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Abstract: *Phoenix dactylifera* fruit, *Cyperus esculentus* nut and *Cocos nucifera* nut are plant materials that are commonly consumed by man in many parts of the world. This research evaluated the liver function and haematological effects of extracts of date fruit, coconut and tiger nut in albino rats. Healthy male albino rats (20) were used and distributed randomly into four groups (n=5). Group 1 rats served as normal control, while groups 2, 3 and 4 animals were administered the corresponding plant ethanolic extracts for twenty-one days and later sacrificed. Serum biochemical indices were analyzed using Cobas C111 chemistry analyzer, while the haematological indices were analyzed using Abacus 380 haematological auto-analyzer. The results showed that AST decreased significantly ($p < 0.05$) in group 2, but increased significantly in groups 3 and 4, compared with the normal control (group 1). ALT decreased non-significantly in groups 2 and 3, but decreased significantly in group 4 compared with normal control. ALP decreased non-significantly in all the test groups compared to the normal control. Total protein and albumin decreased non-significantly ($p > 0.05$) in group 2, but increased non-significantly in groups 3 and 4, while globulin increased non-significantly in group 2, but decreased non-significantly in groups 3 and 4 compared with normal control. WBC count, Hb and PCV increased non-significantly ($p > 0.05$) in all groups compared to control. RBC showed a significant ($p < 0.05$) increase in groups 3 and 4, and a non-significant increase in group 2 compared to group 1. The liver histoarchitectural state of the liver sections of the test animals showed evidence of preserved tissues. This research results showed that ethanolic extracts of *Phoenix dactylifera* fruit, *Cyperus esculentus* nut and *Cocos nucifera* nut do not have apparent negative effect on the liver function and the selected haematological indices in male albino rats.

Keywords: *Cocos nucifera*, *Cyperus esculentus*, haematology, histology, liver, *Phoenix dactylifera*

Introduction

Date palm (*Phoenix dactylifera* L.) is a flowering plant of the date family, Arecaceae. It is known to be rich in sugar (Eissa *et al.*, 2009). In Nigeria, it is commonly called “dabino.” Traditionally, date palm fruits have been used to prepare quite a number of products such as date juice concentrates (spread, syrup and liquid sugar), fermented date products (wine, alcohol, vinegar, and organic acids) and date pastes for different uses (e.g. bakery and confectionary) (Eissa *et al.*, 2009). Date palm has interesting pharmacological properties. The pollen of this plant has been reported to protect the male reproductive system and increase sperm count (Tahvilzadeh *et al.*, 2016). Both fruit and seed extracts of *Phoenix dactylifera* have been reported to significantly improve glycaemic control and to prevent oxidative stress-induced and CCl₄-induced hepatotoxicity *in vivo* (Saafi *et al.*, 2011; Abdelaziz and Ali, 2014). The fruit also has anti-oxidant, anti-inflammatory, anti-tumourigenic and anti-diabetic effects (Rahmani *et al.*, 2014; Salem *et al.*, 2018). Date palm fibres have been used in the removal of phosphate from wastewaters (Riahi *et al.*, 2017). Tiger nut (*Cyperus esculentus* Linn.), also known as yellow nutsedge or nut grass is in the Cyperaceae (sedge) family, subclass Monocotyledoneae, monocots (Defelice, 2013; Schippers *et al.*, 2016). It is a perennial herbaceous sedge widely distributed in the world. The Igbos in Nigeria call it “Aki Hausa”, the Hausas call it “Aya” while the Yorubas call it “Ofio.” It is consumed in different forms. It has been found to be an alternative for diabetics (Gambo and Da’u, 2014). Borham *et al.* (2017) reported *Cyperus esculentus* tuber extracts to show antioxidant effects in an *in vitro* study with liposome peroxidation system. Tiger nut and oil extracted from it have been shown to have antidiabetic, apoptotic and cytotoxic properties (Kilani *et al.*, 2008). Untargeted metabolomic studies revealed that the geographical location determines the nutritional compositions *Cyperus esculentus* tubers (Codina-Torrella *et al.*, 2015). Heat treatment reduces

the nutritional quality of *Cyperus esculentus* tuber (Rubert *et al.*, 2018).

The coconut palm (*Cocos nucifera* L.), is a member of the family Arecaceae (palm family), subfamily Arecoideae and is the only accepted species in the genus *Cocos* (DebMandal and Mandal, 2011). Coconut, found throughout the tropic and subtropic area (Gunn *et al.*, 2011), is known for its great versatility as seen in the many domestic, commercial and industrial uses of its different parts. Coconuts are part of the daily diet of many people (DebMandal and Mandal, 2011). In Nigeria, it is called “Aki Oyibo” or “Aki bekee” in Igbo, “Kwakwa” in Hausa and “Agbon” in Yoruba (Imo *et al.*, 2018). The nut, which some people refer to as the seed has been reported by Imo *et al.* (2018) to be usually eaten in its raw form, and most times consumed alongside other food materials such as date fruit, maize and cassava chips, among others. This plant has been shown to have many beneficial health potentials, amongst which are: antidiabetic (Preetha *et al.*, 2013), cytoprotective, antihyperglycemic (Renjith *et al.*, 2013), antiulcerogenic (Cruz-Vega *et al.*, 2009), antithrombotic, antioxidant, antiatherosclerotic, hypolipidemic (DebMandal and Mandal, 2011), antimicrobial, antiviral (Apraku *et al.*, 2017) properties, etc. It has been involved in treatment of aluminum poisoning (Shadnia *et al.*, 2005). Imo *et al.* (2018) reported that *Cocos nucifera* nuts are a good source of energy and could play immunological, physiological, nutritional and pharmacological roles. Coconut oil is rich in vitamins (folate, B6, B1, B5, and B2) and saturated fatty acids like laurate (Yong *et al.*, 2009).

Phoenix dactylifera fruits, *Cocos nucifera* nuts and *Cyperus esculentus* nuts are commonly sold in many markets and also widely consumed as food in many parts of the world. These three plant materials are commonly used in synergism for the preparation of different types of drinks. In Nigeria, they are used in preparing the drink known as “Kunun aya.” Currently, there is no relevant research data or report on the comparative analysis of these three plant materials in their consumers or as

used in this study. Since these three plant materials are widely consumed, it is important that research findings or data on the effects of the extracts of these plant materials on the liver function and haematological indices in male albino rats should be established. The objective of this study is determination of selected parameters used in accessing liver and haematological status in albino rats administered the various extracts.

Materials and Methods

Plant materials and extraction

Phoenix dactylifera fruits, *Cocos nucifera* nuts and *Cyperus esculentus* nuts were purchased in Wukari, Taraba State, Nigeria. The three plant materials were properly identified at Biological Science Department, Federal University Wukari, Nigeria. The healthy parts were selected and properly cleaned. They were sun-dried and pulverized to powder using a laboratory blender. Powder of each of the samples was macerated in 70% ethanol for two days, with occasional shaking, thereafter filtered. The filtrates were concentrated using a rotary evaporator and the concentrated extracts re-dissolved in normal saline for the experiment.

Experimental animals

Twenty healthy male albino rats of eight weeks old were used in this study. The male rats weighed between 91 to 120 g. The rats were purchased from Department of Animal Production, National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria, and transferred to the animal house, Department of Biochemistry, Federal University Wukari, Taraba State, Nigeria. The male rats were allowed to acclimatize for fourteen days before being used for the experiment. All the male albino rats were allowed free access to feed and water *ad libitum* throughout the experimental period. Standard laboratory protocols for animal studies were maintained as approved by the Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari, Nigeria.

Experimental design

The male albino rats were placed randomly into four different groups. Five animals were distributed to each group. Group 1 animals served as normal control, while groups 2, 3 and 4 animals served as the test animals. The group 1 animals were administered a placebo of normal saline. Group 2 animals were administered ethanolic fruits extract of *Phoenix dactylifera* (200 mg/Kg body weight). Group 3 animals received ethanolic nut extract of *Cyperus esculentus* (200 mg/Kg body weight), while group 4 animals received ethanolic nut extract of *Cocos nucifera* (200 mg/Kg body weight). The different plant extracts were administered to all the test animals through oral route for twenty-one consecutive days.

Blood collection

After the administration of the ethanolic extracts of *Phoenix dactylifera* fruit, *Cyperus esculentus* nut and *Cocos nucifera* nut to the experimental animals for twenty-one days, they were starved overnight and sacrificed after anaesthesia with chloroform. Blood samples were collected through cardiac puncture and dispensed into two different types of tubes. The first part of the blood was dispensed into an anti-coagulant containing sample tube for haematological analysis. The second part of the blood was dispensed into a very cleaned plain tube and allowed to clot for fifteen minutes and then centrifuged for 10 min at 4000 rpm. Serum was separated from the clot using pasteur pipette for the biochemical analysis of the indices of liver function.

Biochemical and histological analysis

The concentrations of serum AST, ALT, ALP, total protein and albumin were determined using Cobas C111 Chemistry analyzer. Globulin was estimated from the results of total

protein and albumin. The level of the selected haematological indices (WBC, RBC, PCV and Hb) were determined with the use of Abacus 380 haematological auto-analyzer. The liver of all the animals were harvested and examined histologically (Stain: Haematoxylin & Eosin). Photomicrographs of liver sections of the rats were taken and examined.

Statistical analysis

Statistical analysis was carried out on the selected biochemical indices results using One-Way Analysis of Variance (ANOVA) and further with Duncan Multiple Comparisons with the use of Statistical Package for Social Sciences (SPSS), version 21. The result means were compared for significance at $p \leq 0.05$ and the group results were then presented as mean \pm standard deviation ($n=5$).

Results and Discussion

The results of the study are presented in the Tables and Figures, respectively. The significant decrease ($p < 0.05$) in serum activity of AST in group 2 is an indication of possible hepatic preservation property of ethanolic extract of *Phoenix dactylifera* fruit on the male albino rats. The non-significant reduction ($p > 0.05$) of ALT and ALP in the group 2 animals is a confirmation of possible non-toxic and hepatoprotective activity of the *Phoenix dactylifera* fruit extract. Cellular leakage of AST, ALT and ALP into the blood have been reported to occur due to malfunctioning of cell membrane of some organs (Imo *et al.*, 2013; Imo *et al.*, 2014), such as the liver. Administration of ethanolic extracts of *Cyperus esculentus* nut and *Cocos nucifera* nut in groups 3 and 4, respectively show that the two extracts possess the ability of reducing the serum activity of ALT and ALP (Table 1). This suggests that these extracts may also possess the ability to preserve liver tissues. However, the administration of the *Cyperus esculentus* nut extract and *Cocos nucifera* nut extracts showed a significant increase in serum activity of AST in the test animals compared to the control animals. This shows that though the extracts may not be affecting the liver negatively, it may possibly be affecting another organ that may predominantly contain AST. Olabiyi *et al.* (2017) evaluated the promoting effect of tiger nut as dietary supplementation on erectile function in healthy male rats. The rats with tiger nut supplemented diet showed significant increase in sexual behaviour, hormonal levels and antioxidant activities. A comparative analysis of the effects of the three plant materials showed that all possess liver preserving effect and as such, may support liver function of the animals. In a research, Saafi *et al.* (2011) reported that *in vivo* date palm fruit extract could be useful in preventing oxidative stress induced hepatotoxicity. The phenolics contents of date palm are documented to exhibit chemopreventive effects against Dimethylbenz(A)Anthracene (DMBA)-induced mammary cancer (Omran *et al.*, 2017).

The concentration of serum total protein, albumin and globulin showed no significant alterations ($p > 0.05$) in all the test animals administered ethanolic extracts of *Phoenix dactylifera* fruit, *Cyperus esculentus* nut and *Cocos nucifera* nut compared to the control animals (Table 2). Total protein and albumin reduced non-significantly in group 2 and increased non-significantly in groups 3 and 4 compared with the normal control. Globulin increased non-significantly in group 2, but decreased non-significantly in groups 3 and 4 compared with normal control. These results show the extracts of these plant materials encourages processes associated with the regulation of protein synthesis and stability. A wide range of phytochemicals have been reported to be detected in ethanolic extract of *Cocos nucifera* nut which possess important industrial, medicinal, physiological and antibiotic properties (Imo *et al.*, 2018). Protein stability is also an index for measuring liver function. Nair (2006) reported that liver

damage and its synthetic function may be assessed using serum protein level. This agreed with the result of this study. This protein regulatory property of the extracts also supports the result of ALT, AST and ALP. This therefore showed that the hepatocytes are functioning properly, since they are

involved in protein synthesis. Imo *et al.* (2017) reported that the hepatocytes are mostly responsible for the synthesis of most proteins that are distributed in plasma.

Table 1: Concentrations of liver enzymes (IU/L) in rats administered ethanolic extracts of *Phoenix dactylifera* fruit, *Cyperus esculentus* nut and *Cocos nucifera* nut

Parameters	Group 1 (Normal control)	Group 2 (<i>Phoenix dactylifera</i> fruit 200mg/kg bw)	Group 3 (<i>Cyperus esculentus</i> nut 200mg/kg bw)	Group 4 (<i>Cocos nucifera</i> nut 200mg/kg bw)
AST	321.13 ± 18.59 ^a	268.13 ± 7.66 ^b	357.27 ± 10.92 ^c	373.03 ± 3.36 ^c
ALT	96.77 ± 3.14 ^a	95.40 ± 1.21 ^a	90.73 ± 2.70 ^{a,b}	81.70 ± 11.26 ^b
ALP	186.57 ± 5.31 ^a	175.97 ± 21.46 ^a	166.80 ± 14.03 ^a	171.17 ± 13.81 ^a

Results represent mean ± standard deviation of group serum results obtained (n=5).

Mean in the same row, having different letters of the alphabet are statistically significant (p<0.05).

The serum activity of AST decreased significantly (p<0.05) in group 2, but increased significantly in groups 3 and 4 compared with the normal control. ALT decreased non-significantly in groups 2 and 3, but decreased significantly in group 4 compared with the normal control. ALP decreased non-significantly in all the test groups compared to the normal control.

Table 2: Concentrations of proteins (g/L) in rats administered ethanolic extracts of *Phoenix dactylifera* fruit, *Cyperus esculentus* nut and *Cocos nucifera* nut

Parameters	Group 1 (Normal control)	Group 2 (<i>Phoenix dactylifera</i> fruit 200mg/kg bw)	Group 3 (<i>Cyperus esculentus</i> nut 200mg/kg bw)	Group 4 (<i>Cocos nucifera</i> nut 200mg/kg bw)
Total protein	54.53 ± 1.69 ^a	54.40 ± 3.97 ^a	55.57 ± 0.78 ^a	56.57 ± 3.33 ^a
Albumin	34.31 ± 3.05 ^a	33.97 ± 1.58 ^a	36.57 ± 0.74 ^a	36.63 ± 0.71 ^a
Globulin	20.22 ± 1.40 ^a	20.43 ± 2.43 ^a	19.00 ± 1.51 ^a	19.93 ± 2.67 ^a

Results represent mean ± standard deviation of group serum results obtained (n=5).

All mean in the same row are statistically non-significant (p>0.05).

The serum total protein and albumin decreased non-significantly (p>0.05) in group 2, but increased non-significantly in groups 3 and 4 compared with the normal control. Globulin increased non-significantly in group 2, but decreased non-significantly in groups 3 and 4 compared with the normal control.

Table 3: Concentrations of selected haematological indices in rats administered ethanolic extracts of *Phoenix dactylifera* fruit, *Cyperus esculentus* nut and *Cocos nucifera* nut

Parameters	Group 1 (Normal control)	Group 2 (<i>Phoenix dactylifera</i> fruits 200 mg/kg bw)	Group 3 (<i>Cyperus esculentus</i> nut 200mg/kg bw)	Group 4 (<i>Cocos nucifera</i> seed 200mg/kg bw)
WBC (cells/mm ³)	84.00 ± 4.90 ^a	95.55 ± 8.55 ^a	95.90 ± 4.80 ^a	97.60 ± 8.60 ^a
RBC (x10 ¹² /L)	0.36 ± 0.05 ^a	0.40 ± 0.04 ^{a,b}	0.47 ± 0.01 ^b	0.51 ± 0.08 ^b
Hb (g/dL)	11.80 ± 0.70 ^a	13.40 ± 0.70 ^a	12.75 ± 0.15 ^a	12.90 ± 1.10 ^a
PCV (%)	35.10 ± 1.80 ^a	40.70 ± 1.60 ^a	38.25 ± 0.45 ^a	38.70 ± 3.30 ^a

Result represent mean ± standard deviation of group result obtained (n=5).

Mean in the same row, having different letters of the alphabet are statistically significant (p<0.05).

White blood cells (WBC) count, Hb and PCV increased non-significantly (p>0.05) in all test groups compared to control. Result of RBC showed a significant (p<0.05) increase in groups 3 and 4, and a non-significant increase in group 2 compared to control.

Concentrations of selected haematological indices in rats administered the ethanolic extracts of *Phoenix dactylifera* fruit, *Cyperus esculentus* nut and *Cocos nucifera* nut showed the three plant materials improved the levels of the haematological parameters. White blood cells count increased non-significantly (p>0.05) in all the test animals compared to the control animals (Table 3). This means the three plant materials may have the ability of stimulating the production of the WBC and have the potency of aiding the animals body system to fight infection or ingestion of foreign materials. Imo *et al.* (2013) reported that WBC is a common immune function index that supports in defense against diseases and pathogens that affects animals, thereby promoting the immune system. White blood cells are also reported by Metcalf (1993)

to originate from pluripotent haemopoietic stem cells. Hb and PCV increased non-significantly (p>0.05) in all test animals compared to the control animals (Table 3), while RBC increased significantly (p<0.05) in the groups administered extracts of *Cyperus esculentus* nut and *Cocos nucifera* nut, but increased non-significantly in the group administered ethanolic extracts of *Phoenix dactylifera* fruit compared to normal control. This study agreed with the report of Morebise *et al.* (2002) that consumption of plant as foods usually promotes the synthesis of haemoglobin due to their high minerals and vitamins contents as observed in this study. This result shows the three extracts have blood boosting property. The increased levels of Hb and PCV shows consumption of the plant materials could aid prevention of anaemia and could

increase the rate of oxygen supply to the body cells. The haemoglobin level usually drops when the number of healthy red blood cells drops (Imo *et al.*, 2016). This usually causes reduction in the delivery of oxygen to tissues and cells, thereby resulting to tiredness, weakness and inability to effectively engage in physical exercise (Janz *et al.*, 2013). This study result is therefore in agreement with the report of Imo *et al.* (2016), since the increase in red blood cells count resulted to increase in haemoglobin level in this study. The increase in RBC counts in the test animals compared to the control animals also support the ability of the plant materials in supporting or enhancing the animals' immune system. The red blood cells are reported to be the animals' principal means of transporting oxygen to the body tissues through blood circulation (Imo *et al.*, 2016). Oguwike *et al.* (2017) reported that consumption of tiger nut by male hyper-cholesteremic subjects showed no negative effects on haemoglobin level, packed cell volume, platelet count and white blood cell count. This agreed with the result of this study.

The histoarchitectural state of the liver sections of the test animals when compared to the control (Fig. 1) showed that the ethanolic extracts of *Phoenix dactylifera* fruit, *Cyperus esculentus* nut and *Cocos nucifera* nut have no apparent toxic effect on the liver. There was evidence of normal sinusoids, hepatocytes, portal tract and central vein in the test animals (Figs. 2 – 4). This agrees with the results of the liver-maker enzymes and proteins, confirming the three extracts have liver supporting function. The implication of this is that consumption of the ethanolic extracts of these plant parts as used in this study may not pose apparent danger to the liver.

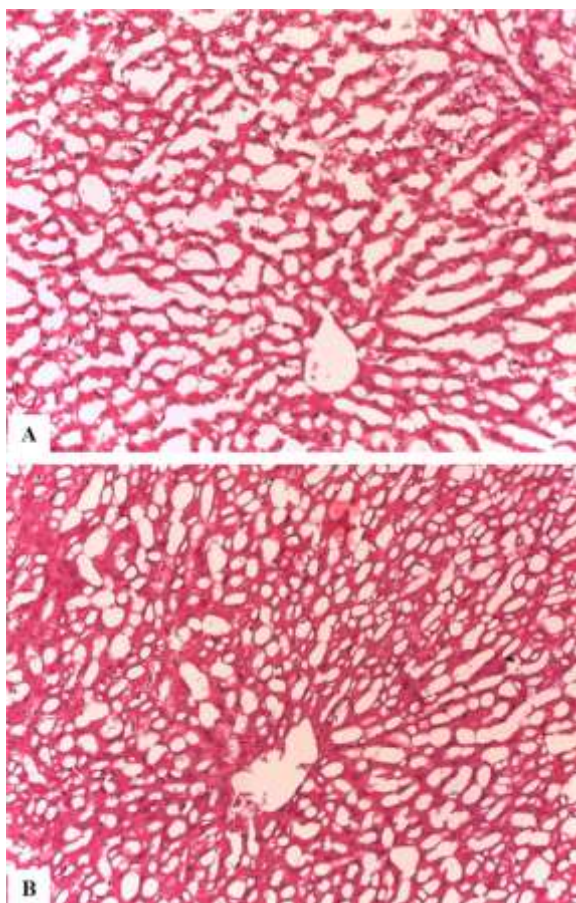


Fig. 1: Photomicrographs of liver section from rat in group 1 (normal control) showing normal features of the hepatic tissue. The hepatocytes, central vein and sinusoids are normal.

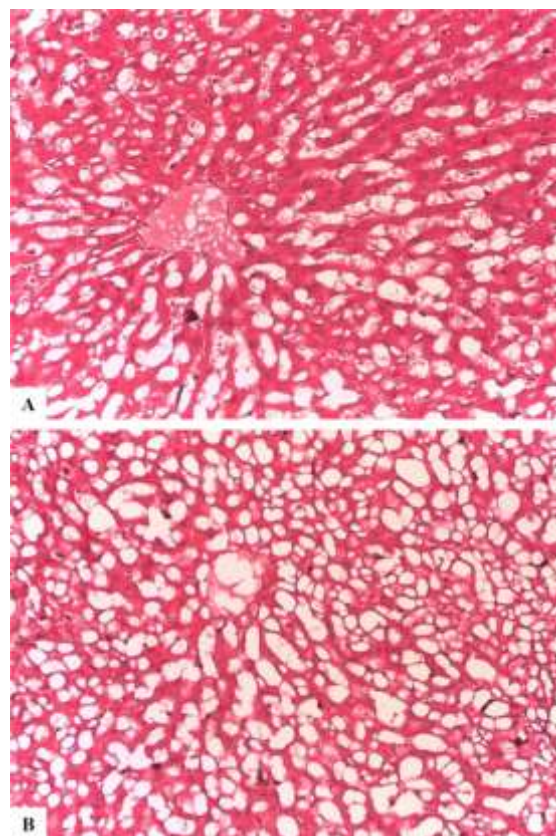


Fig. 2: Photomicrographs from liver section of rat administered ethanolic extract of *Phoenix dactylifera* fruit (200 mg/kg bw) showing normal sinusoids, hepatocytes and central vein.

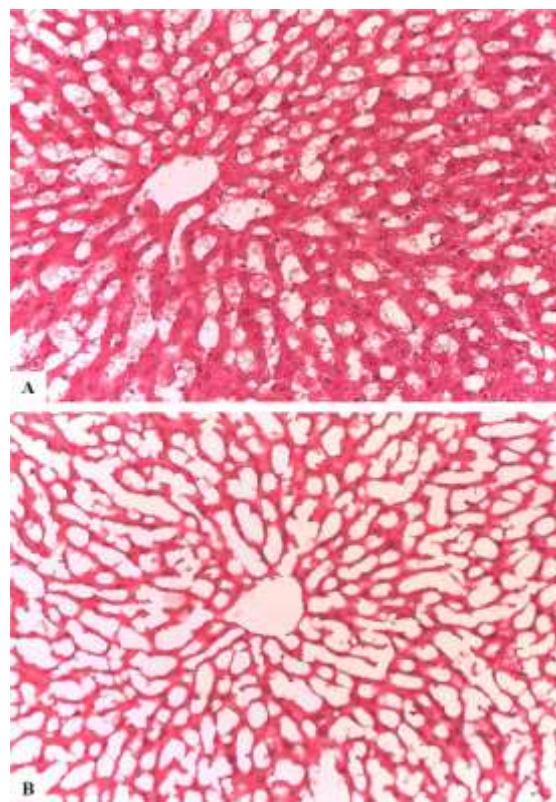


Fig. 3: Photomicrographs from liver section of rat administered ethanolic extract of *Cyperus esculentus* nut (200 mg/kg bw) showing normal sinusoids, hepatocytes and central vein.

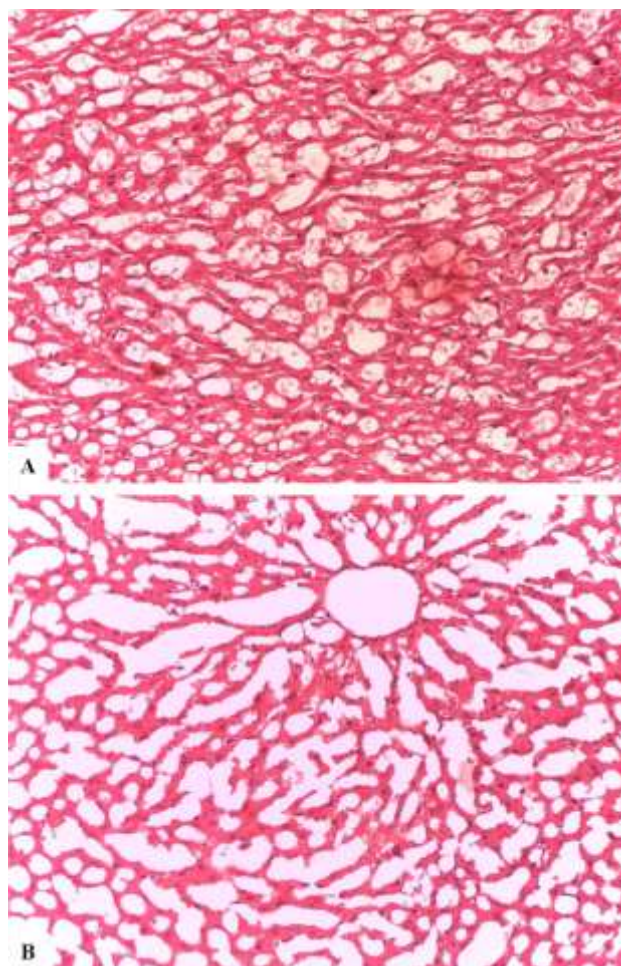


Fig. 4: Photomicrographs from liver section of rat administered ethanolic extract of *Cocos nucifera* nut (200 mg/kg bw) showing normal features of the sinusoids, hepatocytes and central vein.

This study has therefore bridged the existing gap in the literature by providing the required research findings and proof of the comparative evaluation of liver function and haematological effects of ethanolic extracts of *Phoenix dactylifera* fruit, *Cyperus esculentus* nut and *Cocos nucifera* nut in male albino rats.

Conclusion

This research results showed that the ethanolic extracts of *Phoenix dactylifera* fruit, *Cyperus esculentus* nut and *Cocos nucifera* nut did not have apparent negative effect on the liver function and the selected haematological indices in male albino rats. The results also showed that the extracts supported liver function and may have hepatoprotective effect. The liver histoarchitectural state of the liver sections of the test animals showed evidence of preserved tissues when compared to the control animals. The consumption of these three plant materials are therefore encouraged.

Competing Interests

The authors declare that they have no competing interest.

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