



Toxicity of *Balanites aegyptiaca* Seeds Oil in Wistar Albino Rats

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Article History: Received: 15/10/2016

Accepted: 08/01/2017

Abstract

(L.), a multipurpose tree, is used widely in the Sudan for its *Balanites aegyptiaca* nutritional, cosmetic, industrial and medicinal values. The seeds oil has been used by natives to treat rheumatism and as edible oil. The present study was designed to investigate the toxicological status of *B. aegyptiaca* seeds petroleum ether extract in Wistar albino rats in acute and sub-chronic toxicity models. In acute toxicity model, rats were orally administered *B. aegyptiaca* seeds petroleum ether extract at a dose of 2, 4, 8 and 20 ml/kg and observed for signs of toxicity and mortality for 24 hours. In sub-chronic model, rats were given *B. aegyptiaca* seeds oil at a dose of 4 and 8 ml/kg orally for 21 days. Haematological parameters, liver and kidney function tests were taken before and after the administration of each treatment. Histopathology was also performed for different organs. The results indicated that no mortality was seen after administration of seeds oil up to 20 ml/kg in acute toxicity model. On the other hand, oral administration of *B. aegyptiaca* seeds petroleum ether extract for 21 days did not prove any signs of toxicity or organs damage as evidenced by insignificant ($p > 0.05$) change in haematological parameters, serum enzymes activities of ALT, AST, ALP, and urea and creatinine as well as total protein level in treated groups as compared with control group. Furthermore, sections of different organs of treated animals showed minor insignificant histopathological changes, with exception of two kidney sections which showed focal degenerative changes in rats given high dose of extract. In conclusion, the results of the present study indicated that the petroleum ether extract of *B. aegyptiaca* seeds is safe when administered orally in rats in acute and sub-chronic toxicity models.

Keywords: *B. aegyptiaca*, seed, oil, enzymes.

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Introduction

Balanites egyptiaca L. (Hegleeg) is a member of the family *Balanitaceae*. It is a multi-branched, spiny shrub or tree which grows up to 10 m in height (Chothani and Vaghasiya, 2011). The tree is distributed in the dry land areas of Africa, Middle East and South Asia (Chand *et al.*, 2012). The wide range of habitat in which this species is found suggests high pattern of variation among and within locations (Elfeel, 2010). The tree has been extensively used worldwide in folk medicine for various ethno-botanical purposes (Ajayi and Folorunso, 2013).

From ancient times many parts of the plant are used as famine foods in Africa, the leaves are eaten raw or cooked, the oily seed is boiled to make it less bitter and eaten mixed with sorghum (Wufem, 2007). Young leaves and fruits are eaten by all livestock and wildlife. The mesocarp of the fruit is a source of fermentation products (e.g. ethanol) and steroidal saponins. The seed kernel is rich in oil, protein, minerals and is edible as snacks after boiling (Elfeel, 2010). The crude *Balanites* oil is a source of edible vegetable oil used in various industries such as soap production. The kernel cake, after extraction of oil, is a source of protein and carbohydrate for livestock (Maydell, 1986). In Sudanese folk medicine the mesocarp of fruit is used to treat jaundice (Chothani and Vaghasiya, 2011). The fruits are also used in Egyptian folk medicine as an oral hypoglycemic (Kamel, 1998). In Nigeria, the seed oil of *B. aegyptiaca* is used for frying food and adding flavour to the food

and tea. It is also used to treat skin diseases and rheumatism (Obidah *et al.*, 2009).

A number of other effects were demonstrated for the plant as hepatoprotective (Jaiprakash *et al.*, 2003), anthelmintic (Koko *et al.*, 2000 and Anto *et al.*, 2005), anti-inflammatory and analgesic (Gaur *et al.*, 2008) antiviral and antibacterial (Doughari *et al.*, 2007 and Al Ashaal *et al.*, 2010), antioxidant (Meda *et al.*, 2010), anti-diabetic (Mansour and Newairy, 2000) and anticancer activities (Al-Ghannam *et al.*, 2013).

The different parts of the plant contain many constituents (Chothani and Vaghasiya, 2011), and the most important constituent is steroidal saponins which yield diosgenin, a source of steroidal drugs such as corticosteroids, contraceptives and sex hormones (Elfeel, 2010).

Despite the extensive use of oil for various purposes in folk medicine, there are inadequate scientific reports on the possible effects of abundant consumption and extended utilization of the seeds oil. Therefore, this study was conducted to evaluate the acute and sub-chronic toxicities of *B. egyptiaca* petroleum ether extract in albino rats.

Materials and Methods

Plant materials: *B. egyptiaca* fruits were obtained from local market in Omdurman, Sudan. The fruits were then identified and authenticated by the botanists in Medicinal and Aromatic Plants Research Institute (MAPRI),

National Centre of Research (NCR),
Khartoum, Sudan.

Preparation of the extract: The fruits of *B. aegyptiaca* were broken manually and the seeds collected from and powdered. 200 gm of *B. aegyptiaca* seeds were extracted with petroleum ether (40 – 60) °C using soxhlet apparatus. The solvent was then collected and evaporated under reduced pressure using rotary evaporator apparatus. The petroleum ether extract yielded pale yellow colour oil which was stored in dark bottles at room temperature till used (Harborne, 1984).

Experimental animals: Adult albino rats of either sex were obtained from Medicinal and Aromatic Plants Research Institute (MAPRI), National Centre for Research (NCR), Khartoum, Sudan. The animals were kept in polypropylene cages in the laboratory animal house in the College of Veterinary Medicine, Sudan University of Science and Technology. The animals were adapted for 7 days under standard environmental conditions (relative humidity 40 - 60% , controlled temperature 24±2°C, air exchange 12 -15 changes per hour and 12h light and dark cycle) and fed with diet containing flour 70%, meat 18%, edible vegetable oil 10%, sodium chloride 1.5% and vitamins and minerals 0.5% and water *ad libitum*.

Ethical approval: This study was approved by the Scientific Committee of the College of Veterinary Medicine, Sudan University of Science and Technology in accordance with good clinical practice and international guidelines for animal use in experimentations.

Acute toxicity study: The acute toxicity study was achieved in accordance to Organization for Economic Co-operation and Development (OECD/OCDE) guidelines (OECD, 2001).

Twenty albino rats of either sex weighing between 100 – 120 g were divided randomly into 4 groups of 5 rats each. *B. aegyptiaca* seeds petroleum ether extract was administered orally to test groups at a dose rate of 2, 4, 8, 20 ml/kg respectively and mortality was observed for 24h.

Sub-chronic toxicity: Eighteen adult Wister albino rats of either sex, (97 – 150 g) were used; the animals were randomly divided into 3 groups of 6 rats each.

Group 1: Control; animals kept for 21 days without any treatment.

Group 2: Rats were administered orally with petroleum ether extract of *B. aegyptiaca* seeds at a dose of 4ml/Kg/day for 21 days.

Group 3: Rats were given petroleum ether extract of *B. aegyptiaca* seeds at a dose of 8ml/Kg/day for 21 days.

Rats were observed daily for any signs of toxicity and mortality.

Blood samples: Blood samples were obtained before and after administration of plant extract (day0 and day21) for various haematological and biochemical studies. Blood drops were collected gently by puncturing retro-orbital plexus under anaesthesia using capillary tubes for various haematological and biochemical parameters. EDTA was used as an anticoagulant in blood samples for haematological studies and analyzed

immediately while serum was stored at -20°C until analyzed.

Haematological studies: Haemoglobin concentration (Hb), packed cell volume (PCV), red blood cells count (RBC), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC), were measured using Sysmex – KX-21N, Spain.

Biochemical analysis: Biochemical parameters, i.e. alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total proteins, creatinine and urea, were analyzed by Mindray automatic analyzer for biochemistry (Mindray – BS-200 – China) using diagnostic kits (ACCENT-200, PZ COMPANY S.A. POLAND; SPINREACT, S.A./S.A.U. Ctra; Sata Coloma, SPAIN).

Histopathological studies: At the end of the study, tissue specimens from liver, lung, heart, kidney, intestine, stomach and spleen were collected immediately at necropsy, fixed in 10% formalin and processed for histopathology (Drury and Willington, 1967).

Statistical analysis: Data were expressed as the mean \pm SEM. Differences between experimental groups were compared by one way analysis of variance (ANOVA). The results were considered statistically significant when $P < 0.05$ (Gomez and Gomez, 1984).

Results

Extraction of 200 grams of *B. aegyptiaca* seeds by petroleum ether yielded 79.7 gm of oil and the yield percentage was 39.8%.

Acute toxicity:

Petroleum ether extract of *B. aegyptiaca* seeds given to rats was found safe at all doses (2, 4, 8 and 20 ml/kg). No mortality was observed in the treated animals even those received the higher dose of petroleum ether extract up to 20 ml/kg. However, anorexia was noticed in rats received higher doses of the oil.

Sub-chronic toxicity:

There were no obvious clinical signs or mortality observed in the control and treated groups during the course of experiment except the signs of anorexia in the animals received high dose of oil (8ml/kg).

Haematological findings:

There were no significant differences ($P > 0.05$) in haematological parameters between control and rats treated with 4ml/kg of *B. aegyptiaca* seeds petroleum ether extract at day 0 and day 21. Rats receiving 8 ml/kg of the extract had significantly showed lower ($P < 0.05$) values for RBC, PCV and Hb, but significantly high ($P < 0.05$) MCHC values at day 21 of treatment as compared to the control rats and animals treated with 4 ml/kg. The results are presented in Table 1.

Table 1: The effect of administration of *B. aegyptiaca* seed oil on haematological parameters

Groups	RBC (10^6 cells/ μ l)	Hb (mg/dl)	PCV (%)
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	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21
Control	7.55±0.88	7.35±0.18	13.9±0.64	14.3±0.21	44.9±2.35	45.9±0.92
<i>B. aegyptiaca</i> 4ml/kg	6.86±0.43	7.01±0.18	12.5± 0.71	13.5±0.33	40.3±1.75	44.8±1.28
<i>B. aegyptiaca</i> 8ml/kg	7.26±0.10	6.06±0.4*	13.2±0.23	12.0±0.74*	43.0±1.50	38.0±2.75*

Groups	MCV (fl)		MCH (pg)		MCHC (g/l)	
	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21
Control	59.4±0.71	62.6±0.62	18.5±0.14	19.4±0.25	30.7±0.43	30.9±0.47
<i>B. aegyptiaca</i> 4ml/kg	59.4±2.89	63.8±0.27	18.3±0.23	19.3±0.13	31.5±0.16	30.1±0.17
<i>B. aegyptiaca</i> 8ml/kg	60.6±1.67	62.6±0.39	18.2±0.09	19.8±0.19	30.7±0.88	31.6±0.46*

Values are expressed as mean ±SE, * $P < 0.05$, N= 6 rats

Biochemical parameters:

The activities of AST, ALT, ALP and total proteins were insignificantly ($P > 0.05$) increased at day 21 in control and treated animals, except for total protein of 8 ml/kg dose which showed slight

insignificant decrease (Table 2). The levels of urea and creatinine in control were maintained within normal ranges as well as treated groups during the period of experiment (Table 3).

Table 2: Effect of *B. aegyptiaca* seeds oil in rats on liver function tests

Groups	ALT (U/I)		AST(U/I)	
	Day 0	Day 21	Day 0	Day 21
Control	35.2±5.55	63.0±5.84	102.2±9.48	160.0±11.13
<i>B. aegyptiaca</i> 4ml/kg	39.5±7.92	53.5±3.06	112.1±12.68	140.6±6.95
<i>B. aegyptiaca</i> 8ml/kg	34.2±4.13	62.3±8.51	110.8±12.05	152.4±16.84

Groups	ALP (U/I)		Total Proteins (mg/dl)	
	Day 0	Day 21	Day 0	Day 21
Control	75.5±9.15	89.4±2.55	6.45±0.31	6.93±0.34
<i>B. aegyptiaca</i> 4ml/kg	85.1±14.25	100.1±13.54	6.53±0.71	6.67±0.27
<i>B. aegyptiaca</i> 8ml/kg	82.7±6.06	92.1±2.84	7.55±0.52	6.31±0.26

Values are expressed as mean ±SE, N= 6 rats

Table 3: Effect of *B. aegyptiaca* seeds oil in rats on kidney function tests

Groups	Urea mg/dl		Creatinine mg/dl	
	Day 0	Day 21	Day 0	Day 21
Control	28.7±2.62	30.0±1.59	0.55±0.07	0.65±0.11
<i>B. aegyptiaca</i> 4ml/kg	35.0±4.38	32.3±2.32	0.55±0.07	0.69±0.05
<i>B. aegyptiaca</i> 8ml/kg	37.3±2.20	30.5±2.32	0.60±0.04	0.64±0.11

Values are expressed as mean ±SE, N= 6 rats

Histopathological findings:

Heart sections of control and treated groups showed no significant changes in myocardial muscles. In liver sections hepatocytes appeared normal with normal hepatic cords. No inflammatory, degenerative or necrotic changes were observed in all groups. Some sections in

the control and treated animals showed slight dilatation of blood vessels (Figures 1 & 2). In kidney sections of the control animals, glomeruli and bowman spaces appeared normal with some lobulation of glomerular tuft and slight widening of cortical tubules (Figure 3).

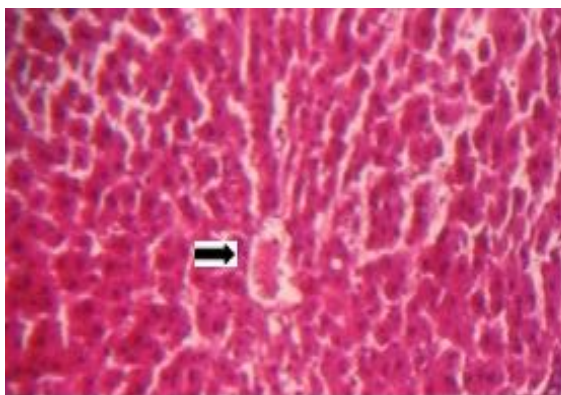


Fig.1. liver section of rat treated with 4ml/kg *B. aegyptiaca* seed oil, showing normal hepatocytes and congestion of central veins (H&E stain) X10.

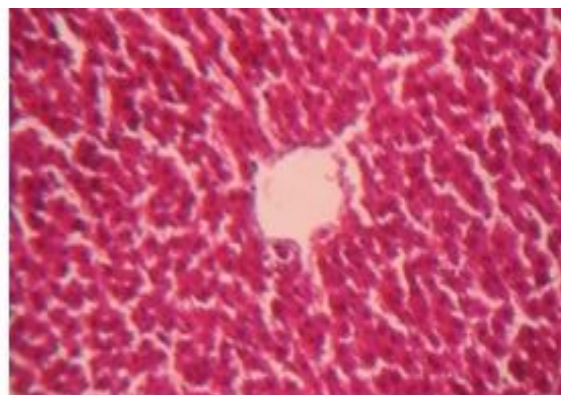


Fig.2 liver section of rat treated with 8ml/kg *B. aegyptiaca* seed oil, showing normal hepatocytes with dilatation in central vein (H&E stain) X10.

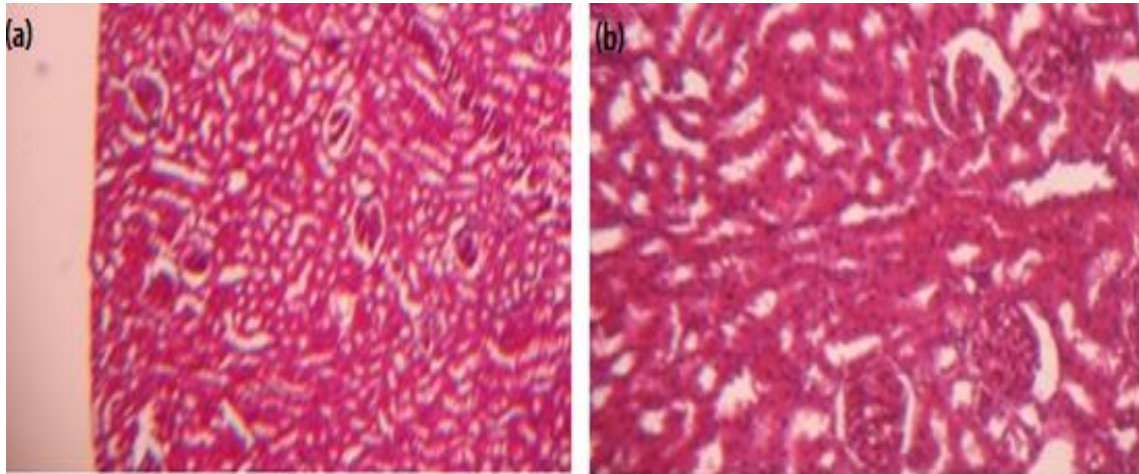


Fig.3 (a) kidney section of control rat, showing normal appearance with slight lobulation of glomerular tuft (H&E stain) X4. (b) High magnification X10 of (a).

Kidney sections of rats given 4 ml/kg *B. aegyptiaca* seed oil showed dilatation of tubules. Some tubules showed detachment of epithelium from the basal

membranes. Generally, cells appeared normal with no evidence of degenerative or necrotic changes (Figure 4).

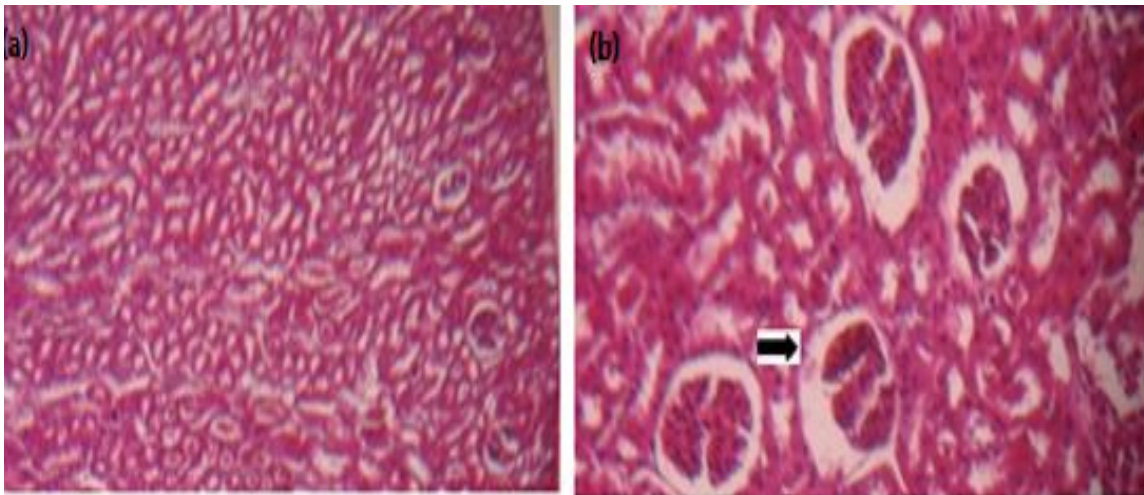


Fig.4 (a) kidney section of treated rat with 4ml/kg *B. aegyptiaca* seed oil, showing normal appearance with slight dilatation of bowman space and lobulation of glomerular tuft (H&E stain) X4. (b) High magnification (X10) of (a).

In kidney sections of rats treated with 8 ml/kg *B. aegyptiaca* seed oil there was slight lobulation in glomerular tuft. In

some sections very few areas of degenerative or necrotic changes were noticed (Figure 5).

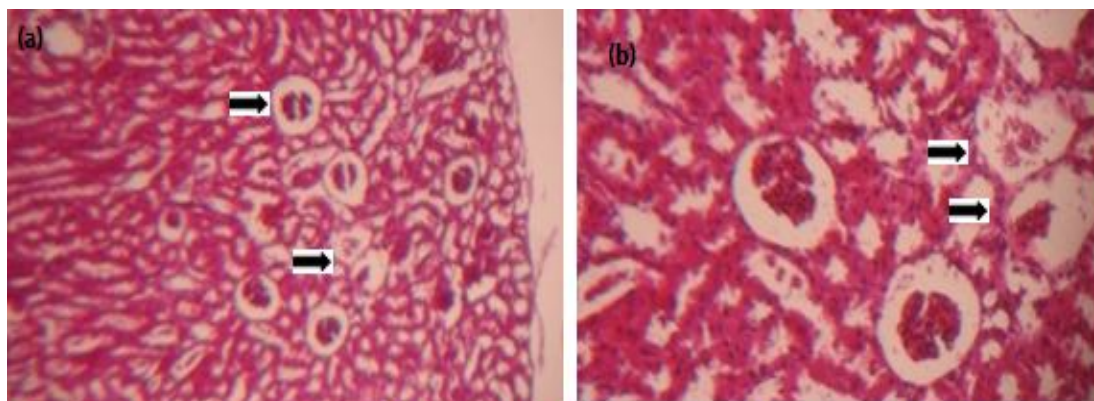


Fig.5. (a) kidney section of treated rat with 8ml/kg *B. aegyptiaca* seed oil, showing normal appearance with dilatation of bowm an space and focal area of degenerative or necrotic changes (H&E stain) X4. (b) High m agnification (X10) of (a).

The white pulps of spleen were distributed normally in the control and treated animals. Very prominent white pulps with dense zone of lymphocytes around central artery surrounded by lighter zone of lymphocytes were seen in some sections in the treated groups. In few sections the white pulps in the treated groups was seen as small collection of condensed lymphocytes around the central artery (Figures 6 & 7). The pancreatic tissue appeared normal in all groups with no evidence of inflammatory or degenerative changes.

Intestine sections in all groups showed normal appearance of villi, glands and lamina propria with marked presence of goblet cells and desquamation of villi. There were no significant changes in

glandular and non-glandular stomach of the control or treated rats.

Emphysema and congestion of alveolar wall capillaries were observed in the lung sections of all groups. Prebronchial lymphoid hyperplasia was also seen in some sections.

Discussion

The safety of seed oil of *B. aegyptiaca* seeds petroleum ether extract was confirmed by acute toxicity study. No clinical signs or mortality were observed in the animals that received petroleum ether extract of up to 20 ml/kg, except that anorexia occurred in rats receiving higher doses of the oil. This may be due to the filling of the stomach with plenty of oil, resulting in anorexia. Anorexia was also observed in rats given high dose of oil (8ml/kg) in sub-chronic

toxicity. According to OECD guidelines the liquid can be administered to rats up to 20 ml/kg (OECD, 2001). Haematological results showed no significant differences in all haematological values between control and rats received 4ml/kg petroleum ether extract of *B. aegyptiaca* during the period of the experiment. In rats treated with the high dose of *B. aegyptiaca* (8ml/kg), results indicated significant decrease in the mean values of RBC, PCV, Hb and increase in MCHC value at day 21 when compared to control rats and animals treated with 4 ml/kg of *B. aegyptiaca* petroleum ether extract. These results may be attributed to the anorexia and reduction of food uptake in this group as a result of administration of high dose of oil. Despite these changes these values are considered within the normal range. The present study showed insignificant change in the activities of serum ALT, AST, ALP and total serum protein level in treated animals compared to control. This indicates that the seeds oil induces no toxic or necrotic hepatic changes and thus is considered safe in rats. These findings were similar to that observed by Ajayi and Folorunso, (2013). The elevation of ALT, AST and ALP levels is taken as markers for hepatocellular injury; these enzymes are released to circulation as a result of destruction of hepatocytes (Wolf, 1999). These findings were also confirmed by microscopic examination of liver sections of treated animals which indicated no evidence of any inflammatory or necrotic changes in treated animals.

In addition, the levels of serum urea and creatinine between treated and control rats were also statistically insignificant. This seems to agree with the results of Obidah *et al.*, (2009) and Ajayi and Folorunso, (2013). Urea and creatinine are metabolic waste products that are freely filtered by the glomeruli of the kidneys. The ability of the kidney to excrete creatinine and urea nitrogen decreases with damage, resulting in increased serum creatinine and urea (Bhuvaneshwari and Krishnakumari, 2012). Serum creatinine and urea levels increase only when significant renal injury has occurred. The insignificant change in serum urea and creatinine is suggestive of normal kidney function. Histopathological investigation of kidney sections provided additional support of seeds oil safety. However, focal degenerative changes were observed in few areas in some kidney sections in rats given high dose (8ml/kg) of seeds oil without modification in kidney functional mechanism.

In conclusion, the safety of *B. aegyptiaca* seeds petroleum ether extract has been confirmed in rats in acute and sub-chronic toxicities models.

Acknowledgements

The authors acknowledge the financial support from Deanship of Scientific Research, Sudan University of Science and Technology.

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