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Aqueous Extract of *Balanites aegyptiaca* Del Fruit Mesocarp Protects against CCl_4 – Induced Liver Damage in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OBO designed the study. Author SM carried out the experiment, author NSO wrote up the work and everyone jointly corrected the write up prior to submission.

Research Article

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ABSTRACT

Aim: Carry out preliminary phytochemical screening of *Balanites aegyptiaca* fruit mesocarp and to evaluate the ability of the aqueous extract to ameliorate carbon tetrachloride-induced hepatotoxicity in rats by determining liver enzymes, blood parameters and histopathology.
Study Design: Sixty albino rats (Wistar rats) were randomly placed into four groups of fifteen animals: Group 1 (normal control), group 2 (Untreated CCl_4 –intoxicated group) while groups 3 and 4 are test groups both administered CCl_4 and 0.08 $mgkg^{-1}$ and 0.19 $mgkg^{-1}$ body weight of concentrated fruit extract respectively orally daily. Administration of CCl_4 was 3 times in a week for 4 weeks at a dose of 1.2 g/kg body weight and plant extract for 3 weeks.
Results: Phytochemical screening indicated the presence of saponin, flavonoids, steroids, alkaloids and cardiac glycosides in the fruit mesocarp. Serum total proteins, albumin and conjugated bilirubin were elevated while the liver enzymes activities (alanine transaminase and alkaline phosphatase) were significantly reduced in the test groups 3 and 4 compared to the untreated rats (Group 2). Histological examinations of the rats'

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tissues showed reduced inflammation in the liver of the CCl_4-damage rats treated with the extracts when compared to the control and the cell architecture on the 21st day of the animal treated with extract resembled that of the normal control.
Conclusion: The mesocarp of desert dates ameliorates hepatotoxicity induced by CCl_4 in rats and this study has further added credibility to the ethnobotanical use of the fruit.

Keywords: Balanites aegyptiaca; phytochemicals; CCl_4; liver enzymes; histology.

1. INTRODUCTION

Most plant secondary metabolites exhibit bioactivity of plants and health-promoting properties [1]. Phytonutrients are used as flavoring agent, natural medicines by humans to cleanse and purify the body by binding chemical carcinogens and activating detoxification enzymes, mostly in the gastrointestinal tract [2]. Bioactive phytochemicals from different part of the plant can be classified chemically as isoprenoid derivatives (terpens, terpenoids, tocotrienols, tocopherol, carotenoids and saponins), phenolics (coumarins, flavones, flavonoids, isoflavones, antrocyanins, lignins and tanins) and sugar derivatives such as vitamin C, non-starch polysaccharide, oligosaccharides) [3]. Other phytonutrients are fatty acid derivatives, amino acid derivatives and minerals. However majority of phytochemicals in fruits have shown radical scavenging or antioxidants activity [4], while those in and vegetables may reduce the risk of cancer, possibly due to dietary fibers, polyphenol antioxidants and antiinflammatory effects [5]. Over production of free radicals are dangerous because they react with vital cellular components such as DNA or cell membranes causing damage to muscles, other tissues and have been implicated in many ailments [6] and antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves [7]. Apart from the antioxidants in the body, foods have naturally occurring antioxidants like vitamin E (tocopherol), urates, vitamin C and some minerals acts as free radical scavengers. Flavonoids are polyphenolic compounds that have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant properties [8].

Consumption of flavonoid-rich foods causes a huge increase in antioxidant capacity of the blood and this is not caused directly by the flavonoids themselves, but most likely is due to increased uric acid levels that result from expelling flavonoids from the body [9]. Furthermore, cardiac glycoside has effects on the heart, stomach, intestines, and nervous system by increasing the force and velocity of myocardial systolic contraction (positive inotropic action), [10]. Saponins could interfere with membrane bilayers leading to red cell lysis and the amphipathic nature of saponins makes them active surfactants that can be used to enhance penetration of macromolecules such as proteins through cell membranes and they have also used as adjuvants in vaccines [11].

Balanites aegyptiaca Del., (Zygophyllaceae) a common wild plant is known commonly in English as Desert date. Fruits are edible and are sometimes mixed with porridge. Aqueous extract of the fruit is used traditionally as vaginal douche since it has spermicidal activity without causing any irritation [12]. Aqueous fruit extract in a previous study (0.75%) had molluscidal activity with cercarricidal penetration inhibition of *schistosoma mansoni* activity, antihelminthic activity as well as fish poison activity [13]. Fruits are planted by the river bank in communities where schistosomiasis is a health challenge and are also used to treat diabetes, jaundice, dysentery, constipation and liver disease [14]. Hepatoprotective activity

of aqueous extract of the stem bark of desert date has been reported in biliary duct ligated rats where there was dose dependent reduction in serum bilirubin levels [15-17] but not with CCl_4 induced liver damage and not with the fruit extract. *Balanite aegyptica* fruit is used traditionally to treat spleen and liver conditions [13] and this informed our decision to ascertain the effect of consumption of Desert date on some of the blood defense parameters, liver enzymes, liver tissue and kidney integrity in CCl_4 intoxicated rats.

2. MATERIALS AND METHODS

2.1 Plant Material

Balanites aegyptiaca fruit was purchased from the market in Ilorin metropolis and was authenticated in Botany Department, University of Ilorin, Ilorin, Nigeria with (F2114). The coat epicarp was gently removed by hand while a cleaned, dried knife was used to peel the mesocarp of the fruit. Cleaned mesocarp (fleshy outer part) was separated from the hard inner shell containing the seed. Mesocarp was air dried at room temperature in the laboratory and was powdered using coffee mill. This was thereafter placed in a dry plastic container and later put inside the refrigerator until required for use. 100g powdered was extracted using 200ml distilled water; it was stirred continuously for 10mins and filtered. Filtrate was concentrated at 60ºC to obtain thick brown viscous semi-solid with a yield of 23.43%.

2.1.1 Phytochemical screening

The phytochemical screening to determine presence of secondary metabolites such as saponins, tannins, flavonoids, steroids, alkaloids, glycosides, phlobatannin, cardiac glycosides and phenols in the fruit mesocarp was done using standard laboratory methods.

2.2 Animals

Sixty albino rats weighing between 150 to 160 g were obtained from the Small Animal Holding Unit of Biochemistry Department, University of Ilorin, Ilorin, Kwara state, Nigeria, Animals were randomly placed in 4 groups of fifteen rats each. Group 1 served as normal control and received only rat chow, Group 2 is disease control group (un-treated CCl_4 intoxicated) while groups 3 and 4 are test groups administered both CCl_4 and 0.08 $mgkg^{-1}$ and 0.19 $mgkg^{-1}$ body weight of concentrated extracts respectively orally daily as shown below.

Group 1 - The normal control rats.
Group 2 – Untreated CCl_4 intoxicated rats.
Group 3 – CCl_4 and fruit extract (0.08 mg/kg body weight) treated rats.
Group 4 – CCl_4 and fruit extract (0.19 mg/kg body weight) treated rats.

Animals were acclimatized for two weeks and feeding experiment with fruit mesocarp was for 21 days, while CCl_4 was for four weeks. The rats (3 each) were bled on different days by jugular puncture with a sharp sterile blade after they were immobilized by anesthetics by placing the rats in a small cardboard in which we had placed cotton wool soaked with chloroform on different days. Blood samples were collected using sterile disposable syringe and needle and it was transferred to ethylenediaminetetraacetic acid (EDTA) anticoagulant bottle for blood cell analysis and visceral organs were excised from representative animals

from each group on different days for histology.

2.2.1 Heamatological parameters

Whole blood collected into EDTA bottles was used to determine white blood count (WBC), platelet, neutrophil and lymphocyte using Automated Heamatocrit Analyzer.

2.2.2 Histology and biochemical analysis

The histopathological study was carried out using Haemotoxylin and Eosin staining techniques as described by Drury and Wallington [18]. The Biuret method was used for total protein [19] while total albumin determination was according to method of Doumas et al. [20]. Alkaline phosphatase and alanine transaminase were determined using Randox kit.

2.3 Statistical Analysis

Data was expressed as mean ± S.D of six determinations and were analyzed using one way analysis of variance (ANOVA) and complimented with student t-test. Values for ($P < 0$ .05) were considered to be statistically significant.

3. RESULTS AND DISCUSSION

Table 1 shows result of the phytochemicals screening of aqueous extract of the mesocarp of *Balanites aegyptiaca*.

Table 1. Phytochemicals of the aqueous extract of *Balanites aegyptiaca* mesocarp

Phytochemical	Inference
Alkaloids	+ve
Saponin	+ve
Phlobatannin	-ve
Anthroquinones	-ve
Tannin	-ve
Steroid	+ve
Phenolics	-ve
Flavonoids	+ve
Cardiac glycosides	+ve
Anthracene derivatives	-ve
Triterpenes	-ve
Cyanogenis glycosides	-ve

Saponins may serve as natural antibiotic as it has been shown to possess antifungal, antimicrobial, antiviral and antitumour effects [21]. In this study we did not test for the presence of oxalate and phytic acid and our result is at variance with Umaru et al. [22], who observed the presence of tannins in the fruit. Plant secondary metabolites vary among species depending on environmental factors, season and handling [22,23].Post harvest treatment such as washing, scrubbing, peeling, trimming, cutting, shredding could cause mechanical damage to plant tissues and mechanical shock which might result in cracks and bruises could elicit biochemical and physiological responses not only in the affected tissues but also in some distal unwounded tissues [24] Tissue wounding could cause the

introduction of pant secondary metabolite synthesis, including a variety of phenolic compounds and tannins are especially affected when browning sets in [25]. Thus our result might be due to the fact that we peeled off the fruit epicarp, used only the fruit mesocarp in our study and tannins might be present in other parts of the fruit as well. However it is noteworthy that in over ten fruits present in Northern Nigeria, Egyptian dates had highest concentration of saponins [26]. Cardiac glycosides are involved in wound healing [27] and the presence of this and saponins might account for the traditional use of the fruit. The absence of cyanogenic glycoside in the sample mesocarp partly makes the plant safe for consumption since cyanogenic glycosides are known to release on hydrolysis, the very poisonous cyanic acid [28]. Steroids in this fruit sample may be as a result of the occurrence of the aglycone moieties of other constituents of the plants like saponins and alkaloids. Alkaloids are basic compounds found in plants [29] while the flavonoids had been reported to inhibit bacterial and viral growth by inhibiting enzymes such as reverse transcriptase, proteases and some pathogenic protozoans [30].

CCl_4 has been widely used as experimental model for hepatic damage in laboratory animals and as an indicator of hepatoprotection for food supplements, herbal remedies and new drugs [31,32]. CCl_4 is biotransformed to give trichloromethyl-free radical and this could go on to trichloroperoxyl radical, induce lipid peroxidation and cell death [31,33]. Increased necrosis in the liver is marked by increase in leakage of enzymes into the blood flow [32,34]. Furthermore entrance of extrogenous substances such as CCl_4, crude oil, radiation elicits changes in the blood cell populations [35]. Rats administered only CCl_4 showed considerable differences in the heamatological paramters compared to those co-administered aqueous extracts of *Balanites aegyptiaca* fruits (0.08 mg/kg and the 0.19 mg/kg) mesocarp and CCl_4 as shown on Table 2.

The white blood cells count for the rats in groups 3 and 4 (i.e. the extracts treated groups) were observed to be significantly reduced ($p<0.05$) compared to group 2 (untreated CCl_4 rats). High WBC might imply presence of infections; thereby predisposing the animals to reduced immunological responses and to infections. This may indicate amongst others that the extracts may have some components, which are useful in preventing cellular inflammations / damage (30 previous). Platelet, percentage lymphocyte and neutrophil were higher on the second day of dosing and progressively reduced with increased treatment regimen in rats on fruit extract compared to CCl_4 only untreated group 2. Moreover the decrease in these parameters increased over time with administration of fruit mesocarp.

Rats administered CCl_4 only showed significant hepatic damage as observed from increased serum ezymes and reduction in serum proteins. Table 3 has result for serum marker of liver toxicity and drug clearance in the animals in this study. Alkaline phosphatase and alanine transaminase are not present in the cellular flow and are only released after tissue damage. ALP and ALT were significantly lower in the test groups 3 and 4 compared to untreated CCl_4 intoxicated group and the values compared favourably with normal control group. Biochemical observations were supplemented with histopathological examination of rat liver sections. The hispathology of liver was done, Plates 1 – 5 shows the liver tissue histographs of the various groups of experimental rats

Table 2. White blood cells and platelets count parameters of rats administered with aqueous extracts of mesocarp of *Balanites aegyptiaca* fruit following CCl_4 - induced liver damage

Parameter	Animals Grouping	2nd day	4th day	8th day	14th day	21st day
WBC (x $10^{9\mu}$)	Group	8.3 ± 0.01 ^{a}	8.4 ± 0.03 ^{a}	8.6 ± 0.04 ^{a}	8.5 ± 0.04 ^{a}	8.6 ± 0.01 ^{a}
	Group 2	25.1 ± 0.01 ^{d}	52.7 ± 0.14 ^{d}	44.2 ± 0.06 ^{d}	39.0 ± 1.06 ^{d}	37.6 ± 0.04 ^{d}
	Group 3	19.1 ± 0.32 ^{b}	19.9 ± 0.02 ^{b}	19.2 ± 0.10 ^{b}	13.0 ± 0.41 ^{b}	12.7 ± 0.01 ^{b}
	Group 4	22.5 ± 0.02 ^{c}	21.6 ± 0.06 ^{c}	22.1 ± 0.01 ^{c}	17.2 ± 0.03 ^{ca}	19.3 ± 0.32 $^{c\,b}$
Platelet (x $10^{9\mu}$)	Group	503.5 ±11.10 ^{ca}	503.0±32.26 ^{ca}	501.3±34.19 ^{ba}	505.3±41.2 ^{a}	506.0 ±15.15 ^{a}
	Group 2	361.0±3.63 ^{a}	361.0±16.52 ^{a}	488.3±16.11 ^{a}	529.0±10.92 ^{b}	513.3 ±18.11 ^{b}
	Group 3	469.0±12.47 ^{ba}	469.0±18.70 ^{ba}	472.3±11.29 ^{a}	501.3±16.27 ^{a}	496.0 ±20.20 ^{a}
	Group 4	721.3±14.94 ^{db}	721.3±20.16 $^{d\,b}$	608.7±33.60 $^{c\,a}$	593.3±13.20 ^{c}	590.0 ±13.18 ^{c}
Neutrophil (%)	Group	16.0 ± 0.56 ^{a}	16.3 ± 0.52 ^{a}	16.7 ± 0.02 ^{a}	16.7 ± 0.33 ^{a}	16.8 ± 0.02 ^{a}
	Group 2	25.3 ± 1.02 ^{b}	21.3 ± 1.00 ^{b}	20.0 ± 0.02 ^{b}	22.3 ± 0.03 ^{b}	22.4 ± 1.10 ^{b}
	Group 3	36.0 ± 1.07 ^{c}	30.7 ± 0.69 ^{db}	26.7 ± 1.06 $^{d\,a}$	29.0 ± 0.99 ^{cb}	29.6 ± 0.82 ^{cb}
	Group 4	35.7 ± 1.00 ^{c}	25.3 ± 0.71 ^{cb}	23.3 ± 0.69 $^{a\,c}$	22.3 ± 0.06 ^{ba}	22.5 ± 0.07 ^{ba}
Lymphocyte (%)	Group	79.7 ± 1.53 ^{c}	82.3 ± 2.00 ^{d}	74.7 ± 1.86 ^{b}	76.0 ± 0.86 ^{b}	75.4 ± 0.54 ^{b}
	Group 2	71.7 ± 1.53 ^{b}	75.3 ± 0.86 ^{b}	74.3 ± 0.21 ^{b}	72.7 ± 0.04 ^{a}	71.0 ± 0.72 ^{a}
	Group 3	66.0 ± 1.61 ^{a}	71.7 ± 0.05 ^{ab}	72.3 ± 0.06 ^{ab}	78.7 ± 0.93 ^{c}	79.8 ± 1.71 ^{c}
	Group 4	93.0 ± 1.01 ^{d}	78.0 ± 1.02 $^{c\,a}$	76.0 ± 0.19 $^{c\,a}$	82.3 ± 1.24 ^{cb}	86.2 ± 1.00 ^{db}

Each value is a mean ± SEM (n = 3 in a group)

Values along the same column and row with different superscripts a, b, c, d. are significantly different (p< 0.05)

Table 3. Activities of enzymes and proteins of liver of CCl_4-induced experimental rats treated with aqueous extract of the mesocarp of *Balanites aegyptiaca*

Parameter	Animals Grouping	2nd day	4th day	8th day	14th day	21st day
Alkaline phosphatase (Ul/l)	Group 1	26.43 ± 0.94[a]	25.16 ± 4.01[a]	25.62 ± 1.21[a]	25.06 ± 3.01[a]	25.09 ± 2.81[a]
	Group 2	39.61 ± 3.01[c]	40.02 ± 6.11[c]	43.96 ± 1.36[c]	42.39 ± 1.77[d]	42.80 ± 5.03[d]
	Group 3	34.22 ± 0.86[b]	32.96 ± 0.93[b]	30.06 ± 3.41[b]	28.93 ± 1.82[b]	27.09 ± 3.38[b]
	Group 4	31.17 ± 1.00[b]	30.83 ± 2.16[b]	31.02 ± 2.66[b]	33.06 ± 2.05[c]	34.91 ± 4.17[c]
Alanine transaminase (U/l)	Group 1	29.60 ± 3.02[a]	29.81 ± 2.9[a]	29.64 ± 3.16[a]	29.93 ± 1.26[a]	28.39 ± 2.00[a]
	Group 2	42.02 ± 2.98[c]	52.34 ± 3.02[c]	53.26 ± 4.01[c]	51.07 ± 4.28[d]	50.27 ± 3.62[d]
	Group 3	39.68 ± 0.91[b]	32.86 ± 0.17[b]	33.02 ± 2.14[b]	36.47 ± 3.20[b]	30.93 ± 2.94[b]
	Group 4	38.77 ± 0.83[b]	32.92 ± 1.22[b]	32.76 ±0.79[b]	35.41 ± 2.8[c]	36.65 ± 1.63[c]
Total protein (µmol/l)	Group 1	35.68 ± 2.05[a]	32.92 ± 1.76[a]	35.06 ± 2.14[a]	34.97 ± 3.6[b]	35.06 ± 4.12[c]
	Group 2	46.08 ± 3.34[d]	42.20 ± 2.66[c]	37.27 ± 2.41[b]	32.13 ± 2.33[a]	31.11 ± 1.76[a]
	Group 3	41.16 ± 0.62[b]	41.09 ± 1.94[b]	41.43 ± 1.6[c]	42.01 ± 5.14[d]	42.14 ± 2.14[d]
	Group 4	43.61 ± 1.78[c]	41.78 ± 5.02[b]	40.03 ± 4.02[c]	36.81 ± 3.16[c]	33.92 ± 4.62[b]
Albumin (µmol/l)	Group 1	28.96 ± 2.10[c]	27.98 ± 1.17[b]	28.60 ± 1.22[c]	28.60 ± 1.91[c]	28.70 ± 0.92[c]
	Group 2	24.42 ± 3.21[a]	23.14 ± 4.32[a]	22.56 ± 2.66[a]	21.13 ± 3.20[a]	20.66 ± 2.19[a]
	Group 3	25.17 ± 2.02[a]	28.61 ± 2.91[c]	29.94 ± 3.14[c]	29.13 ± 1.71[c]	31.55 ± 3.32[d]
	Group 4	26.09 ± 1.33[b]	29.03 ± 4.27[c]	26.10 ± 3.99[b]	25.11 ± 3.02[b]	22.92 ±1.09[b]

Each value is a mean ± SEM (n = 4 in a group)

Values along the same column with different superscripts a,b,c and d are significantly different (p< 0.05)

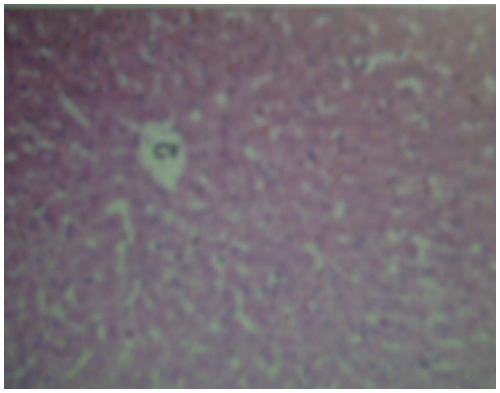

Plate 1. Histograph (x 400) of Grp 1 rat liver tissue on 21st day

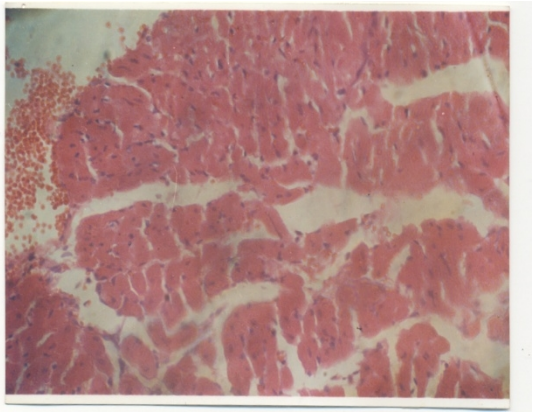

Plate 2. Histograph(x400) of Grp 3 rat liver tissue on 21st day

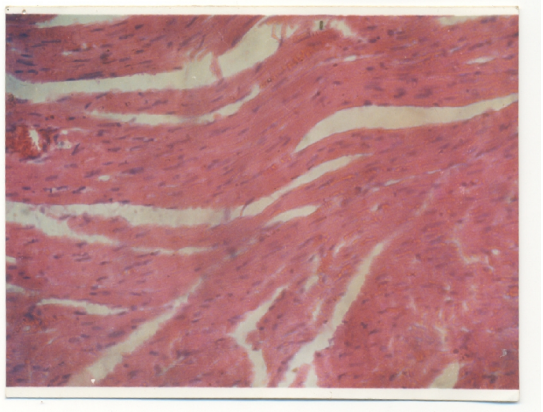

Plate 3. The histograph (x 400) of Grp 3 rat liver tissue on 8th day

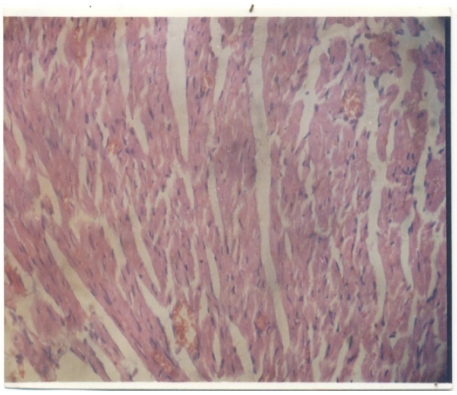

Plate 4. The histograph (x 400) of Grp 4 rat liver tissue on 8th day

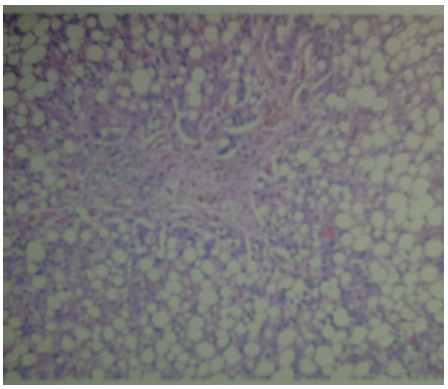

Plate 5. Histograph (x 400) of Grp 2 Rat liver after 21st day

The Group 1 i.e. the normal control animals liver cells were denser with the nuclei more prominent and the blood vessels were seen. This indicates an apparent normal liver cell while the cells in the Group 2 (Plates 2 and 5) i.e. the CCl_4 induced and not treated rats liver cells were scattered and the liver architecture were much distorted and the nuclei were rarely visible, this is observable in Plates 2 and 5. But with the rats in the group 3 i.e. the CCl_4 induced and raw extract treated animals, the liver cells were seen slightly at the end of the 14th day treatment and it closely resembling that of the control but the nuclei staining were not so prominent as in the normal control animals as could be observed in Plate 3. However, the group 4 i.e. the CCl_4 induced and treated with the concentrated extract had their nuclei prominently shown but slightly enlarged while the cell architecture were seen distorted (Plate 4). Thus, the moderate infiltration of the pericentral, sinusoids and periportal areas by lymphocytes suggest vascular disorders of the liver in the CCl_4 - induced rats [36]

4. CONCLUSION

It can therefore be concluded from the results of this study which indicated that *Balanites aegyptiaca* may be relatively safe when consumed at moderate concentrations and the fruit mesocarp can be used for short term management of liver disorder as it has shown hepatoprotective properties in CCl_4 induced rats.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors declare that no competing interests exist.

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