Effect of *Balanites aegyptiaca* (L.) Del.) Seed Oil on Liver and Kidney Biomakers of Albino Rat

Jude Nwaogu¹, Isah Musa Fakai¹ and Ibrahim Abdullahi²*

¹Department of Biochemistry, Kebbi State University of Science and Technology Aliero, Kebbi State, Nigeria.
²Department of Science Laboratory Technology Federal Polytechnic, Kaura – Namoda, Zamfara State, Nigeria.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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(1) Dr. Ashish Anand, GV Montgomery Veteran Affairs Medical Center, University of Mississippi Medical Center, William Carey School of Osteopathic Medicine, USA.
(2) Wilson Obidah, Modibbo Adama University, Nigeria.
Adegbite Adesola, Ladoke Akintola University of Technology, Nigeria.
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ABSTRACT

Vegetable oil has been used for cocking and ethnomedicinal purpose in developing countries. The aim of this research was to determine the toxicological effect of *Balanites aegytiaca* seed oil in albino rats. Acute and sub chronic toxicological evaluations were carried out on albino rats. The albino rats were divided into six experimental groups (n = 6 albino rats/group). Five different doses of *Balanites aegytiaca* seed oil; 1000mg/kg, 2000mg/kg, 3000mg/kg, 4000mg/kg and 5000mg/kg were administered orally (gavage) to groups 2 – 6 respectively, once per day for 28 consecutive days. While control group received saline with Tween® 80 2%. Standard laboratory methods were used to assess liver and kidney functions indices. The acute toxicity screening revealed that the Balanite aegyptiaca seed oil (BASO) was non-toxic with LD 50 above 5000mg/kg. The liver function test parameters revealed non-significant (p>0.05) difference in serum AST and ALT activities between control group and treated groups. However, there is significant (p<0.05) increased of serum ALP activity in treated groups when compared to normal control group. Serum albumin concentration significantly (p<0.05) decreased in treated groups when compared to normal control group. No significant difference (P>0.05) in the concentration of total protein, total and direct bilirubin observed in treated groups when compared to normal control group. While kidney
function test parameters showed alterations in serum creatinine and Potassium concentrations in treated groups compared to normal control group. Oral administration of B. aegyptiaca seed oil for 21 days did not displayed signs organs damage. The results suggest that prolonged consumption of Balanites aegyptiaca seed oil could not cause adverse effects on the liver and kidney organs.

Keywords: Balanites aegyptiaca seed; liver biomarker; kidney biomarker.

1. INTRODUCTION

Balanites aegyptiaca (L.) Del., is a perennial tropical plant used in food preparations and herbal medicine, especially in Africa and some developing countries [1]. It is also called desert date (English), adua (Hausa, Nigeria), tanni (Fulfulde, Nigeria) and heglig (Arabic) [2]. The entire genus of Balanites is consisting of nine species and eleven intra-specific taxa [3]. All of this plant has ethnomedicinal uses including fruits, seeds, barks and roots [4]. B. aegyptiaca have contained a number of phytochemical compounds including flavonoids [5]. These phytochemical compounds such as flavonoids, phenolic acids, tannins and lignans can scavenge reactive oxygen species (ROS), and quench free radicals hence, phytochemical can serve as effective natural agents for preventing and treating free radical-mediated diseases [6]. Higher intake of oil with prominent content of saturated long-chain free fatty acid (FFAs) demonstrated moderate but significant toxicity both in human beta-cells and rat islets. Hence, saturated FFAs obtained from animal based oil are thought to be harmful due to lipotoxicity potential of the oil [7].

Vegetable oil has been used for cocking and ethnomedicinal purpose in developing countries especially in Nigeria, because it is relatively inexpensive. The rate of vegetable oil consumption in Northern Nigerian is alarming, so there is need for toxicological studies to determine the Balanites aegyptiaca seed oil toxicity and thus establish criteria for safe consumption.

2. METHODS AND MATERIALS

2.1 Collection and Identification of Plant Materials

Balanites aegyptiaca seed was collected from Dundaye village in Wamakko Local Government Area of Sokoto State, Nigeria. The samples were carried to Herbarium section of Plant Science and Biotechnology Department, Kebbi State University of Science and Technology Aliero, Kebbi State, Nigeria. The samples were authenticated by Professor Dharamendra Singh and the voucher number (291) was deposited at Herbarium.

2.2 Experimental Animals

Forty- two apparently healthy albino rats of both sexes weighing about 120-180g were used for this study. The rats were kept at animals’ house of the biological science department, faculty of science, Kebbi State University of Science and Technology Aliero, Kebbi State, at room temperature and maintained with free access to pelleted growers feed, and access to water ad libitum.

2.3 Methods

2.3.1 Preparation of plant sample

The Balanites aegyptiaca fruits were soaked in water for 10 hrs, washed and dried. The seed kernel was obtained by using hammer and grounded into powder for the extraction.

2.4 Oil Extraction

About 50g of grounded powder of the kernel was weighed into an extraction thimble. The thimble was placed inside the Soxhlet apparatus. A dried pre-weighed solvent flask was connected beneath the apparatus. 200ml of petroleum ether was poured in to the flask and connected to condenser, the extraction process was conducted for 16 hr. At the end of the extraction period, the flask was disconnected from the unit. The thimble was removed and the petroleum ether was reclaimed using the rotary evaporator. The removal of petroleum ether was completed on a water bath and the flask dried at 105°C for 30 min. The crude oil extract was cool in a desiccator and weighed. The crude lipid extract was stored in air-tight container prior to use. The percentage yield of crude oil was determined as follows:

\[
\text{Crude fat} = \frac{W_2 - W_3}{W_3} \times 100
\]

Where:

\[
W_2 = \text{Weight of the flask and ether extract}
\]

\[
W_3 = \text{Weight of the crude extract}
\]
2.6 Sub-acute Toxicity

The albino rats were divided into six experimental groups (n = 6 albino rats/group). Five different doses of BASO: 1000, 2000, 3000, 4000 and 5000 mg/ kg, were administered orally to groups (2-6) once per day for 28 consecutive days. The control group received only the vehicle (saline with Tween® 80 2%). The doses were chosen based on the Guideline 407 from OECD (Repeated Dose 28-Day Oral Toxicity Study in Rodents) [8]. At the end of the treatment period, rats were fasted overnight at the completion of the treatment period and blood collected by heart puncture under diethyl ether anesthesia for serum chemistry. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and Alkaline phosphatase activity were determined by the method of Reitman and Frankel [9] and that of Rec (1972) respectively. Serum albumin and total protein concentration were determined according to methods of Doumas et al., [10] and Tietz, [11] respectively. Serum total and direct bilirubin concentration were evaluated according to methods described by Tietz, [11]. Serum creatinine and urea concentration were also determined using standard method described by (Bartels et al., 1972; Weatherburn, 1957). Serum sodium, potassium and chloride were also determined using standard methods described by (Maruna, 1958; Terry and Sensin, 1958).

2.7 Histopathological Examination

Histopathological examination of the liver, kidney and spleen was carried out using the method described by Drury et al., [12]. The liver was fixed in 10% buffered formalin solution. Then, the formalin-fixed organ specimens were embedded in paraffin wax and serially sectioned (3–5 μm) and further stained with haematoxylin and eosin. The stained tissues were observed for pathological changes using light microscopy.

2.8 Statistical Analysis

Results were presented as Mean and Standard error (Mean ± S. E), analysed using the statistical package SPSS 20.0 version software. The differences between means were carried out using one- way analyses of variance (ANOVA) followed by Duncan multiple comparison test. Values were considered statistically significant at P<0.05.

3. RESULT

The result of chronic and sub-acute toxicity test revealed no clinical signs or mortality observed in the control and treated groups during the course of experiment except the signs of body weakness in the animals received high dose of BASO (5000mg/kg).

Table 1 shows the effect of administration of different doses of Balanites aegyptiaca seed oil on liver function indices. There was no significant difference (P<0.05) in the concentration of AST, TB and DB in all the groups when compared to the normal control group. As for the ALT concentration, Group 1 was significantly higher than all the treated groups. There was no significant difference (P>0.05) in the concentration of TP when the control group was compared to group 3, group 4 and group 6. As for the ALP concentration, the control group had the lowest concentration which was significantly lower (P<0.05) than all the other groups. The highest concentration was recorded in group 6 which was significantly higher than those of the other groups. As for the ALB concentration, the highest concentration was recorded in the control which was significantly higher than that of the other groups.
Table 1. Effect of administration of different doses of *Balanites aegyptiaca* seed oil on liver function indices

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/l)</th>
<th>ALT (U/l)</th>
<th>TP (U/l)</th>
<th>ALP (g/dl)</th>
<th>ALB (g/dl)</th>
<th>TB (mg/dl)</th>
<th>DB (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water (2ml/kg)</td>
<td>57.13±0.17a</td>
<td>25.50±1.50ab</td>
<td>7.29±0.20b</td>
<td>127.70±3.21b</td>
<td>4.47±0.22a</td>
<td>0.90±0.29a</td>
<td>0.20±0.03a</td>
</tr>
<tr>
<td>BASO 1000mg/kg</td>
<td>57.20±9.30a</td>
<td>22.07±0.74ab</td>
<td>6.52±0.16a</td>
<td>135.32±2.80ab</td>
<td>4.10±0.06ab</td>
<td>1.00±0.32a</td>
<td>0.21±0.02a</td>
</tr>
<tr>
<td>BASO 2000mg/kg</td>
<td>53.87±4.04a</td>
<td>21.97±2.16ab</td>
<td>7.13±0.24ab</td>
<td>136.13±0.93c</td>
<td>4.03±0.09ab</td>
<td>1.13±0.37a</td>
<td>0.23±0.03a</td>
</tr>
<tr>
<td>BASO 3000mg/kg</td>
<td>53.70±6.01a</td>
<td>20.73±1.29a</td>
<td>6.76±0.24ab</td>
<td>136.13±0.93c</td>
<td>4.03±0.09ab</td>
<td>1.13±0.37a</td>
<td>0.23±0.03a</td>
</tr>
<tr>
<td>BASO 4000mg/kg</td>
<td>57.13±1.08a</td>
<td>22.50±0.10ab</td>
<td>7.57±0.55b</td>
<td>155.47±2.44d</td>
<td>3.87±0.20a</td>
<td>1.17±0.17a</td>
<td>0.17±0.02a</td>
</tr>
<tr>
<td>BASO 5000mg/kg</td>
<td>58.30±6.92a</td>
<td>25.03±1.23ab</td>
<td>7.19±0.29ab</td>
<td>186.73±3.67e</td>
<td>3.63±0.13a</td>
<td>0.90±0.25a</td>
<td>0.18±0.03a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error of mean, n = 3. Mean values having common superscript letters in a column are not significantly different (P<.05) (one-way ANOVA followed by Duncan’s multiple range test). ALP- Alkaline Phosphatase, AST- Aspartate Amino Transferase, ALT-Alanine Amino Transferase, ALB-Albumin, TP-Total Protein, DB-Direct Bilirubin and TB-Total Bilirubin, *Balanites aegyptiaca* (L.). Seed Oil (BASO)

Table 2. Effect of administration of different doses of *Balanites aegyptiaca* seed oil on kidney function indices

<table>
<thead>
<tr>
<th>Doses (mg/kg)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Na⁺ (meq/l)</th>
<th>K⁺ (meq/l)</th>
<th>Cl⁻ (meq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water (2ml/kg)</td>
<td>24.70±3.04a</td>
<td>0.66±0.02a</td>
<td>150.09±9.70a</td>
<td>4.27±0.20ab</td>
<td>98.37±2.83a</td>
</tr>
<tr>
<td>BASO 1000mg/kg</td>
<td>29.77±0.29a</td>
<td>0.80±0.01b</td>
<td>143.50±3.25a</td>
<td>3.70±0.15a</td>
<td>101.20±4.05a</td>
</tr>
<tr>
<td>BASO 2000mg/kg</td>
<td>22.83±2.88a</td>
<td>0.75±0.05b</td>
<td>150.37±4.42a</td>
<td>3.73±0.24a</td>
<td>91.20±2.31a</td>
</tr>
<tr>
<td>BASO 3000mg/kg</td>
<td>28.23±2.85a</td>
<td>0.65±0.03a</td>
<td>152.00±3.26a</td>
<td>4.10±0.17ab</td>
<td>102.47±6.06a</td>
</tr>
<tr>
<td>BASO 4000mg/kg</td>
<td>25.30±3.14a</td>
<td>0.67±0.02a</td>
<td>139.47±6.82a</td>
<td>4.30±0.17ab</td>
<td>105.60±4.30a</td>
</tr>
<tr>
<td>BASO 5000mg/kg</td>
<td>25.97±2.70a</td>
<td>0.82±0.02b</td>
<td>146.33±4.25a</td>
<td>4.43±0.15b</td>
<td>100.13±5.12a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error of mean, n = 3. Mean values having common superscript letters in a column are not significantly different (P<.05) (one-way ANOVA followed by Duncan’s multiple range test). Na⁺- Sodium, K⁺- Potassium and Cl⁻- Chloride
Table 2 shows the effect of administration of different doses of *Balanites aegyptiaca* seed oil on kidney function indices. The concentrations of urea, sodium ion, and chloride ion were not significantly different (P<0.05) when the control group was compared with other treated groups. The creatinine concentration showed that group 1, group 4 and group 5 were significantly lower (P<0.05) than group 2, group 3 and group 6 when compared. The concentration of K+ followed a similar trend to that of creatinine.

4. DISCUSSION

ALT, AST and ALP activities are commonly measured to monitor potential plant and drug induced hepatic injury in both pre-clinical studies and human patients and thus, they serve as markers of liver toxicity (Salawu et al., 2009). ALT is localized primarily in the cytosol of hepatocytes, AST is normally found in the cytoplasm and mitochondria of many cells, primarily in cardiac muscle, liver and skeletal muscle with low concentrations in the kidney, pancreas and erythrocytes. ALP is found in most tissues, including bone, liver and kidney [13]. Changes in ALT and AST activities may indicate alteration of cellular permeability or cellular injury and necrosis. Elevated activities of AST also observed in myocardial infarction Low (Crook, 2006) [14,15]. Non-significant increase of serum ALT activity in albino rats administered with BASO is an indication that BASO has no significant effect on cellular integrity of the liver, hence BASO cannot caused liver necrosis.

Increased ALP level is usually a characteristic finding in cholestatic liver disease and biliary obstruction [16]. AST is less specific than ALT as an indicator of liver function and ALT activity is usually greater than AST activity at early or acute hepatocellular disease [17] although both are useful indices for identifying inflammation and necrosis of the liver [18]. Decrease in ALT and AST activities following administration with crude BASO suggests that BASO might possess hepatoprotective potentials [19].

Albumin and total proteins are globular proteins found in the serum and they are synthesized by the liver. A decrease in albumin and total protein is a sign of reduced synthetic ability of the liver or might be due to impaired hepatocellular function [20]. In the present study, absence of alteration in serum total protein is suggestive that the physiology of the liver was not affected. Bilirubin results from the breakdown of haemoglobin and it is excreted out of the body by the liver [20]. Higher levels of bilirubin may indicate different types of liver problems as well as an increased rate of destruction of RBC. In this study, no significant changes observed in serum total and direct bilirubin concentration between control group and groups treated with BASO, indicating that BASO did not interfere with the metabolism of bilirubin in the liver [21].
Kidneys play important roles in the elimination of metabolic waste products and toxic substances. When a toxic substance affects the kidney, it can lead to the leakage of biochemical substances into the blood circulatory system (Young, 1997). Therefore, the deviation of biochemical values in blood from the normal range is a sign of abnormal kidney function due to the accumulation of metabolic waste products. Serum electrolytes, creatinine and urea can be used as biomarkers for assessing the functional capability of the kidney. Urea and uric acid are the major nitrogen containing metabolic end product of protein catabolism. Creatinine is a waste product of muscle energy metabolism. They are found in the liver and conveyed through the blood to the kidney for excretion [22]. Healthy kidneys remove these compounds from the blood to be excreted in the urine. Since the results of serum levels of urea and creatinine of this study were not altered in all the BASO treated groups, it could be deduced that BASO might not have exerted any hazardous effect on the kidneys and hence it can possibly be considered as safe for consumption.

Serum electrolytes such as sodium, potassium, bicarbonate and chloride play a major role in intercompartmental water balance and the exchange of gases [23]. Significant alteration in the concentration of these electrolytes is indicative of poor renal functions or renal impairment [24]. Of these, sodium is the most abundant extracellular ion, and it plays an important role in muscle contraction. Increased sodium in the blood may result due to kidney disease, too little water intake, and loss of water due to diarrhoea or vomiting [25]. Decrease in sodium levels can result from relative increase in the amount of body water relative to sodium [26]. This could lead to respiratory arrests, neurogenic pulmonary oedema, and hypoxic exacerbation of cerebral oedema leading to fatal herniation [27]. Potassium ion is a cation of the intracellular fluid which plays a vital role in muscle contraction [28]. It is normally excreted by the kidneys, so disorders that impair kidney function can lead to hyperkalaemia [29]. The most common cause of high potassium is kidney disease. Other causes may result from dehydration, uncontrolled diabetes and injuries that cause severe bleeding [30,31]. Chronic kidney disease, excessive alcohol intake and diarrhoea are some of the causes of low potassium in serum [32]. The effect of BASO even at higher doses investigated on the renal function biomarkers suggests that the normal functionality of the nephrons at the tubular and glomerular levels were not altered. Bicarbonate buffer system is the most important amongst blood buffers when the pH of the blood is considered [33]. Chloride ion is a major extra cellular anion. It is involved in maintaining proper water distribution, osmotic pressure and normal acid-base balance in the extracellular fluid compartment. Histological (Plates 1-6) result of Kidney, Liver and Spleen revealed that BASO at highest concentration (5000mg/kg) has no toxicological effect on these vital organs. In liver sections portal triad, central vein and hepatocytes appeared normal. No inflammatory, degenerative or necrotic changes were observed in all groups. While in the spleen sections of control and treated groups normal red and white pulp were observed. Hence there is no obvious light microscopic evidence of cellular injury.

5. CONCLUSION

In conclusion, oral administration of B. aegyptiaca seeds oil to albino rats proven non obvious sign of acute and clinical toxicity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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