African Journal of Pharmaceutical Research & Development

Vol. 8 No. 2; pp. 93-99 (2016)

EVALUATION OF ACUTE AND CHRONIC TOXICITIES OF AQUEOUS STEM BARK EXTRACT OF *BALANITES AEGYPTIACA* L DELILE

Ugwah OM[1*], Ugwah-Oguejiofor CJ[2], Etuk EU[1], Bello SO[1], Aliero AA[3]

[1]Department of Pharmacology & Therapeutics, College of Health Sciences, Usmanu Danfodiyo University P.M.B. 2346, Sokoto, Nigeria
[2]Department of Pharmacology & Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University P.M.B. 2346, Sokoto, Nigeria
[3]Department of Botany, Faculty of Biological Sciences, Usmanu Danfodiyo University P.M.B. 2346, Sokoto, Nigeria

ABSTRACT

The stem bark of *Balanites aegyptiaca* is used in traditional medicine in the North-western Nigeria for the treatment of peptic ulcer and other diseases. Previous studies showed the antidiabetic and anti-inflammatory properties of the plant. The present study was to analyse the phytochemical constituents and evaluate the acute and chronic toxicity profile of the aqueous stem bark extract of *Balanites aegyptiaca* in albino rats. Phytochemical analysis was performed following standard procedures. The Up and Down method of acute toxicity testing was used to assess the LD_{50} of the plant at an oral limit dose of 3000 mg/kg in female rats. Chronic toxicity study was assessed using albino rats of both sexes housed separately (n=8) at oral doses of 750, 1500 and 2250 mg/kg of *Balanites aegyptiaca* for 12 weeks. Weekly weights were recorded. At the end of the 12 weeks the rats were anaesthetised and blood samples for haematological and biochemical studies collected. Their small intestine, stomach, heart, kidney and liver were excised for histological examination. Phytochemical analysis of the extract revealed the presence of tannins, saponins, alkaloids flavonoids, steroids and glycosides. It showed no mortality and no observed symptoms of toxicity at the limit dose of 3000 mg/kg. This suggests that the LD_{50} of *Balanites aegyptiaca* is greater than 3000 mg/kg. In the chronic study, the extract treated groups, had no significant weight gain compared with control and no behavioural effects. Liver enzymes, AST and ALT, were significantly increased ($p<0.01$) at doses of 1500 and 2250 mg/kg. Urea level significantly increased ($p<0.05$) at the dose of 750 mg/kg compared with control. Histopathological studies revealed evidence of microscopic lesions in the liver which correlated with biochemical disturbances. Aqueous stem bark extract of *Balanites aegyptiaca* may be safe but could have toxic effects in the liver on prolong use.

KEYWORDS: *Balanites aegyptiaca, Toxicity, Liver enzymes, Urea, Haematological studies*

INTRODUCTION

Traditional medicines are widely utilised in our world today for the treatment of various ailments. About eighty percent of the world's population depend on traditional medicine for health sustenance [1, 2]. Many of these medicines have been scientifically validated to possess such biological activities and can be used to treat various ailments. Several studies have documented the toxic effects of some of these medicinal plants [3-5]. Nevertheless, some of them still lack available evidence of their toxicity profile and how safe that medicinal plants may be to the consumers.

Balanites aegyptiaca L. Delile (Zygophyllaceae) is a medicinal plant tree used in North West Nigeria. It is commonly known as the Desert date, 'Aduwa' in hausa, 'enyi-ndi- mmuo' in Igbo, and 'Tanni' in Fulani. It is highly adapted to the drier parts of Africa and south Asia and distributed

***Corresponding author:** mikeugwah@yahoo.com; +234 8035978754

in most adverse arid desert environments [6,7]. Balanites is a multipurpose tree known by its many uses as fuel wood, charcoal, timber and fodder. The fruits are edible and the seeds are crushed to produce oil. The tree grows naturally up to a height of 6–8 m. The first fruiting is at 5–6 years, the tree lives for more than 100 years, for 75 years annually producing crop of 125 kg of fruit [8]. Extensive literature survey revealed that 'desert date' has a long history of traditional uses for wide range of diseases. It has been experimentally proven that *B. aegyptiaca* Del possess antioxidant, antimicrobial, anticancer, diuretic, hypocholesterolemic, wound-healing, antiviral, antidiabetic, hepatoprotective, mosquito larvicidal, anti-inflammatory and analgesic, antivenin, anthelmintic, cardioprotective and antinociceptive properties [9]. Bark, fruits, seeds, seed oil, and leaves of this plant are widely used in folk medicine [10]. Despite the wide use of this plant, adequate safety information is lacking. Therefore, acute and chronic toxicity studies were evaluated in rats in this study.

MATERIALS AND METHODS

Preparation of plants extract

The stem bark of *Balanites aegyptiaca* was collected in December from Wammakko Local Government area, Sokoto state. The plant material was identified and authenticated by Alhaji Umar Moh'd of the Department of Botany, Usmanu Danfodiyo University, Sokoto. Specimen voucher of the plant (004B) was deposited at the herbarium. The stem bark of the plant was air dried to a constant weight, pulverised to a dry powder and was extracted in distilled water using soxhlet apparatus. The extract was evaporated to dryness in a hot air oven at 45^{o}C.

Animals

Adult albino rats of both sexes weighing 150–180g bred in Mike Ugwah animal house were used for the study. The animals were housed in separate metallic cages of female and male animals and allowed free access to standard feed and water. They were allowed to acclimatize for two weeks before the commencement of the study. Animal experiments were performed according to the National Institute of Health Guide for Care and Use of Laboratory Animals guidelines (Pub No. 85 – 23, revised 1985). The study was approved by the Animal Research Ethical Committee, Usmanu Danfodiyo University, Sokoto.

Methods

Phytochemical analysis

Phytochemical screening of stem bark extracts of *B. aegyptiaca* was conducted to determine the presence or absence of alkaloids, tannins, flavonoid, anthraquinones, saponins, carbohydrates, steroids, and glycosides following standard methods [11, 12].

Acute toxicity test

Acute toxicity study was performed as described in "Guideline for Testing of Chemicals - Acute Oral Toxicity – Up and Down procedure." [13]. The Up and down procedure was adopted to evaluate the acute toxicity of the extract after oral administration in the rats. Five adult, female, non-pregnant rats were randomly selected for this experiment. They were fasted overnight, but allowed free access to water before the administration of a freshly prepared extract at a single oral dose of 3000 mg/kg. After the administration, the animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. The animals were observed for signs of toxicity or death. Similar dose level was administered to the next rat in each group after 48h if no death occurs and similar observations made. Food and water were provided throughout the experiment. The procedure continued till the five animals in each group were dosed and observed. The toxicological effects were assessed on the basis of mortality which was expressed as Median Lethal dose (LD_{50}) and calculated using up and down procedure [13].

Chronic toxicity studies

Treatment of animals

A total of 32 white albino rats were used for the chronic toxicity studies. The rats were randomly divided into four groups with 8 rats each (4 females and 4 males) The males and females in each group were kept in different cages.

Group 1: Received low dose 750 mg/kg (25% of LD_{50}) body weight orally for 12weeks.

Group 2: Received medium dose 1500 mg /kg (50% of LD_{50}) body weight orally for 12weeks

Group 3: Received high dose 2250 mg/kg (75% of LD_{50}) body weight orally for 12weeks

Group 4: Receive 10 ml/kg of distilled water orally for 12 weeks.

The rats were weighed at the beginning, and then subsequently weekly for the period of treatment. After 24 hours of the last dose, the animals were weighed, and anaesthetized with

chloroform. Blood samples were collected by cardiac puncture from all the animals. The blood sample was collected in EDTA bottles for Haematological analysis and plain sample bottles for biochemical analysis following standard procedures.

Histological Analysis

Fresh stomach, heart, kidney and liver tissue samples were excised, weighed and washed with saline and preserved in Bouin's solution for histopathological studies. The sections of stomach, heart, kidney and liver were stained with haematoxylin and eosin and were assessed for histopathological changes such as congestion, oedema, haemorrhage and necrosis [14].

Statistical Analysis

The results were expressed as Mean ± SD. Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons. The significance of difference was accepted at $p < 0.05$.

RESULTS

Phytochemical Analysis

The result showed the presence of alkaloid, tannins, saponins, glycosides, carbohydrates and steroids in varying degrees (Table 1).

Table 1: Phytochemical Analysis of aqueous stem bark extract of *Balanites aegyptiaca*

Phytochemical Constituents	Relative presence
Alkaloids	++
Tannins	++
Flavonoids	++
Anthraquinone	–
Saponins	+++
Carbohydrate	+
Steroids	++
Glycosides	+

+ (trace), ++ (moderate), +++ (high), - (absent)

Acute Toxicity LD_{50}

At a limit dose of 3000 mg/kg, all the rats in the short and long term observations survived. There was no mortality; however, there were signs of drowsiness in all the rats for about 1 to 2 hours.

Chronic Toxicity Studies of aqueous stem bark extract of *Balanites aegyptiaca*

Effect of *Balanites aegyptiaca* on Weight of Rats Following Weekly Dosing

There was a gradual increase in the weight of the rats over time in both the *B. aegyptiaca* treated groups and the control group (Table 2). However, there was no significant difference ($p>0.05$) between the net weight gained by extract treated rats and the control (Figure 1).

Table 2: Effect of *Balanites aegyptiaca* on Weight of Rats Following Weekly Dosing

Treatment		*B. aegyptiaca*			Distilled water
Dose mg/kg		750	1500	2250	10 ml/kg
Mean ± SD Weekly Weight (g)	1	179.29 ± 9.34	171.33 ± 6.15	188.35 ± 5.52	183.01 ± 7.15
	2	184.86 ± 12.96	179.85 ± 10.57	191.95 ± 9.71	188.36 ± 9.15
	3	190.43 ± 14.15	190.30 ± 17.67	206.73 ± 21.04	194.13 ± 18.15
	4	196.06 ± 19.94	194.47 ± 17.01	212.85 ± 27.72	197.6 ± 18.91
	5	201.56 ± 18.93	202.34 ± 20.66	223.81 ± 30.53	204.41 ± 21.21
	6	206.94 ± 23.22	209.04 ± 24.24	231.085 ± 31.42	214.88 ± 21.91
	7	212.41 ± 23.74	211.64 ± 24.97	235.97 ± 30.92	221.24 ± 22.4
	8	223.08 ± 26.11	221.19 ± 24.95	244.14 ± 30.08	228.47 ± 25.03
	9	231.07 ± 30.60	229.67 ± 30.62	250.74 ± 31.61	238.78 ± 29.42
	10	239.35 ± 30.77	236.02 ± 34.53	255.16 ± 32.30	247.06 ± 29.99
	11	248.01 ± 35.74	241.62 ± 35.85	260.00 ± 31.03	250.69 ± 30.57
	12	254.55 ± 37.04	246.37 ± 35.20	263.39 ± 30.11	258.39 ± 34.22

Results expressed as mean ± SD, n=8, statistical analyses were performed using ANOVA followed by Dunnett's test. No significant difference ($p>0.05$) between *Balanites aegyptiaca* treated groups compared with the control.

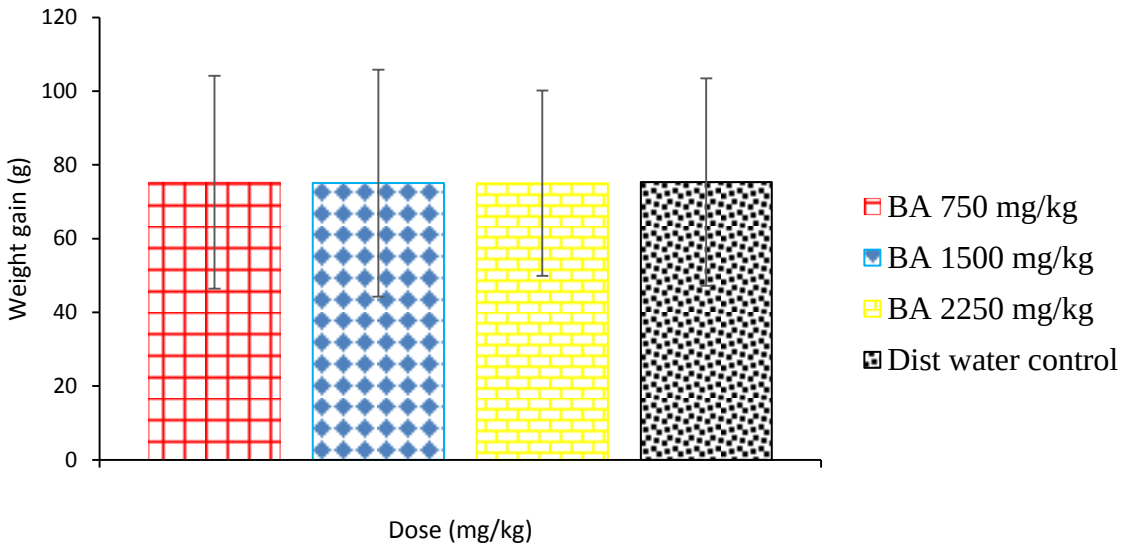

Figure 1: Effect of *Balanites aegyptiaca* on Net Weight Gain After 12 weeks Dosing.
Results expressed as mean ± SD, n=8, statistical analyses were performed using ANOVA followed by Dunnett's test. No significant difference ($p > 0.05$) between *Balanites aegyptiaca* treated groups compared to the control.

Table 3: Effect *Balanites aegyptiaca* on Biochemical Parameters in Rat

Electrolyte/Enzyme/molecule	750 mg/kg	1500 mg/kg	2250 mg/kg	Distilled water 10 ml/kg
Na^+	135.33 ± 2.48	133.00 ± 2.08	133.85 ± 2.40	135.43 ± 1.77
K^+	5.00 ± 0.25	4.67 ± 0.22	5.16 ± 0.17	5.49 ± 0.34
Cl^-	95.33 ± 2.48	93.29 ± 2.10	93.85 ± 2.40	95.43 ± 1.77
HCO_3^-	24.83 ± 0.6	25.71 ± 0.56	25.57 ± 0.37	24.71 ± .97
Urea	7.63 ± 0.27*	6.69 ± 0.47	5.25 ± .22	5.89 ± 0.59
Creatinine	0.43 ± 0. 07	0.51 ± 0.09	0.49 ± 0.07	0.6 ± 0.11
Albumin	2.66 ± 0.07	2.66 ± 0.05	2.45 ± 0.05	2.57 ± 0.12
AST	55.85 ± 1.67	93.07 ± 11.66**	105.79 ± 12.41**	40.47 ± 1.75
ALT	131.48 ± 4.02**	145.60 ± 3.20**	212.37 ± 13.57**	51.14 ± 9.75
CHO	137.03 ± 5.84	146.77 ± 6.38	125.57 ± 5.62	132.76 ± 16.23
ALP	184.95 ± 44.3	155.40 ± 37.63	167.93 ± 30.68	80.40 ± 17.39
T-Bilirubin	3.51 ± 0.59	8.09 ± 0.70**	16.49 ± 2.12**	1.06 ± .30
Conj Bilirubin	0.88 ± 0.21	3.03 ± 0.28	7.52 ± 1.50**	0.27 ± 0.04
UnConj Bilirubin	2.63 ± 0.54	5.06 ± 0.67*	8.12 ± 1.96**	0.84 ± 0.27

Results expressed as mean ± SEM, n=8, statistical analyses were performed using ANOVA followed by Dunnett's test. Significant at *$p<0.05$ compared with control, highly significant at **$p<0.001$, when compared with the control.

Table 4: Effect of *Balanites aegyptiaca* on haematological parameter

Blood Parameters	750 mg/kg	1500 mg/kg	2250 mg/kg	Distilled water 10 ml/kg
RBC x 10^{12}/L	6.34± 2.45	6.93 ± 0.54	6.91 ± 0.54	6.78 ± 1.06
PCV %	32.86 ± 9.96	35.29 ± 2.06	35.71 ± .138	34.29 ± 5.20
WBC X 10^6/L	7.72 ± 3.59	6.79 ± 1.91	6.80 ± 2.64	7.76 ± 4.23
Hb g/DL	11.07 ± 3.53	12.23 ± 0.90	12.43 ± 0.65	11.76 ± 0.63
Platelet X 10^6/L	357.86±174.27	406 ± 61.55	364.29 ± 56.76	303.57 ± 33.28

Results expressed as mean ± SEM, n=8, statistical analyses were performed using ANOVA followed by Dunnett's test. No significant difference when compared with the control. WBC= White blood cell, PCV= Pack cell volume. RBC= Red Blood cell Hb= Haemoglobin.

Effect of *Balanites aegyptiaca* on biochemical parameters in rat

The results of the oral administration of *Balanites aegyptiaca* in various doses of 750, 1500 and 2250 mg/kg for 12 weeks on different biochemical parameters in rats are presented in Table 3. There was a significant ($p<0.05$) elevation of urea in the *Balanites aegyptiaca* treated rats at 750 mg/kg. Significant differences were also noted in the value of the liver enzymes, aspartate aminotransferase (AST) ($p< 0.001$) at the doses of 1500 and 2250 mg/kg and Alanine aminotransferase (ALT) ($p<0.001$) at all doses. Total bilirubin ($p< 0.001$) at 1500 and 2250 mg/kg, conjugated bilirubin ($p<0.001$) at dose of 2250 mg/kg, unconjugated ($p<0.05$) at 1500 mg/kg ($p< 0.001$) and at 2250 mg/kg in the *B. aegyptiaca* treated were all elevated significantly (Table 3).

Effect of *Balanites aegyptiaca* on Haematological Parameters in Rat

The results of the oral administration of *Balanites aegyptiaca* at various doses of 750, 1500 and 2250 mg/kg for 12 weeks on different haematological parameters in rats are presented in Table 4. There was no significant haematological parameter effect between *Balanites aegyptiaca* treated groups and control. However, there was increase in the value of RBC, Hb, PVC and platelets in rats treated with 1500 mg and 2250 mg/kg of *Balanites aegyptiaca*. The RBC and platelets were highest in rats treated with 1500 mg/kg of *B aegyptiaca* while WBC decreased as the doses increased (Table 4).

Effect of *Balanites aegyptiaca* on White Blood Cell Differentials

There was dose dependent increase in the values of Neutrophil% and Eosinophil% in rats treated with *B. aegyptiaca*. The values of Monocyte % and Basophil % are zero for all the rats treated with *B. aegyptiaca.* These are shown in Table 5

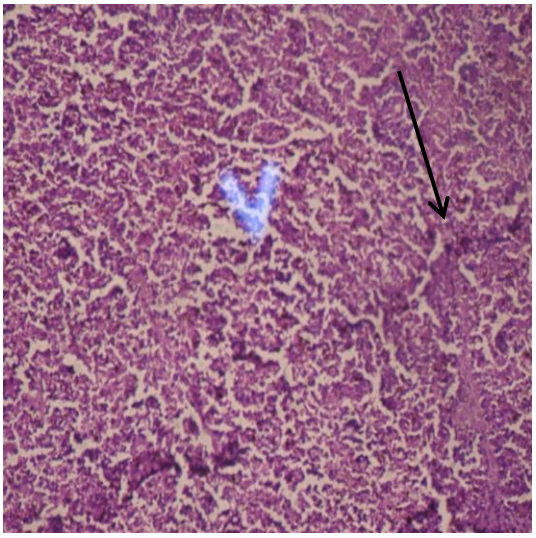

Figure 2: Photomicrograph of Liver treated with 1500 mg/kg *Balanites aegyptiaca* showing mild distortion of Hepatic Architecture X 200

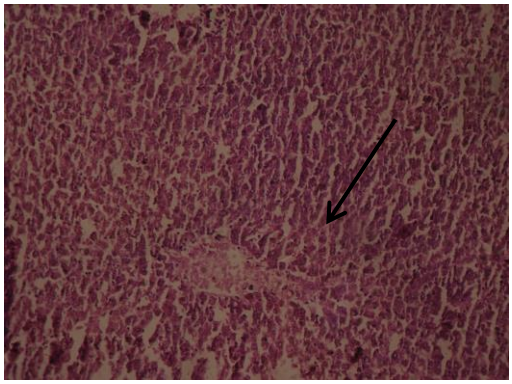

Figure 3: Photomicrograph of Liver treated with 2250 mg/kg *Balanites aegyptiaca* showing complete distortion of Hepatic Architecture X 200

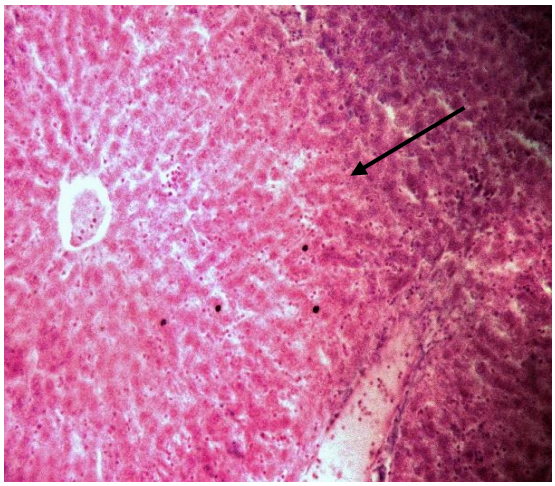

Figure 4: Photomicrograph of the Control Liver showing Normal Portal Triad surrounded by Normal Hepatocyte X 200

Effect of *Balanites aegyptiaca* on Histology of various organs

There was no significant effect of *Balanites aegyptiaca* on histology of the Heart, Kidney, Intestine, and Stomach (Photomicrograph not shown). However, there was moderate distortion of hepatic architecture at 1500 mg/kg (Figure 2) and complete distortion of hepatic architecture at 2250 mg/kg (Figure 3) as compared to the control (Figure 4).

DISCUSSION

The stem bark extract of *Balanites aegyptiaca* is used traditionally in the Northwest Nigeria for the treatment of many ailments. In this study the toxicological profile was assessed using the acute and chronic toxicity study methods.

The oral acute toxicity test of the stem bark extract of *B. aegyptiaca* carried out in female albino rats at a single dose of 3000 mg/kg body weight showed no major toxic effects. However, drowsiness was observed in all the animals for the first two hours. This suggests that the extract of *B. aegyptiaca* may be relatively safe with LD_{50} greater than 3000 mg/kg. This is because any compound or pharmaceutical with an oral LD_{50} greater than 1000 mg/kg is considered safe or to be of low toxicity [13, 15, 16].

Again, the stem bark extract of *B. aegyptiaca* is used for the treatment of many ailments some of which are chronic disorders hence the need to ascertain the effect of the extract on chronic administration. A chronic toxicity study was therefore carried out at the doses of 750, 1500 and 2250 mg/kg body weight. After 12 weeks of treatment, there were no obvious changes in the animals on gross observation. Body weight increase or decrease is associated with the toxicity of compounds [17].

Table 5: Effect of *Balanites aegyptiaca* on White Blood Cell Differentials

WBC Differential (%)	750mg/kg	1500 mg/kg	2250 mg/kg	Control
Lymphocytes	81.43 ± 8.46	63.43 ± 31.89	58.29 ± 35.95	71 ± 27.78
Neutrophil	15.43 ± 8.06	35.14 ± 32.02	40.29 ± 35.77	25 ± 27.84
Monocyte	0.00 ± 0.00	0± 0.00	0 ±.00	0.86 ± 1.57
Eosinophil	3.14 ± 3.02	1.42 ± 0.98	1.43 ± 1.51	2.86 ± 2.27
Basophil	0.00 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

Results expressed as mean ± SEM, n=8, statistical analyses were performed using ANOVA followed by Dunnett's test. No significant difference when compared with the control. WBC= White blood cell.

There was also no difference in changes in weight of the animals within the period. This suggests that the extract did not adversely affect growth in the rats. Estimation of the serum biochemical parameters in the animals showed a significant elevation ($p<0.001$) in the transaminases enzymes AST and ALT at doses of 1500 and 2250 mg/kg, increase in urea (750 mg/kg) and conjugated and unconjugated bilirubin at higher doses. The use of several screening tests improves the detection of hepato-biliary abnormalities, helps differentiate the basis for clinically suspected disease and determine the severity of liver disease [18]. It is a well-known fact that any damage to the parenchymal liver cells results in elevations of AST and ALT in the blood [19]. ALT and AST are measures of liver homeostasis and are markers of hepatocellular injury [20]. They are indications of the concentration of hepatic intracellular enzymes that have leaked into the circulation. They aid in recognizing acute hepatocellular diseases such as hepatitis. From the result obtained, the extract of *B. aegyptiaca* may be toxic to the liver. This was further confirmed by histopathological examination which showed

moderate to complete distortion of hepatocytes in the treated groups. This result differed from an earlier observation documented by Mohammed *et al.,* 1999 in which the total bilirubin was significantly dose dependently reduced by aqueous bark extract of *Balanites aegyptiaca* and was proposed as hepatoprotective [21]. The dose and duration administration may account for this disparity. Effect of the extract of *B. aegyptiaca* on haematological parameters were evaluated. There was no significant difference in the parameters when compared to the control. In the histological views of the hearts, intestines, stomachs and kidneys, there were no changes observed. The toxicity observed in the liver could be because of some phytochemical constituents that may be present in the plant.

CONCLUSION

This study provides valuable data on the acute and chronic oral toxicity profiles of the stem bark extract of *B. aegyptiaca* that could be very useful in its future clinical study. The acute toxicity study suggests that the extract could be safe while the chronic toxicity studies indicate that the plant extract may be hepatotoxic on repeated administration. Further studies such as teratogenic, mutagenic and carcinogenic studies are needed to complete the safety profile of this plant.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist

REFERENCES

1. Lin J, Puckree T, Mvelase TP. Anti-diarrhoeal evaluation of some medicinal plants used by Zulu traditional healers. J Ethnopharmacol, 79: 2002; 53–56.

2. Akah PA. Indigenous Knowledge and Medical Practice, In: Akah PA Ed. Ethnopharmacology, Research Signpost, Kerela, India, 2008, 1-13.

3. Yuet Ping K, Darah I, Chen Y, Sreeramanan S, Sasidharan S. Acute and subchronic toxicity study of Euphorbia hirta L. methanol extract in rats. BioMed research international, 2013: 2013.

4. Kifayatullah M, Mustafa MS, Sengupta P, Sarker MM, Das A, Das SK. Evaluation of the acute and sub-acute toxicity of the ethanolic extract of Pericampylus glaucus (Lam.) Merr. in BALB/c mice. J Acute Disease, 4(4): 2015; 309-315.

5. Olumese FE, Onoagbe IO, Eze GI, Omoruyi FO. Safety assessment of Uvaria chamae root extract: acute and subchronic toxicity studies. J Afr Assoc Physiol Sci, 4(1): 2016; 53-60.

6. Sagna MB, Niang KS, Guisse A, Goffner D. *Balanites aegyptiaca* (L.) Delile: geographical distribution and ethnobotanical knowledge by local populations in the Ferlo (north Senegal)/*Balanites aegyptiaca* (L.) Delile: distribution géographique et connaissances ethnobotaniques des populations locales du Ferlo (nord Sénégal). Biotechnologie, Agronomie, Société et Environnement, 18(4); 2014:503.

7. Ibrahim EE. Phytochemical, antioxidant activity and cytotoxicity of methanolic extract of *Balanites aegyptiaca* (L.) DELILE. Pharmacie Globale, 7(1); 2016:1.

8. Abdel-Rahim EA, El-Saadany SS, Wasif MM. Chemical and physical studies on Balanites aegyptiaca oil. Grasas y aceites, 37(2);1986: 81-85

9. Mohamed AH, Eltahir KE, Ali MB, Galal M, Ayeed IA, Adam SI, Hamid OA. Some pharmacological and toxicological studies on Balanites aegyptiaca bark. Phytotherapy research: PTR, 13(5): 1999:439-41.

10. Chothani DL, Vaghasiya HU. A review on Balanites aegyptiaca Del (desert date): phytochemical constituents, traditional uses, and pharmacological activity. Pharmacognosy reviews. 5(9); 2011:55.

11. Harborne JBC. Phytochemical methods. Chapman and Hall, London, 1973; 279.

12. Trease GE, Evans WC. Drugs of Biological origin. In: Pharmacognosy, 12th ed., Balliere Tindall, United Kingdom, 1983; 309-540.

13. Organization for Economic Development. Guideline for testing of chemicals. Guideance no. 425. Up and Down procedure, Adopted: 17th December 2001.

14. Shah AH, Khan ZA. Gastroprotective effects of pretreatment with *Ziziphus sativa* fruits against toxic damage in rats. Fitoterapia. 3; 1997: 226–234.

15. Clarke EGC, Clarke ML. Veterinary Toxicology, Cassel and Collier Macmillan Publishers, London, 1977; 268-277.

16. Ibrahim H, Williams FE, Salawu KM, Usman AM. Phytochemical screening and acute toxicity studies of crude ethanolic extract and flavonoid fraction of Carissa edulis leaves. Biokemistri. 27(1); 2016:39-43.

17. Sireeratawong S, Lertprasertsuke N, Srisawat U, Thuppia A, Ngamjariyawat A, Suwanlikhid N, Jaijoy K. Acute and subchronic toxicity study of the water extract from Tiliacora triandra (Colebr.) Diels in rats. Sonklanakarin Journal of Science and Technology. 30(5); 2008:611.

18. Al-Jumaily EF. The effect of chronic liver diseases on some biochemical parameters in

patients serum. Current Research Journal of Biological Sciences. 4(5); 2012:638-642.
19. Anderson N, Borlak J. Molecular mechanisms and therapeutic targets in steatosis and steatohepatitis. Pharmacological reviews. 60(3); 2008:311-357.
20. Mukhopadhyay P, Horváth B, Zsengellér Z, Bátkai S, Cao Z, Kechrid M, Holovac E, Erdélyi K, Tanchian G, Liaudet L, Stillman IE. Mitochondrial reactive oxygen species generation triggers inflammatory response and tissue injury associated with hepatic ischemia–reperfusion: therapeutic potential of mitochondrially targeted antioxidants. Free Radical Biology and Medicine. 53(5); 2012:1123-1138.
21. Mohamed AH, Eltahir KE, Ali MB, Galal M, Ayeed IA, Adam SI, Hamid OA. Some pharmacological and toxicological studies on Balanites aegyptiaca bark. Phytotherapy research: PTR. 3(5); 1999:439-441.