



RESEARCH ARTICLE

Hepatoprotective activity of aqueous extract of *Balanites aegyptiaca* L. Delile (Balanitaceae) roots bark.

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Abstract

Balanites aegyptiaca (L.) Del (Balanitaceae) is traditionally used for the treatment of various ailments such as syphilis, jaundice and liver disorders, epilepsy. This study was designed to evaluate acute toxicity and hepatoprotective effect of aqueous extract of *Balanites aegyptiaca* on CCl₄ induced hepatotoxicity in rats. Acute toxicity was assessed with the extract at a dose of 2000 mg / kg bw. The extract at doses of 25, 50 and 100 mg / kg b.w. was orally administered respectively to CCl₄-induced hepatotoxicity (0.5 ml / kg) animals. Silymarin (100 mg / kg) was given as a reference. Biochemical parameters such as ALT, AST, PT, ALB and ALP were assayed as well as enzymatic antioxidant activities SOD, CAT and MDA. Nitrogen monoxide (NO) involved in inflammation was also measured. Activities of liver marker enzymes, ALT, AST and ALP, total protein, albumin and showed a significant hepatoprotective effect. Regarding antioxidant enzymatic activities in vivo (SOD, CAT and MDA) of aqueous extract exhibited a significant effect showing increasing levels of SOD, CAT and reducing malondialdehyde (MDA) levels. The production of NO is significantly reduced compared to the batch intoxicated by CCl₄. *Balanites aegyptiaca* is endowed with hepatoprotective properties that can be attributed to antioxidant potential which could justify its use in traditional medicine in liver disorders.

Keywords: *Balanites aegyptiaca*; Aqueous extract; Acute toxicity; Hepatotoxicity; Carbon tetrachloride

Introduction

The main causes of liver damage are viral infections (B and C), parasitic infections, mycotoxins, alcohol, drugs, autoimmune diseases and metabolic syndromes (obesity, insulin resistance and diabetes) [1]. These liver lesions cause the activation of several endogenous mediators, in particular the metabolism of reactive oxygen species (ROS) by cellular damage [2]. These ROS can cause deleterious lipid peroxidation of cell membranes lead-

ing to a number of liver diseases such as fibrosis, cirrhosis and hepatocellular carcinoma [3]. This production of ROS may become excessive or result from exogenous toxic phenomena and the body will have to protect itself from these excesses by different antioxidant systems. Endogenous antioxidant enzymes fail to reduce the excessive production of ROS resulting in their accumulation in the body [4]. This can lead to disorganization of biochemical parameters and massive production of nitric oxide (NO) involved in inflammation.

To overcome this deficit, the use of exogenous antioxidants is more than necessary thanks to the drugs used in the management of hepatitis allowing the liver to recover to rebalance the internal imbalance. People in developing countries use herbal

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medicines to treat liver disease. Increasingly, it is necessary to evaluate natural compounds as effective alternatives. The more and more studied herbal medicines have shown their proofs in the use of natural antioxidants in the improvement of antioxidant enzymatic activities and the restoration of biochemical parameters [5]. It has been found that hepatoprotective effects are directly associated with phytoconstituents.

Balanites aegyptiaca L. Delile, (Balanitaceae), known for its many therapeutic properties, is a shrub native to Asia and North Africa and located in arid regions [6]. These fruits are of great nutritional interest and all parts of the plant are used in the treatment in traditional medicine of various pathologies such as cancer, hemorrhoids, stomach aches, jaundice, yellow fever, [7]. In Burkina Faso, the whole root is used in the treatment of chronic hepatitis in traditional medicine [8]. Despite this strong use in traditional medicine, some studies on this part of the plant have not yet been studied. The objective of this study was to study the hepatoprotective effects of aqueous extract of *Balanites aegyptiaca* on hepatotoxicity induced by carbon tetrachloride in rats.

Material and methods

Chemical reagents

Sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), ethylenediamine tetra acetic acid (EDTA), silymarine, N-(1-naphthyl) ethylenediamine dihydrochloride, Sulfanilamide and Carbon tetrachloride (CCl_4) are from SIGMA aldrich (Steinheim, Germany). Iron chloride (FeCl_3), Sodium carbonate (Na_2CO_3) and phosphoric acid (H_3PO_4) are from Fluka chemic reagents (Buchs, Switzerland) and prochimic. They were all of analytical grade. Kits for examining albumin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), Total protein, Albumin obtains by SpinReact, Girona, Spain. Other products and reagents such as ethanol, hydrogen peroxide (H_2O_2) were also used.

Animals

NMRI male mice 1-2 months old weighing 25-30 g from the Institute of Research in Health Science (IRSS) pet shop were used for the acute toxicity studies. Normal male and female rats of 3-4-month-old WISTAR strains weighing between 150 and 170 g were used for the hepatotoxicity activity. They were fed wheat cake (29% protein) and running water. They were raised under air conditioning (23-25 °C) and 60% humidity. All experiments were conducted in the morning in accordance with the Laboratory Animal Care Guidelines and the Ethical Guidelines for Painful Experimentation on Conscious Animals [9].

Plant materials and extraction

Root barks of plant were harvested in Ouagadougou in 2015. Specimen was identified by Pr Amado OUEDRAOGO and was deposited at the herbarium of the university Joseph KI-ZERBO against a code number T4263. Drying was carried out under dust-free ventilation, then the plant material was ground and stored in a vacuum plastic bag in a desiccator.

Fifty grams of the powder were extracted in 500 ml of distilled water and extract has been frozen and lyophilized. The lyophilizate obtained (12.5 g) was stored in a vacuum desiccator for later use.

Acute toxicity test

Acute toxicity of aqueous extracts of root bark of *Balanites aegyptiaca* was performed on male NMRI mice according to the OECD test guideline 423 [10]. After 4 h of fasting, a single dose of 2000 mg / kg body weight of extract was administered orally by gavage. Animals were observed individually during the first 2 h post-treatment for signs of toxicity and after that, fed was restored. They were then observed at least once a day for 14 days to detect mortality [11].

Hepatoprotective activity

Experimental protocol

Wistar rats weighing between 150 and 170 g were placed in cages in groups of seven animals with access to fed pellets and tap water. The rats were divided into 7 groups of 7: Lot I (control) received a single dose of olive oil (0.5 ml / kg body weight p.o.); lot II, carbon tetrachloride (CCl_4) at the dose of 0.5 ml / kg body weight i.p; lot III, a reference (silymarin) antioxidant at a daily dose of 100 mg / kg body weight p.o. with CCl_4 [12]. The test lots (IV, V and VI) received variable doses (25, 50 and 100mg / kg of bw) of the aqueous extracts of *Balanites aegyptiaca* once daily. A lot VII was constituted to receive only the extract at the dose of 100 mg / kg of bw. All treatments were administered orally for 6 days. On day 7, exception of lots I and VII, animals from the other lots were injected with CCl_4 (0.5 mg / kg, i.p, body weight). Animal weight, food consumption and water were recorded daily during the 7 days.

After administration of CCl_4 , the rats were fasted overnight for 12 h. All rats were anesthetized using ketamine (150 mg/kg, i.p) and blood was collected directly by heart puncture and was allowed to clot before centrifugation at 3000g for 10 min to separate serum. The sera obtained were stored at -20°C for later biochemical analysis (ALT, AST, PT, ALB and ALP) and inflammatory marker levels (NO). All animals were then sacrificed and the liver were collected in ice cold and divided in two parts: a fine part was washed twice with ice cold 0.1 M PBS

(phosphate buffer saline), pH 7.2, blotted and dried. A 10% homogenate of the tissues of each rat were prepared in PBS. This homogenate was centrifuged at 12,000 g for 1 h at 4 ° C. The resulting supernatant was used for the determination of antioxidant activity *in vivo* (MDA, CAT, SOD).

Assay of biochemical analyses and determination of antioxidant enzyme activities

ALAT / GPT, ASAT / GOT, PAL, Albumin, and total proteins were measured using a specific diagnostic kit (SpinReact, Girona, Spain). Catalase [13], SOD [14] and MDA [15] was evaluated. The NO₂ levels in serum were determined [16].

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test using GraphPad Prism 6 (Graph Pad Software Inc., USA). Differences were considered statistically significant at p value < 0.05.

Result

Acute toxicity

The aqueous extract of root barks showed not symptoms of toxicity or mortality at the single dose of 2000 mg / kg body weight during the two-week post-treatment observation period.

Hepatoprotective activity assay

Effect of plant extract on animal physical parameter

Experimentation during seven days was not influenced on animals' weight (figure 1). The consumption of food (Figure 2) and water (Figure 3) were not affected equally by the administration of *B. aegyptiaca* aqueous extract.

Assay of biochemical parameters

Administration of CCl₄ to rats resulted in a considerable increase in Alt, Ast and PAL and a decrease in protein and albumin (Table 1). On the other hand, for treated groups with the extract (25, 50 and 100 mg/Kg bw), its parameters decreased with respect to the parameters of negative group. The 50 mg / kg extract give the best biochemical parameters compared to the 25 and 100 mg / kg doses after the CCL₄ injection. It is noted that the extract alone allowed a protection of the liver through the biochemical parameters comparable to those of control.

Antioxidant enzymatic activity

The action of CCl₄ significantly reduced the activity of CAT, SOD while increasing the content of MDA (Table 2). The in-

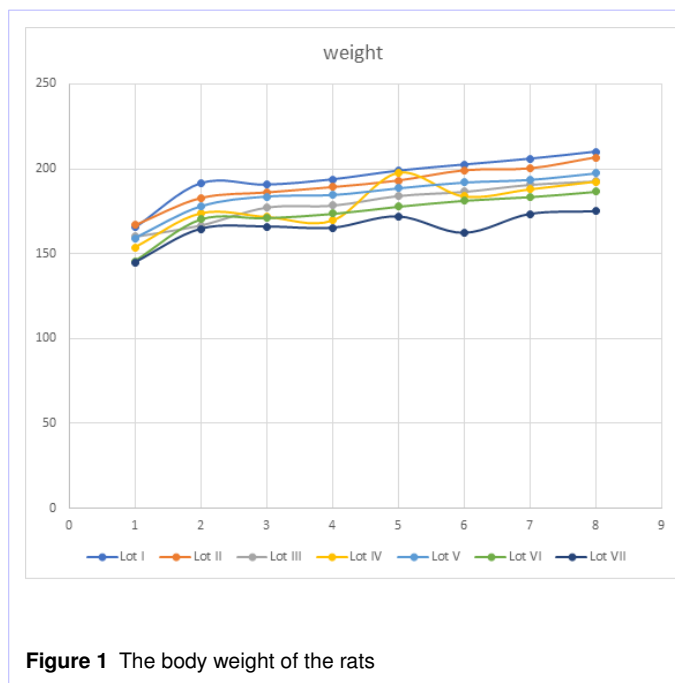


Figure 1 The body weight of the rats

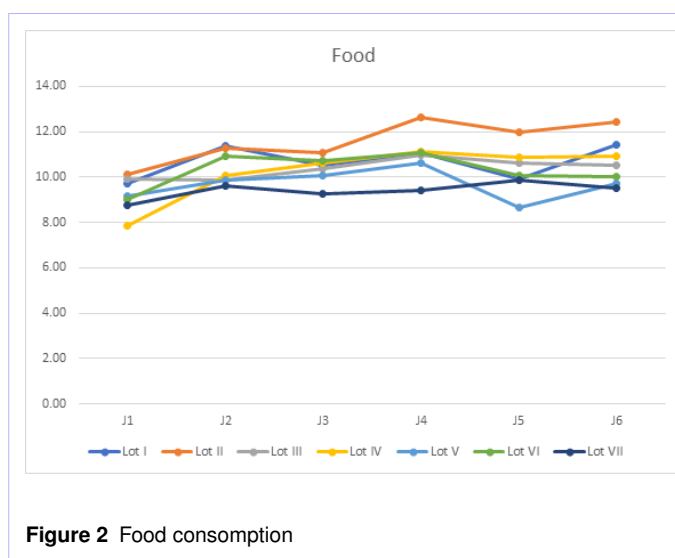


Figure 2 Food consumption

crease in MDA was attenuated by the administration of extract at different doses (25, 50 and 100 mg / kg bw) as well as the increase in CAT and SOD. Serum NO was elevated in the CCl₄ group compared to the other groups.

Discussion

In the acute toxicity test, according to OECD guideline 423, the LD₅₀ was estimated at 5000 mg / kg of body weight and this allows the extract to be classified in category 5 of the Globally Harmonized System (GHS) of the OECD and the United

Table 1 Effect of ethanolic extract of *Balanites aegyptiaca* on serumbiochemical parameters on CCl₄ Induced hepatotoxicity in rats.

	ALT (UI/L)	AST (UI/L)	Creatinin (umol/L)	Protein T (g/L)	Albumin (g/L)	PAL (UI/L)
Control	31,43±4,08	99±5	65,3±3,4	64,63±3,24	43,32±2,14	133,8±24,96
CCl ₄ 0,5 ml/Kg bw	790±65	697,5±52,5	69,68±2,10	55,6±0,95	34,23±3,02	357,8±5,12
Silymarin 100 mg/ Kg + CCl ₄	84,6±18,32	182,5±59	65,2±5,2	58,67±1,87	40,89±3,75	237±21,5
Extract 25 mg/ Kg + CCl ₄	72±20	163±73,5	65,34±4,66	58,4±2,1	34,49±1,47	256,86±56,69
Extract 50 mg/ Kg + CCl ₄	46±0,12	103±1,25	59,96±7,79	55,22±4,05	35±1,4	210,17±44,56
Extract 100 mg/ Kg + CCl ₄	95±25	158,89±35,44	64,14±2,35	60,6±2,86	36,06±0,93	234±37
Extract 100 mg/ Kg	53±7,33	174,67±40,44	66,68±4,54	66,63±3,1	41,16±0,87	203±16,22

Values are means ± SEM (n=7). ^aP < 0.05, significant change with respect to Control; ^bP < 0.05, significant change with respect to CCl₄ 0,5ml/Kg

Table 2 Effect of aqueous extract of *Balanites aegyptiaca* on liver antioxidant parameters on CCl₄ induced injury in rats

	Cat mmol/mg	MDA mmol/mg	SOD mmol/mg	NO (µg/ml)
Control	12,58±0,61	0,17±0,01	9,66±0,98	0,40±0,11
CCl ₄ 0,5 ml/Kg	4,77±1,95 ^a	0,20±0,03 ^a	7,06±1,01 ^a	0,53±0,13 ^a
Silymarin 100 mg/ Kg + CCl ₄	10,42±2,24 ^{a,b}	0,16±0,03	9,37±2,32	0,47±0,15
Extract 25 mg/ Kg + CCl ₄	11,75±2,54 ^{a,b}	0,17±0,03	9,46±1,79	0,43±0,16
Extract 50 mg/ Kg + CCl ₄	12,55±1,71 ^{a,b}	0,18±0,05	9,29±1,15	0,44±0,11
Extract 100 mg/ Kg + CCl ₄	12,27±0,82 ^{a,b}	0,16±0,03	9,61±0,56	0,51±0,09
Extract 100 mg/ Kg	14,85±2,47 ^{a,b}	0,17±0,02	9,46±1,19	0,49±0,13

Values are means ± SEM (n=7). ^aP < 0.05, significant change with respect to Control; ^bP < 0.05, significant change with respect to CCl₄ 0,5ml/Kg

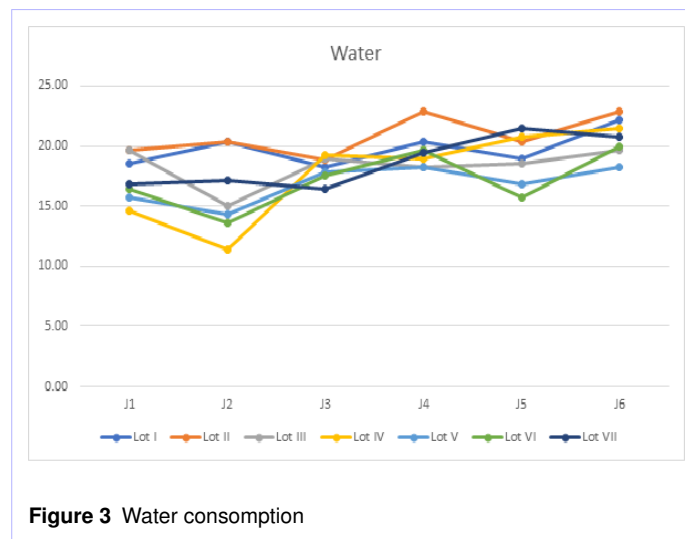


Figure 3 Water consumption

Nations [10, 17]. This result suggests that the plant extract has relatively low acute oral toxicity [10].

The extract and silymarin do not interfere with the behavior and eating habits of the animals. The elevation of AST is attributed to a hepatocellular lesion in rats, that of ALT linked to the necrotic state and the action of ALP is linked to hepatocyte function (evaluation of obstructive hepatic lesion) [18]. In this study, the increase in the level of its biochemical parameters in rats is due to liver failure leading to their release into the blood compared to normal rats. Rats treated with the extract at different doses saw their levels of ALT, AST and ALP decrease depending on the dose used. This analysis demonstrated

the hepatoprotective effects of *Balanites aegyptiaca* root bark extract on liver damage by induction of CCl₄ [19]. Reduction in total protein and albumin levels is considered a useful indicator of the severity of hepatocellular dysfunction [20]. Stimulation of protein biosynthesis has been suggested as a hepatoprotective mechanism that improves the regenerative process of hepatocyte production [20]. The action of the extract would stimulate the biosynthesis of these parameters.

The action of CCl₄ on rats led to a decrease in SOD and CAT activities which could be linked to a hyperproduction of radicals exceeding the production of its enzymes. Its results are in line with the results of the work carried out by Eidi A and al., [19], Wang R and al., [21] and Al-Olayan EM and al., [22]. The action of the different doses of the extract on the liver tissue cells led to improve the production of SOD and CAT. Its results are in agreement with the results of Suky TMG and al., [23] that found that the extract of the aerial parts of *Balanites aegyptiaca* was able to prevent the decrease of SOD and CAT activities that could be correlated with neutralization radicals. They also corroborate from those of Ali BH and al., [24] who had found a modest hepatoprotective activity of *Balanites aegyptiaca* against damage caused by paracetamol.

CCl₄-induced liver damage causes liver levels of MDA and serum nitric oxide (NO) to increase. The LPO caused by the administration of CCl₄, leads to a large production of free radicals followed by a decrease in the antioxidant defense causing oxidative stress. Hepatic MDA was evaluated to estimate the hepatoprotective effect of *Balanites aegyptiaca* extract in rats treated

with CCl₄ [25]. In this study, MDA levels were reduced by different doses of extracts and silymarin (reference substance). NO production in CCl₄-induced liver injury at animal is released by inducible nitrite oxide synthase (iNOS) expression [26]. A decrease in the production of NO in the serum in rats treated with the extract of *Balanites aegyptiaca* would show the capacity to block the inflammatory activity induced by CCl₄ on the liver. Al-Olayan *et al.*, found in their study an increase in NO production in rats dependent on CCl₄ and a decrease in rats treated with CCl₄ with their extract [22].

Conclusion

The results of this study indicated that the aqueous extract of the root bark of *Balanites aegyptiaca* had a preventive effect against liver damage induced by CCl₄ in rats. Its action has inhibited the production of oxidative stress in rats, restoring the level of biochemical indicators in serum and liver tissues and proteins in liver tissues. The extract prevented liver damage caused by the oxidative stress of CCl₄. Additional studies will be carried out to characterize and discover the mechanism of action of the active compounds in the aqueous extract of *Balanites aegyptiaca* which are at the origin of the hepatoprotective activity observed.

Authors' contributions

All authors had similar contributions regarding the manuscript writing, literature research, review design, literature analysis and final text approval

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