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Hepatoprotective effect of the aqueous leaf extract of *Balanite aegyptiaca* on carbon tetrachloride-induced liver damage in albino rats

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Abstract
The aqueous leaf extract of *Balanite aegyptica* (BA) was evaluated for its hepatoprotective effect on carbon tetrachloride-induced hepatic damage in albino rats using liver enzymes activities. Preliminary phytochemical screening showed the presence of flavonoids, alkaloids, saponins, glycosides and sterols. Evaluation of the serum marker enzymes indicated significant increase in the serum activity of the marker enzymes - AST, ALT, ALP in rats administered with the hepatotoxin when compared to the treatment groups (P<0.05). There was a substantial decline in the levels of these enzymes and bilirubin after treatment with BA extract thus indicating its hepatoprotective activity. Silymarin (standard drug) exhibited significant hepatoprotective effect in the study animals. These findings suggest that BA extract possess hepatoprotective activity judging by the reduced levels of the serum markers even more than silymarin. Thus, the enhanced hepatoprotective activity of BA extract might be attributed to the synergistic effect of the phytochemicals present in the plant.

Keywords: *Balanite aegyptiaca*, hepatoprotective, carbon tetrachloride, hepatotoxicity, serum marker enzymes

Introduction
The liver is a vital organ and the principal site for the body's metabolism. It is involved in almost all the biochemical pathways of the body, combat diseases, nutrient supply, energy provision, reproduction and regulate homeostasis (Marieb and Hoehn, 2007) [1]. Liver damage is a serious health challenge globally, it encompasses all the potential factors that predispose and cause the liver to fail to perform its designated function optimally (Kumar *et al*., 2005) [2]. Usually, more than 75% of the liver tissue need to be impaired before a decrease in function occurs (Guyton and Hall, 1996) [3]. The liver is responsible for many critical functions within the body and should it become diseased, the loss of function can cause significant damage to the body.

Hepatotoxicity entails injuries to the liver that is associated with impaired liver function caused by exposure to drugs and other non-infectious agents (Navarro and Senior, 2006) [4]. It also implies liver damage usually by chemical insults. Both overdose and therapeutic dose of drug may elicit hepatotoxicity. Other chemicals such as those widely used in research laboratories and industries, herbal preparations and natural chemicals e.g microcystin produced by cyanobacteria, are also potential hepatotoxins (Pandit *et al.*, 2012) [5]. Over 900 drugs have been associated with liver injury and it is the most common justification for drug recall from market circulation. Chemicals often cause subclinical injury to the liver which manifests only as abnormal liver enzyme tests (Ostapowicz *et al.*, 2002) [6].

Balanite aegyptica is an evergreen woody and xerophytic tree known for its enormous medicinal significance (Yadav *et al*., 2010) [7]. It belongs to the family Balanitaceae and is commonly found growing wild in Borno and Adamawa states of Nigeria (Kubmarawa *et al*., 2008) [8]. It is locally known as 'Aduwa' in Hausa, Adowa (Yoruba), Tanni (Fulfude), Cungo (Kanuri) (Wilson *et al*., 2009) [9]. The fruit is popularly referred to as Desert date and eaten along with the whole plant for its medicinal value in Africa and some developing countries (Wilson *et al*., 2009) [9]. *B. aegyptica* has been utilized in folkloric medicine for the treatment of various ailments such as syphilis, jaundice, liver and spleen problem, epilepsy, yellow fever.

The plant also possess insecticidal, antiheminthic, molluscidal and contraceptive activities (Yadav *et al*., 2010) [7]. Other pharmacological properties of *B. aegyptiaca* include antifungal (Runyoro *et al*., 2006) [10], anticancer, antioxidant (Gnoula *et al*., 2008) [11], antiparasitic (Fatima *et al*., 2005) [12], anti-inflammatory (Speroni *et al.*, 2005) [13]. The plant has been documented to yield several products for human benefits. These include food source, fodder, fuel, fibre, timbre, gum or resin, oil for cooking, poison to aquatic lives such as snail and fish. It also offer shade and shelter and act as a barrier for fencing livestock enclosures (Orwa *et al*., 2009) [14].

The ethanol extract of *B. aegyptiaca* have been reported to display hepatocurative activity by lowering the level of serum marker enzymes in paracetamol-induced hepatotoxicity (Babandi *et al*., 2016) [15]. The objective of this study is to evaluate the hepatoprotective effect of the aqueous extract of *B. aegyptiaca* against CCl_4-induced liver damage model in albino rats.

Materials and methods

Collection of plant sample

The leaves of *B. aegyptiaca* were collected from Panisau in Ungogo Local Government Area, Kano State. The plant was authenticated in the Department of Biological Sciences, Bayero University Kano and a voucher specimen was deposited at the herbarium.

Preparation of Extract

The leaves of *B. aegyptiaca* were shade-dried at room temperature, pulverized using mortar and pestle. 200g of the powdered leaves was soaked in water for 48 hours and filtered. The filtrate was evaporated using rotary evaporator to obtain the aqueous extract which was stored for subsequent use.

Experimental animals

Albino rats were purchased from the Animal House Facility, University of Jos. The weight of the animals ranges from 110 – 170g, and they were made to acclimatize with their new environment and fed with animals' feeds and water *ad libitum* for 5 weeks.

Phytochemical screening

Qualitative chemical tests were carried out on the aqueous extract of *B. aegyptiaca* using standard procedures to identify the secondary metabolites present according to the standard methods of Evans, 2002[16].

Experimental design

The rats were randomly assigned to five experimental groups of 4 four rats each. Group 1 served as control and received only feed and water. Group 2 rats received 150 mg/kg CCl_4 intraperitoneally. Group 3 received 150 mg/kg CCl_4 and 100 mg/kg extract once daily for 2 weeks. Group 4 rats received 150 mg/kg CCl_4 and 200 mg/kg extract once daily for 2 weeks, while group 5 received 100 mg/kg silymarin for 2 weeks.

Evaluation of biochemical parameters

In the first phase, two animals from each group were sacrificed after one week of treatment with the BA extract and the blood taken for analysis of biochemical parameters (aspartate aminotransferase, alanine aminotransferase (Reitman and Frankel, 1957) [17] alkaline phosphatase (Rec, 1972) [18], bilirubin, and protein as described by Jendrassik & Grof (1935) [19] and Lowry (1957) [20] respectively. Similarly, in the second phase of the experiment, at the end of 2 weeks, all the remaining animals in the various groups were sacrificed and their blood samples taken for biochemical analyses.

Statistical analysis

Data are expressed as mean ± standard deviation. Statistical analysis was performed using GraphPad InStat version 3.00, GraphPad Software, San Diego California USA by one-way analysis of variance (ANOVA). $P<0.05$ were considered significant.

Results and discussion

Phytochemical screening

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, anthraquinones and sterol as shown in table 1. A possible explanation is provided in the report by Parvati and Naryana (1978) [21] in which the presence of tannins and phenol in the leaves might be partly due to the difference in the solvent used for the extraction.

Table 1: Phytochemical screening of *B. aegyptica*

Phytochemicals	*B. aegyptiaca*
Alkaloid	+
Flavonoids	+
Tannins	_
Saponins	+
Glycosides	+
Sterol	+
Anthraquinone	_

Key: + = present
- = absent

Evaluation of biochemical parameters

The results of the serum levels of AST, ALT, ALP, protein and bilirubin levels in albino rats challenged with *B. aegyptiaca* leaves extract and silymarin for seven and fourteen days are shown in tables 2 and 3 respectively. In the evaluation of hepatic damage by hepatotoxins such as CCl_4, the measurement of the levels of serum marker enzymes such as AST and ALP are usually used (Dobbs *et al*., 2003, Xu *et al*, 2002) [22, 23]. CCl_4-induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts (Rubinstein, 1962; Suja *et al*., 2002) [24, 25]. Hepatic structure deformations liberate these enzymes into circulation thereby making its detection in the serum possible. High level of AST is indicative of liver damage, ALT catalyze the conversion of alanine to pyruvate and glutamate, and is released in a similar fashion. Therefore, ALT being more specific to the liver, is a better parameter for the detection of liver injury (Williamson *et al*., 1996) [26]. In contrast, serum ALP played its role in hepatic cell functionality. An increase in serum level of ALP is attributed to its increased synthesis in the presence of increasing biliary pressure (Moss and Butterworth, 1974) [27].

In the present study, CCl_4-induced liver damage was confirmed by elevated levels of bio-chemical parameters, namely, ALT, AST, and ALP. Specifically, elevation of these enzymes in serum is indicative of hepatocytic damage (Ben-Porath *et al.*, 2008) [28]. Reactive oxygen species generation and lipid peroxidation of cell membranes leads to loss of membrane integrity, changes in membrane potential, and an increase in membrane permeability, which in turn results in

leakage of the enzymes from liver cells into circulation (Ben-Porath *et al.,* 2008) [28] and thereby resulting in increased serum levels

Table 2: Mean serum AST, ALT, ALP, protein and bilirubin activities in albino rats 7days post-treatment

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Protein (g/dL)	Bilirubin (µmol/L)
I	54.57±6.26^{abc}	22.3±0.594^{abc}	211±12.73^{abcd}	2.45±0.21^{abc}	12.76±1.31
II	105±7.07^{adef}	33.5±2.12^{ade}	721±29.69^{aef}	0.07±0.01^{adef}	24.5±6.36
CCl_4 + 100 mg/kg BA	76±1.414^{bd}	28.3±1.061^{bd}	565±21.21^{beg}	1.4±0.141	15.65±0.919
CCl_4 + 200 mg/kg BA	63.05±1.48^{e}	25.6±0.565^{e}	495±7.07^{cfh}	1.75±0.71^{e}	15.25±0.354
CCl_4 + 100 mg/kg Silymarin	74.7±3.96^{cf}	28.95±1.34^{c}	675±35.35^{dgh}	1.35±0.354^{cf}	17.95±2.899

Results are expressed as mean±SD. Values in the same column having the same superscript are significantly different at $p<0.05$

The toxicity of CCl_4 begins with the change in the endoplasmic reticulum, which eventually results in the loss of metabolic enzymes located in the intracellular structures (Recknagel, 1990) [29]. The toxic metabolite, trichloromethyl radical, produced further react with oxygen to give trichloromethyl peroxy radical. Both radicals covalently bind to macromolecules and induce peroxidative degradation of the membrane lipid of endoplasmic reticulum which is rich in polyunsaturated fatty acids (Recknagel, 1990) [29]. This results in lipid peroxidation accompanied by pathological changes such as decline in protein synthesis, elevation of serum marker enzymes etc. These enzymes have been reported to be higher than normal in serum where there is existing liver necrosis (Keith and Robert, 2001; Price and Steven, 2003) [30, 31]. CCl_4 provoked hepatic damage in rats and elevated levels of serum marker enzymes (Wolf, 1999) [32]. These changes in the marker enzymes level is a reflection of the integrity of the liver structure, and the rise in AST is usually accompanied by elevated ALT which is responsible for the conversion of amino acids to keto acids (Sallie and Tredger, 2001) [33].

In the present study, the values of AST, ALT and ALP were significantly higher ($P<0.05$) in group II compared to group I, and this is an indication of possible induction of liver damage (Obi and Uneh, 2003) [34]. This finding tallies with the work of Alhassan *et al* (2009) [35]. The serum level of group II was found to decrease when compared to group I, this decrease is associated with hepatic damage. However, the values for bilirubin increases slightly suggesting the existence of hyperbilirunaemia

Table 3: Mean serum AST, ALT, ALP, protein and bilirubin activities in albino rats 14 days post-treatment.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Protein (g/dL)	Bilirubin (µmol/L)
I	53.4±0.282^{ab}	22.59±0.827^{a}	206.5±3.54^{a}	2.2±0.283^{a}	13.6±1.44^{a}
II	92±2.83^{acde}	29.1±1.273^{ab}	325±35.35^{ab}	0.08±0.033^{abcd}	17.5±0.72^{a}
CCl_4 + 100 mg/kg BA	59±1.414^{c}	25.9±1.34	232.5±31.89	1.9±0.141^{b}	15.45±0.64
CCl_4 + 200 mg/kg BA	54.05±1.34^{d}	23.75±1.061^{b}	210±14.14^{b}	2.3±0.141^{c}	14.4±0.56
CCl_4 + 100 mg/kg Silymarin	62.8±3.82^{be}	26±0.000	235.8±21.21	1.83±0.177^{d}	15.7±0.13

Results are expressed as mean±SD. Values in the same column with the same superscript are significantly different at $p<0.05$

The aqueous extract of BA attenuated the levels of serum markers (AST, ALP, and ALT) especially after two weeks of treatment as shown in table 3 when compared to CCl_4 untreated group. This conforms to the previous work of Zaahkouk *et al* (2003) [36]. The findings were also in agreement with the work of Babandi *et al.,* 2016 [15]; Ruckmani *et al.,* 1998 [37]. This could be attributed to the presence of flavonoids and other bioactive components seen in the extract. It has been suggested that plants containing flavonoids possess hepatoprotective activity (Di Carlo *et al*., 1999) [38]. The flavonoids act as anti-oxidant and may contribute to the hepatoprotective effect of the plants amongst other pharmacological activities (Joy and Kuttan, 1998; Iwu, 1993; Kasuya *et al*., 2003; Adeneye *et al*., 2006) [39, 40, 41, 42]. Previous studies have shown the extract of BA possess flavonoids, alkaloids and saponins in high concentration (Ajayi and Ifedi, 2015) [43]. These phytochemicals exhibit diverse pharmacological and biochemical actions and may have contributed to the earlier report of the medicinal properties of the plant (Ajayi and Ifedi, 2015) [43]. The protein content increases which is due to the promotion of ribosome assembly on endoplasmic reticulum which facilitate uninterrupted protein biosynthesis after treatment with natural product (Rajesh and Latha, 2004) [44].

A comparison between the serum activities in weeks 1 and 2 is carried out to ascertain the effectiveness of time duration, which shows that AST in both weeks of groups III and IV showed a significant difference ($p<0.05$) except in group V (74.7±3.96, 62.8±3.82) which shows no statistical significance. The ALP in both weeks of groups III, IV and V was significant while ALT, protein and bilirubin were not statistically significant in all the groups, and this may be due to haemolysis of the red blood cells or dosage of the extract administered during the study

The silymarin-treated groups exhibited lower levels of the marker enzymes as compared to the CCl_4-treated group. Silymarin is hepatocurative because it contains groups of flavonoids. Flavonoids are proactive polyphenolics found in most plants and cannot be synthesized or produced by humans (McCuglough *et al.,* 2012) [45]. They have been found to be effective in controlling various biological activities; they possess anti-inflammatory, anti-angiotic, antimicrobial, antioxidant, antihypertensive, and anti-cholesterol properties (Liu *et al.,* 2014) [46]. There is tremendous research interest in polyphenols and flavonoids due to their antioxidant capacity which is largely attributed to the redox properties of their hydroxyl groups and the structural relationship between different functional groups in their structure. This enables these phytochemicals to actively act as free radical scavengers, reducing agents, singlet oxygen quenchers metal chelators, and hydrogen donors (Materska and Perucka, 2005) [47].

The aqueous extract of BA (200 mg/kg) showed more hepatocurative activity than the standard drug silymarin. This may be due to the synergistic effect of the different phytochemicals present in the plant. This phenomenon result

to maximum therapeutic effect as observed in group IV (AST: 54.05±1.344, ALT: 23.6±1.06, ALP: 210±14.14; protein: 3.6±0.849 and bilirubin: 14.4±0.566) and are close to the negative control values. As mentioned earlier, the hepatoprotective potentials of *B. aegyptiaca* may be attributed to its flavonoid content. However, tannins may also play a role in hepatoprotection as suggested by Ojo *et al* (2006) [48] in *V. paradoxa* plant extract.

Conclusion and recommendation

The aqueous leaves extract of *Balonite aegyptiaca* showed hepatoprotective effect on liver damage which was due to the phytochemicals present in the extract principally flavonoids which possess antioxidant property. Administration of the extract showed promising effect in normalizing level of serum marker enzymes, thus suggesting its potentials as a hepatoprotective agent. This study therefore provided empirical basis for the folkloric application of *B. aegyptiaca* for liver associated ailments such as jaundice. However, further study is required for the isolation, characterization and mechanism of action of the hepatocurative active compound. In addition, histopathological studies should be carried out to support the outcomes of the serum markers investigation.

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