated with 700 ml of analytically graded methanol for 72 hours with occasional stirring using a stirring rod. The extract was sieved using a fine sieving cloth. The filtrate was passed through Whatman No 4 filter paper, concentrated by rotary evaporator and dried at room temperature.

2.3 Method of Induction of Liver Damage

The minimum dose of paracetamol that causes death in rats is 1060 mg/kg and the median dose (LD_{50}) is 765 mg/kg [11]. Paracetamol hepatotoxicity was induced by single administration of solution of paracetamol at 750 mg/kg b. w. orally. After 4 days of administration, only rats with AST levels above 65 U/l were considered hepatotoxic and used for the study.

2.4 Experimental Design

A total of twenty male albino rats weighing between 180-220 g were used for the study, the rats were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. The animals were used according to the NIH animal care guidelines with approval of the Departmental Animal Committee (AIFP/AFK-EB/1-07). They were acclimatized for seven days in the Department of Science laboratory technology animal house with regular feeding and water *ad libitum*. The rats were divided into five different groups with four animals per group (n=4):

Group I: Negative control
Group II: Positive control (Paracetamol exposed).
Group III: Paracetamol exposed rats treated with 25 mg/kg body weight of silymarin
Group IV: Paracetamol exposed rats treated with 200 mg/kg body weight of the extract.
Group V: Paracetamol exposed rats treated with 400 mg/kg body weight of the extract.

2.5 Acute Toxicity and Lethality (LD50) Test

The acute toxicity and lethality of methanol extract of the *Cocos nucifera* stem bark was determined using the modified method of Lorke [12]. The test was divided into two stages. In stage one, nine (9) randomly selected adult mice were divided into three groups, three per group (n=3) and received 10, 100 and 1000 mg/kg body weight of the methanol extract and the signs of toxicity and number of death for a period of 24-hours were recorded. After 24 - hour observation, the doses for the second phase were determined based on the outcome of the first phase. Since there was zero death, a fresh batch of animals were used following the same procedure in phase I but with higher dose ranges of 1900, 2600 and 5000 mg/kg body weight of the extract. The animals were also observed for 24-hours for signs of toxicity and possible number of death. The LD50 was calculated as the geometric mean of the high non-lethal dose and lowest lethal dose [12].

2.6 Phytochemical Test

Basic quantitative phytochemical screening of the methanol extract of the *Cocos nucifera* stem bark sample was carried out by testing for the concentration of the following plant constituents: flavonoids, tannins, saponnins, steroids, alkaloids, reducing sugar, cyanogenic glycosides and soluble carbohydrate. The phytochemical

analysis of the sample was carried out using procedures outlined by Harborne, [13] and Pearson [14].

2.7 Biochemical Assay

The activity of aminotransferase (AST and ALT) was determined by the method of Reitman and Frankel, [15], Plasma alkaline phosphatase activity was assayed spectrophotometrically according to the method described by King and King, [16]. The concentration of bilirubin was determined by the method of Mallony and Evelyn, [17] as described in Randox assay kits.

2.8 Liver Histology

The animals were sacrificed and the abdominal cavity of each rat opened, the liver taken out. The organ was fixed in 10% formalin. After complete fixation the blocks was embedded in paraffin and sections cut at 5µm (micron) which was then stained with haematoxylin and eosin and mounted in Canada balsam. Microscopic examinations of the sections were then carried out under a light microscope [18].

2.9 Statistical Analysis

The data obtained was analyzed using One Way Analysis of Variance. The data was further subjected to LSD post hoc test for multiple comparisons and differences between Means regarded significant at $P<0.05$. The results were expressed as Mean±SEM.

3. RESULTS

3.1 Yield of the Methanol Extract of *Cocos nucifera* Stem Bark

The yield for the extract was 7.4 g (4.40%).

3.2 Acute Toxicity and Lethality (LD50) Test

Intraperitoneal administration of up to 5000 mg/kg body weight of methanol extract of *Cocos nucifera* stem bark to mice caused no death in the two stages of the test. Thus, the intraperitoneal LD_{50} of methanol extract in the mice was estimated to be greater than 5000 mg/kg body weight.

3.3 Phytochemical Test

Results of the quantitative phytochemical components of methanol extract of *Cocos nucifera* stem bark (Table 1) showed presence of alkaloids, flavonoids, soluble carbohydrate tannins and steroids. The results indicated the relative abundance as: tannins > soluble carbohydrates > flavonoids > alkaloids > saponnins > steroids.

Table 1. Quantitative phytochemical compositions of methanol extract of *Cocos nucifera* stem bark

Phytochemical compounds (mg/100 g)	Methanol extract of *Cocos nucifera* stem bark
Alkaloids	3.420±0.030
Flavonoids	3.590±0.014
Saponins	0.230±0.043
Steroids	0.006±0.001
Tannins	6.732±0.031
Soluble carbohydrate	5.372±0.001

Values expressed in mean ± SEM

3.4 Effect of Methanol Extract of *Cocos nucifera* Stem Bark on Liver Function Parameters of Paracetamol Intoxicated Rats

As shown in Table 2, there was non-significant ($P>0.05$) increase in AST and total bilirubin level of hepatic damaged rats administered 0.5 ml of distilled water compared with normal control rats. The hepatic damaged rats administered 0.5 ml of distilled water showed significant ($P<0.05$) increase in ALT activity compared with normal control rats while the ALP level of rats in group B decreases non-significantly ($P>0.05$) compared with the normal control rats. Hepatic damaged rats administered 200 and 400 mg/kg b. w. of the plant extract showed significant ($P<0.05$) decrease in ALT and total bilirubin level compared with the hepatic damaged rats administered 0.5 ml of distilled water. The animals in group IV administered 400 mg/kg b. w of the extract showed significant ($P<0.05$) decrease in AST, ALT and ALP activity compared with group III rats treated with 200 mg/kg b. w of the extract after hepatic damage (Table 2).

4. RESULT OF THE LIVER HISTOLOGY

Liver from the normal control rats showed normal hepatic histo-architecture, rats treated with 25 mg/kg b.w of silymarin revealed a mild to moderate hepatitis. Rats treated with 200 mg/kg and 400 mg/kg b. w. of C. nucifera bark after paracetamol exposure showed vacuolar

degeneration of the hepatocytes around the portal areas.

5. DISCUSSION

Cocos nucifera has been found to have an important dietary and medicinal role for centuries. Most of its prophylactic and therapeutic effects were ascribed to specific oil, water soluble compounds and secondary metabolites present in *Coco nucifera* including steroids, flavonoids and alkaloids which may have been responsible for its reported therapeutic effects [19].

Table 2. Effect of methanol extract of *Cocos nucifera* stem bark on liver function parameters of paracetamol intoxicated rats

Group	Treatment	AST (iu/l)	ALT (iu/l)	ALP (iu/l)	T.BIL (mg/dl)
I	Normal control	34.25±4.75	11.82±1.22	40.82±2.79	7.07±1.52
II	Hepatic damage administered 0.5 ml of distilled water	54.00±7.67	17.85±1.70*	36.08±2.86	11.23±3.82
III	Hepatic damage administered 25 mg/kg b. w. of silymarin	48.25±3.94	15.33±1.76	29.33±1.20	5.33±0.19
IV	Hepatic damage treated with 200 mg/kg b. w. of extract	58.00±6.00*	14.70±1.58	37.70±3.19	6.15±0.64
V	Hepatic damage treated with 400 mg/kg b. w. of extract	43.50±3.74	12.03±1.58	31.68±1.65*	10.42±3.10

*n = 4; *P<0.05 compared with the control (one way ANOVA; LSD post hoc test); values represented in Mean±SEM*

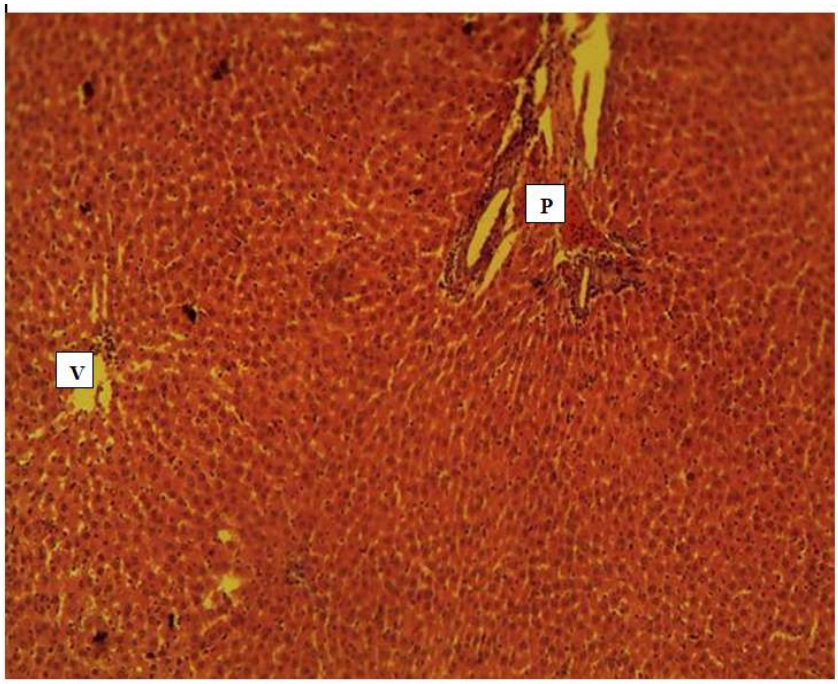

Plate 1. Sections of the liver histo-architecture of the Rats in the control group that were given distilled water without paracetamol administration. Liver from this group showed normal hepatic histo-architecture, it showed normal hepatocytes arranged in radiating chords around the central veins, diverging towards the portal areas with normal bile ducts, hepatic arteries and hepatic veins

Photomicrographs of the section of the liver showing normal hepatocytes (black arrow) arranged in radiating chords, around the central vein (V), Portal area (P) (H&EX100)

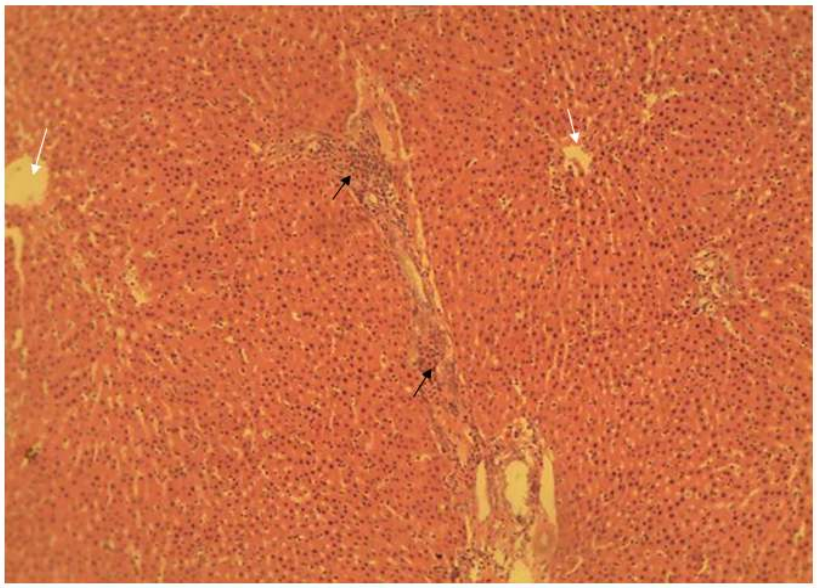

Plate 2. Sections of the liver histo-architecture of the Rats administered with paracetamol, treated with 25 mg/kg b.w of silymarine. Sections of the liver from this group revealed a mild to moderate hepatitis. Like the others, normal lobular outlines with individual lobules showing normal hepatocytes arranged in interconnecting chords were observed. At the periphery of most of the lobules mild to moderate aggregations of mononuclear leukocytes were observed. Also observed were multifocal areas of coagulative necrosis with mild infiltrations of mononuclear leukocytes. Central vein (white arrow). H&Ex100

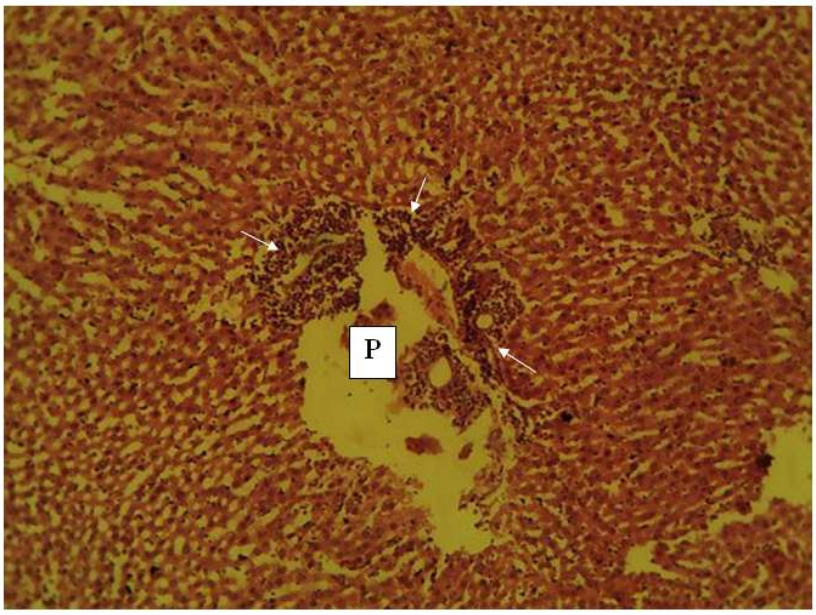

Plate 3. Sections of the liver histo-architecture of the rats that were given distilled water after paracetamol administration. Sections of the liver from this group showed multiple, mild to moderate, periportal infiltration of mononuclear leucocytes (primarily lymphocytes and macrophages). A few random aggregates of these mononuclear cells were also observed

A photomicrograph of liver showing a severe infiltration of monocuclear leucocytes (arrow) around the portal area (P) (H&E X 100).

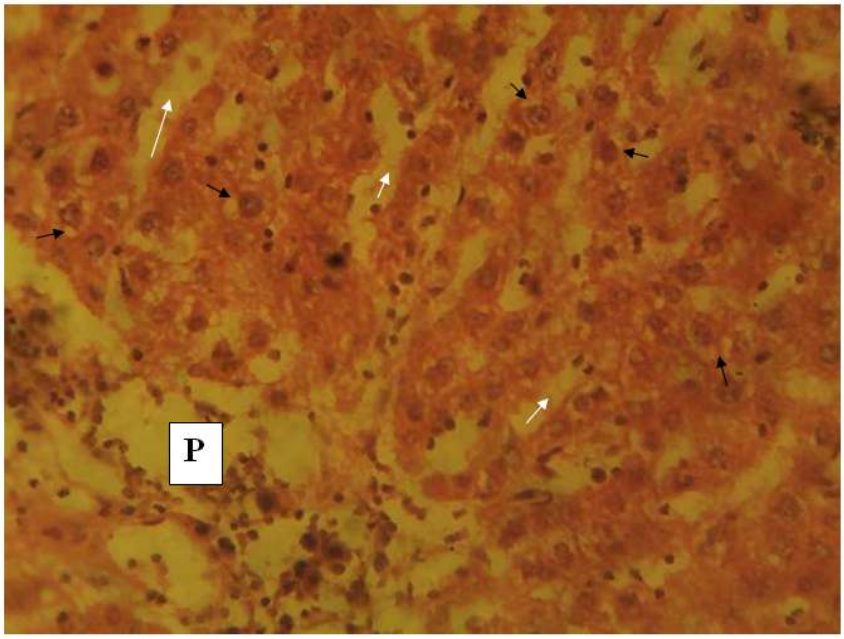

Plate 4. Sections of the liver histo-architecture of the rats that were treated with 200 mg/kg body weight of methanol extract of *Cocos nucifera* bark after paracetamol administration. Sections of the liver from the rats in this group showed primarily, vacuolar degeneration of the hepatocytes around the portal areas

A photomicrograph of the liver showing cytoplasmic vacuolations in the hepatocytes around the portal triad (arrow). Portal area (P), sinusoids (white arrow) (H&EX400).

The liver is an organ of paramount importance which plays an essential role in the metabolism of foreign compounds entering the body. Human beings are exposed to these comp1ounds through environmental exposure, consumption of contaminated food or during exposu1re to chemical substances in the occupational environment. In addition, human beings consume a lot of synthetic drugs during disease conditions which are alien to body organs. All these compounds produce a variety of toxic manifestations [20].

The liver gets damaged after high dose of paracetamol which produces hepatotoxicity; it leads to leakage of cellular enzymes into the plasma [21]. The increased level of serum enzymes such as ALT, AST, ALP and bilirubin observed in hepatotoxic rats are indicator of liver damage, increase permeability and necrosis of the hepatocytes [22]. The significant ($P<0.05$) 1increase observed in the level of plasma aminotransferase (AST and ALT) in high dose paracetamol treated rats compared with the normal rats in this study could be due to hepatocellular damage because these enzymes are normally located in the cytoplasm and released into the circulation at high concentration after cellular damage. The result from the phytochemical studies of the methanol extract of *C. nucifera* stem bark indicated the presence of tannins, alkaloids and flavonoids that may play a role in plant extract metabolism. The mechanism of action of *C. nucifera* could be by the prevention of the intracellular enzyme release and its membrane stabilizing effect. Ippoushi et al. [23] and Lee et al. [24] have reported the antioxidant properties of *C. nucifera* stem bark. This may also enhance the hepato-curative properties of this plant extract observed in this study. The reduction in ALP activity by the extract may suggest repairing of the rats' liver by *C. nucifera* stem bark extract. This may be ascribe to the bioactive compounds present in *C. nucifera* which could have possibly increase the levels of glutathione which binds to the toxic metabolites of paracetamol such as N-acetyl-p-benzoquinone imine (NAPQ1) and increased its rate of metabolism and excretion from the body system. It might also result in inhibition of cytochrome P-450 enzyme system which decreased the formation of NAPQ1 from ingested paracetamol [25].

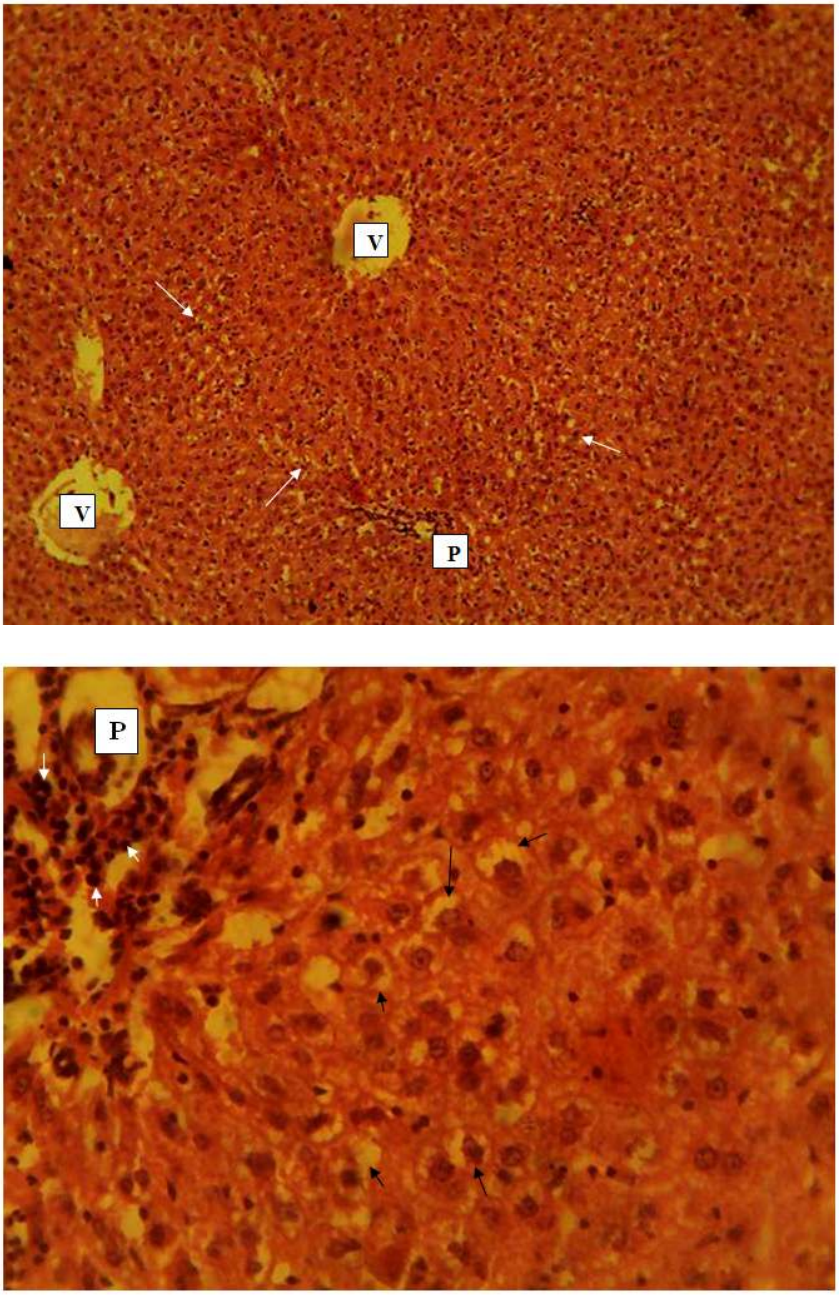

Plate 5 (A&B). Sections of the liver histo-architecture of the rats that were treated with 400 mg/kg body weight of methanol extract of *Cocos nucifera* bark after paracetamol administration. Sections of the liver from this group showed a mild to moderate hepatocellular vacuolar degeneration which tend to involve mainly the periportal hepatocytes. Also evident were periportal infiltrations of mononuclear leucocytes comprised mainly of lymphocytes and macrophages

Plate 5(A). *A photomicrograph of the liver showing a mild to moderate cytoplasmic vacuolations of the periportal hepatocytes (arrow). Central vein (V), Portal area (P) (H&EX100)*

Plate 5(B). *Higher magnification of figure 4.7(A) above, showing cytoplasmic vacuolations in the periportal hepatocytes (black arrow) and a moderate periportal infiltration of mononuclear leucoccytes (white arrow). Portal area (P) (H & E X400).*

The elevation in total plasma bilirubin observed in paracetamol hepatotoxic rats suggested abnormal conjugation of bilirubin by the liver due to generalized hepatocellular damage [26]. Total plasma bilirubin was decreased in paracetamol hepatotoxic rats after treatment with *C. nucifera* stem bark extract. The possible mechanism of action of *C. nucifera* extract may be through their anti-oxidative effect. This is because *C. nucifera* possess bioactive compounds that are capable of free radical scavenging in living system [27].

The results obtained in this study are consistent with the findings of Ozougwu and Eyo, [5]. It is well documented that flavonoids are good hepato-protective agents because they can effectively inhibit lipid peroxidation, scavenge free radicals and enhance anti-oxidant enzyme activities [28,29].

The primary histopathological change observed was vacuolar degeneration of the hepatocytes. This lesion varied in its distribution from periportal to mid-zonal and pan-lobular across the groups. The control group showed normal hepatic micro-architecture, the group administered paracetamol only showed a severe periportal infiltration of inflammatory leucocytes without evident degenerative change in the hepatocytes. This is not consistent with the commonly reported paracetamol induced hepatocytes damage. This could be due to incomplete induction of liver damage or poor collection of organs for histopathology. The other slides from the groups treated with tested extract showed changes in the hepatic histo-architecture which are consistent with paracetamol induced hepatic damage. The rats treated with methanol extract of *Cocos nucifera* stem bark at 200 and 400 mg/kg b. w. similar histopathological changes were observed in the liver and these changes were both mild (only periportal vacuolar degeneration of the hepatocytes). These could mean that this extract at the test doses have hepato-protective effect on the liver.

6. CONCLUSION

The results demonstrated that *Cocos nucifera* stem bark acted as anti-hepatotoxic agent because of the significant ($P<0.05$) reduction in the elevated level of plasma total bilirubin concentration and liver enzyme activity in the rats exposed to paracetamol. The observed anti-hepatotoxic activity might be linked with the presence of flavonoids, alkaloids and other bioactive compounds in the plants.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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