# Hepatoprotective effect of *Balanites aegyptiaca* (L.) Delile leaves against carbon tetrachloride-induced hepatic damage in rats

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**Received: December 28th, 2015; Accepted: March 10th, 2016**

**Abstract:** Methanolic extract of *Balanites aegyptiaca* (L.) Delile (leaves), was evaluated for its hepatoprotective activity against carbon tetrachloride ($CCl_4$)-induced hepatic damage in Wistar rats; by measuring levels of serum marker enzymes like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin. Histological studies were also carried out to support our hypothesis. Administration of the extract (200 and 400 mg/kg) orally markedly prevented $CCl_4$-induced elevation of serum GPT, GOT, ALP and total bilirubin levels. A comparative histopathological study of liver in treated groups exhibited similarity to normal tissue architecture, compared to $CCl_4$-treated group.

**Keywords**: *Balanites aegyptiaca* (L.) Delile; Hepatoprotective

## Introduction

Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any injury to it or impairment to its functions may lead to many implications on one's health. Management of liver disease is still a challenge to the modern medicine. Conventional medicine is now pursuing the use of natural products such as herbs to provide the support that the liver needs on a daily basis (Sherlock S., Dooley J. 2002). *Balanites aegyptiaca* Del. (Zygophyllaceae), known as 'desert date', is spiny shrub or tree up to l0 m tall, widely distributed in dry land areas of Africa and South Asia (Hall, J.B. and Waljer D.H. 1991). This tree is native to much of Africa and parts of the Middle East. (Mohamed A.H., *et al*., 1999).This is one of the most common trees in Senegal. (Ndoye M., *et al.,* 2004) It can be found in many kinds of habitat, tolerating a wide variety of soil types, from sand to heavy

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clay, and climatic moisture levels. It is traditionally used in the treatment of various ailments i.e. jaundice, intestinal worm infection, wounds, malaria, syphilis, epilepsy, dysentery, constipation, diarrhea, hemorrhoid, stomach aches, asthma, and fever. It contains protein, lipids, carbohydrate, alkaloid, saponin, flavonoid, and organic acid. In Ayurvedic, Fruits are described to have a bitter sharp taste, and are used in alternative medicine as anthelmintic and analgesic preparation (Kirtikar B.D., Basu B.D. 1988). And in Unani system, it is used for treatment of skin diseases (Pandey C.N. 2005) while in Sudanese and Egyptian folk medicines it is used for treatment of jaundice (Koko, W.S., *et al.* 2008) and the fruit (after removal of the apocarps) is used as an oral antidiabetic drug (Staerk D., *et al*. 2007) Its antimalarial and molluscidal activity is well studied (Kamel M.S., *et al.* 1991). Root is used in various folk medicines for treatment of abdominal pain and as purgative, while the bark is employed as a fish poison. The root, bark, kernel, and fruit have been shown to be lethal to mollusks (Neuwinger H.D. 2004). In Senegal, Nigeria, Morocco, and Ethiopia, *B. aegyptiaca* is taken as a purgative for colic and stomach ache. In Libya and Eritrea, the leaves are used for cleaning infected wounds (Breyer J.M., Brandwijk, M.G. 1982). It was also reported to possess immune modulating properties, anti-inflammatory, antinociceptive, antioxidant and hypocholesterolemic actions (Khan F.M. 2009). Hepatoprotective activity of the park as well as fruit pulp of this plant has been reported. Therefore, in view of these reasons, we have selected it to study the Hepatoprotective activity of its leaves.

## Materials and methods

### *Plant Material and Extraction*

Fresh leaves of the plant *Balanites aegyptiaca* were collected in the morning from Khartoum area in the year 2015, dried and powdered. Methanolic extract was prepared by maceration of leaves powder (1000g) with methanol (3L) for 48 hours with intermittent stirring. After extraction, the solvent was filtered and concentrated under reduced pressure. The extract (yield: 27%) obtained was stored at -20°C until being used.

### Animals

Experiments were performed in healthy male Wistar Albino rats (11-12 weeks old). Rats were housed within the premises of the Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum, Sudan, under illumination at night and early morning with

feed and drinking water provided *ad libitum*. The rats were divided randomly to four groups (n=5). Animals were fasted for 16 h prior to the administration of $CCl_4$. The study protocol was approved by the Institutional Ethical Committee.

**Chemicals**

Assay kits for the estimation of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) were purchased from Randox, UK. 5, 5' dithio bis-(2-nitrobenzoic acid) was purchased from Sigma Chemical Co., USA. All other chemicals were of analytical grade.

**Acute toxicity study**

Wistar Albino rats of either sexes weighing between 100 and 150 gms were used in present investigation. The animals were fasted overnight prior to the experimental procedure. The methanolic extract was administered orally only once in doses of 1000 and 2000 mg/kg to two groups of rats (n=5), and percentage mortality was reported after 7 days (Kamel, M.S. *et al.*, 1991).

**Hepatoprotective activity**

Hepatic injury was induced in rats by subcutaneous administration of a single dose of 3.0 ml/kg $CCl_4$ mixed with equal volume of olive oil on the $7^{th}$ day (Salwa, A., *et al.*, 1988). Animals were divided in to 5 groups of 5 animals each: Group 1: Control group, treated with 2% w/v gum acacia in water at the dose of 2.0 ml (orally) for 7 days, then injected with olive oil treatment (3.0 ml/kg, s.c.) on day 7. Group II: Treated with vehicle (2.0 ml, orally) for 7 days followed by $CCl_4$ on day 7. Group III: Treated with the standard drug silymarin (100 mg, orally) for 7 days followed by $CCl_4$ on day 7. Group IV and V: treated with methanolic extract of *Balanites aegyptiaca* suspended in 5% gum acacia in water at doses of 200 and 400 mg/kg (orally) for 7 days followed by $CCl_4$ on day 7, respectively.

**Estimation of Biochemical Parameters**

The rats were sacrificed 24 hours after the administration of the last dose under anesthesia using halothane. The blood was collected and the serum was separated by centrifugation. The serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate trasnsaminase

(SGPT), serum alkaline phosphatase (ALP) and total bilirubin were estimated by the spectrophotometer (Hatapakki B.C., *et al.,* 2006).

**Statistical analysis**

All values are expressed as means ± S.D. the data were subjected to Student's t-test; and one-way ANOVA followed by Newman-Keuls multiple comparison test; and $P<0.05$ were considered significant (Thakur V.D., Mengi S.A. 2005).

**Histopathological Studies**

Liver was rapidly excised immediately after the sacrifice, and was washed with normal saline (0.09%), fixed in formalin (10%) and embedded in paraffin wax. Sections of 4-5µ thickness were made and stained with haematoxylin-eosin. Histological observations were made under light microscope (Sethuraman M.G., *et al*., 2003).

**Results and discussion**

***Results***

***Acute toxicity study***

Oral administration of the methanolic extract of *Balanites aegyptiaca* at doses of 1000 and 2000 mg/kg did not produce any overt changes in behavior or symptoms of toxicity. The extract was found to be safe up to a dose 2000 mg/kg in rats. Thus two doses (200 and 400 mg/kg) which were found to be safe, were employed for further pharmacological studies.

**Biochemical estimations**

The results for the effect of Balanites aegyptiaca on serum enzymes SGOT, SGPT, ALP and Total serum bilirubin are shown in table **1**. The induced hepatic damage by $CCl_4$ in rats caused significant rise in marker enzymes SGOT, SGPT, ALP and Total serum bilirubin. Oral administration of *Balanites aegyptiaca* extract 400 mg/kg was observed to significantly lower the levels of marker enzymes SGOT, SGPT and Total bilirubin ($P < 0.001$). It also lowered ALP ($P < 0.05$). While, *Balanites aegyptiaca* extract 200 mg/kg was observed to lower significantly the levels of SGOT ($P < 0.01$), SGPT ($P < 0.05$) and Total bilirubin ($P < 0.01$) but did not lower ALP. The effect of Silymarin seemed dose dependent and offered relatively

greater protection. The toxic effect of $CCl_4$ was significantly controlled in the animals treated with methanolic extract of *B.aegyptiaca* by way of restoration of the levels of liver function biochemistry similar to that of standard drug silymarin.

**Histopathology**

Histopathological profile of liver sections of control group showed normal cellular architecture with distinct hepatic cells, cenusoidal spaces and central vein (Figure 1a). Group II animals exhibited disarrangement of normal hepatic cells with intense centrilobular necroses, vacuolization of cytoplasm and fatty degeneration (Figure 1b). The liver sections of the rats treated with methanolic extract of *B. aegyptiaca* and silymarin followed by $CCl_4$ intoxication showed a sign of protection as it was evident by the absence of necrosis and vacuoles (Figure 1c,1d,1e).

**Discussion and Conclusion**

Hepatotoxicity of liver is due to the consequence of $CCl_4$ activation by cytochrome P-450 to trichloromethyl free radical ($CCl_3$) and which in turn disrupts the structure and function of lipid and protein macromolecules in the membrane of the cell organelles (Obidah W., *et al*., 2009) Carbon tetrachloride is the one of the most commonly used hepatotoxins in the experimental study of liver diseases (Johnson D.E., Kroening C., 1998). It induces liver cell necrosis and apoptosis and can be used to induce hepatic fibrosis or cirrhosis by repetitive administration (Shi G.F., Q. Li, 2005). The increased level of SGOT, SGPT, ALP and total bilirubin is sensitive indicators of liver injury (Vadivu R.1., *et al*., 2008). The increase in the levels of serum bilirubin reflected the depth of jaundice. The increase in transaminases and alkaline phosphatase was the clear indication for the loss of functional integrity of the cell membrane (Watt J.M., Breyer-Brandwijk M.G. 2002). The leaves of *Balanites aegyptiaca* were reported possessing flavonoids. It was suggested that the plants containing flavonoids possess hepatoprotective activity (Reitman S., Frankel S. 1957). In previous studies, The extracts of leaf, stem, stem bark and root of *B. aegyptiaca* were screened for hepatoprotective activity in Wistar albino rats. The stem bark extracts of the plant showed significant ($P < 0.05$) hepatoprotective effects as revealed by a decrease in the activity of serum transaminase and alkaline phosphatase enzymes as compared to control rats. The effect of lyophilised extracts of *B. aegyptiaca* (1 g/kg) and silymarin (0.1 g/kg), a standard heptoprotective agent, given for 5 consecutive days, was tested on liver damage induced by paracetamol (0.6 g/kg) in

the mice. *B. aegyptiaca* had a relatively modest hepatoprotective activity (27%) while silymarin protected about 92% of the treated mice. These results suggest that the extract could protect the paracetamol-induced liver damages perhaps by eliminating the deleterious effects of toxic metabolites from the drug (Ali B.H., *et al*., 2001).

In the present study, also it was seen that administration of $CCl_4$ elevates the levels of serum marker enzymes SGOT, SGPT, ALP and total bilirubin. The Sylimarin-treated groups exhibited lower levels of marker enzymes as compared to $CCl_4$-treated groups. The stabilization of marker enzyme levels by *Balanites aegyptiaca* extract is a clear indication of the improvement of the functional status of the liver cells (Valeer J.D. 2003). These findings were further confirmed with histopathological studies. The histopathological examination clearly reveals that the hepatic cells and central vein were similar to normal tissue in the group treated by *Balanites aegyptiaca* extract (400 mg/kg) in contrast to the group which received $CCl_4$.

Thus, *Balanites aegyptiaca* can be considered an effective hepatoprotective drug as it restores liver damage caused by $CCl_4$. Hence, this extract can be used in poly herbal formulations to provide a synergistic effect with other hepatoprotective drugs and thereby preventing the process of initiation and progress of hepatocellular diseases (Mujumdar A.M. *et al*., 1998).

**Table 1.** Effect of using *Balanites aegyptiaca* extract as treatment on different biochemical parameters in the serum of rats.

| Parameters | Control | $CCl_4$ | Silymarin (100 mg/kg) | B.aegyptiaca (200 mg/kg) | B.aegyptiaca (400 mg/kg) |
|---|---|---|---|---|---|
| **SGOT (U/ml)** | 42.56±6.51 | 113.27± 9.17* | 54.28± 7.84** | 95.40± 8.16*** | 67.81 ±9.25** |
| **SGPT (U/ml)** | 35.19± 7.10 | 128.73± 7.21* | 45.16± 5.82 | 118.65± 6.46** | 92.76 ±6.52** |
| **ALP (KA/unit)** | 13.56± 2.85 | 27.11± 3.61* | 19.12 ±2.48** | 25.68± 2.14 | 21.19± 2.14** |
| **T. Bilirubin (mg/dl)** | 0.62± 0.05 | 1.42± 0.06* | 0.51± 0.05** | 1.29± 0.07*** | 0.89± 0.07** |

Values expressed as mean ± S.D. of five animals in each group.
*P < 0.001 as compared with the group I.
**P < 0.05, ***P < 0.01 as compared with the group II.

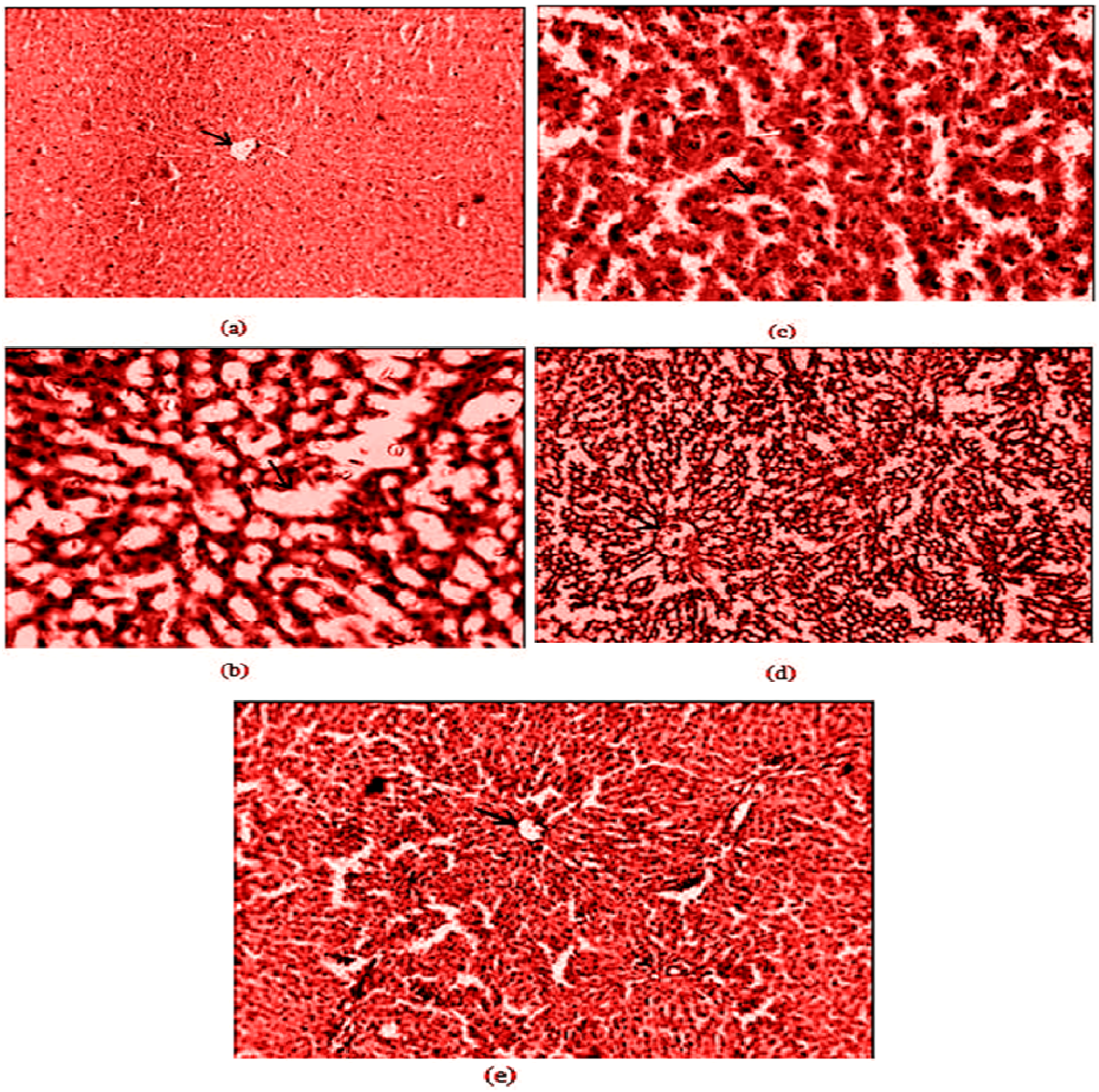


**Figure 1.** a. Section through the liver tissue of control rats showing normal histology.
b. Section of the liver tissue of rats treated with $CCl_4$ showing necrosis and fatty vacuoles.
c. Section of the liver tissue of silymarin- treated rat showing normal hepatocytes.
d. Section of the liver tissue of methanol extract (200mg/kg) treated rat showing normal arrangements of hepatocytes around the central vein.
e: Section of the liver tissue of methanol extract (400mg/kg) treated rat showing normal arrangements of hepatocytes around the central vein, absence of necrosis.

## Acknowledgement

We gratefully acknowledge the Medicinal & Aromatic Plants Research Institute, Khartoum, Sudan for providing necessary facilities to carry out this work.

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Arabian Journal of Medicinal and Aromatic Plants

www.ajmap.org ISSN 2458-5920