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Biochemical and histopathological effects of the aqueous stem-bark extract of *Balanite aegyptiaca* (Del) on the liver and kidney of adult Wister rats

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Abstract

Herbs has been accepted and widely used by large population of individuals as an alternative source of medicine in Africa and other parts of the world. However, the major concern is that, most of the medicinal plants used have not been evaluated for safety and toxicity, thus affecting the major organs of the body such as liver and kidney. This study investigated the effects of the aqueous extract of *Balanite aegyptiaca* stem-bark on the liver and kidney in rats. A total of 28 rats (both sexes) were randomly divided in to four groups with group I serving as the normal control group, while groups II, III and IV are the experimental groups and were administered with 150 mg/kg, 300 mg/kg and 450 mg/kg body weight of the extract respectively for period of 21 days. The level of the serum urea and creatinine increased significantly (P<0.05) in the groups treated with 300 mg/kg and 450 mg/kg. There was a significant (P<0.05) increase in the level of liver enzymes (ALT, AST and ALP) in all the treated groups which was found to be dose dependent. There was a decrease in the LDH level in groups treated with 150 mg/kg and 300 mg/kg while a significant increase in the group treated with 450 mg/kg was observed. The level of G-6-PDH decreased significantly (P<0.05) in the group treated with 450 mg/kg. There is a gross alteration in the histomorphology of the liver and kidney in the treated groups. The alterations in both biochemical parameters and histomorphological features provide a supportive evidence for functional alteration of liver and kidney. Therefore chronic intake of aqueous stem -bark extract of *Balanite aegyptiaca* resulted to functional and morphological alterations in liver and kidney of wister rats.

Keywords: *Balanite aegyptica,* Toxicity, Medicinal plant, liver and kidney.

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1. Introduction

Medicinal plants help in alleviating human suffering and are widely used for subsistence, home remedies, and trade [1]. Much of the medicinal use of plants is seem to have been developed through studies using animals. The global demands of herbal medicine is growing and its market is expanding annually especially in developing countries [2]. Numerous drugs were developed through exploration of ethnopharmacology and traditional medicine [3]. Traditional therapies is widely accepted and use in most developing countries and this has been associated with believe and low socio-economic status [4]. Thus, due to high increase in use of traditional therapies, it is essentials to establish a more

scientifically sound evidence for the principles behind the therapies, safety and effectiveness of medicinal plants [5]. The traditional therapies are often criticized due to inadequate research and critical evaluation. There is limited number of *in vivo* studies that predicts clinical trials validations to support the safety uses of most of the medicinal plants [6]. Thus research on medicinal product is important as it help to improve the quality and to avoid any potential toxicity [7].

Balanite aegyptiaca is found in many kinds of habitat tolerating a wide variety of soil types and climatic moisture level [8]. The tree is multi-branched and can grow to 6-10m high. The trunk and the bark are grey in colour, and are deeply fissured longitudinally, while the leaves are spirally arranged on the shoot [9]. The ellipsoid fruit is green when unripe and brown or pale brown when ripped with little coat enclosing pulp and a hard stone seed [8].

Medicinally, various parts of the *Balanite aegyptiaca* have been used for folk medicine [9]. The plant is traditionally used to treat many illnesses including laxative, diarrhoea, stomach ache, jaundice, syphilis and epilepsy, vermifuge, febrifuge, malaria, emetic and can also cure other types of ailments like skin boils, leucoderma, liver and spleen disorders, [8, 9].

Several studies have revealed the phytochemical composition of the different parts of the *Balanite aegyptiaca*. The leaves contains saponin, furanocoumarin, and flavonoid, while the fruits contains protein and sugar [10]. The roots contains saponin and glycosides and the stem-bark was reported to contains *furanocoumarin bergapten* [9].

Evidences from the previous studies has shown that, the aqueous leaf extract of *Balanite aegyptiaca* and saponin isolated from its kernel cake have antibacterial activity [11], anti-inflammatory and analgesic effects [12]. Moreover, various extracts of the plant were reported to have molluscicidal activities [13], anti-helminthic [14] and antidiabetic and antioxidants activities [15].

Considering the traditional believe and the high level of consumption of different parts of the plant by the individuals, there is great need to scientifically evaluate the safety dose of stem-bark of this plant. This study was designed to evaluate the effects of the aqueous stem-bark extract of *Balanite aegyptiaca* on the liver and kidney of rats. It is hypothesized that, chronic intake of aqueous stem–bark extract of *Balanite aegyptiaca* affects the morphological and functional activities of liver and kidney in wister rats.

2. Materials and method

2.1 Plant materials, Ethical approval and Animal husbandry

This study was approved by the Animal Care and Use Committee (ACUC), University of Ilorin, (Ref. number: UIL/ACUC/2010/0115). A fresh stem-back of *Balanite aegyptiaca* was collected from Akko local government area of Gombe State, Nigeria. It was identified and authenticated at the herbarium unit of Biological Sciences Department, Gombe State University, Nigeria. A total number of twenty eight presumable healthy adult rats of the Wistar strain weighing 170 g - 200 g (of both sexes) were used for the study. Animals handling was performed in accordance with ACUC guidelines. The animals were purchased from the animal house of the Department of Physiology, University of Ilorin, Nigeria. Following acclimatization period of 2 weeks, the rats were individually identified by colour tattoo, weighed and kept in plastic cages of dimensions 155 cm x 95.5 cm x 86 cm with cleaning done twice daily, and under a uniform husbandry condition at room temperature, with 12hr light/dark cycle. They were fed with standard laboratory diet (Bendel feeds, Ilorin) and drinking water *ad libitun.*

2.2 Plant extract preparation

The stem-bark of *Balanite aegyptiaca* was shade-dried for five days and pulverized using a pestle and mortar. The powdered stem-bark was stored in a cellophane bag at room temperature. 200 g of the powder was soaked in 1000 ml of distilled water in a glass jar. This was stirred for one hour and allows settling for 48 hours at room temperature. The extract was filtered through muslin cloth (Sieve) and the process was repeated three times. The filtrate obtained was concentrated in a water bath at a lower temperature (< 50 ^{0}C), at this temperature, the solvent gradually evaporated, until a constant dark sticky residue was obtained and this was further over dried. Aqueous solution of the extract was prepared by dissolving 3000 mg weight of the extract in 30 ml of Phosphate Buffered Saline (PBS) and the concentration used was 100 mg/ml

2.3 Administration of plant extract

A total of twenty eight rats were randomly assigned into four groups of seven rats per group. Group I served as control and were administered phosphate buffered saline (PBS) equivalent to the volume administered to the highest dosed of treated rats; while groups II, III and IV (treatments) served as low, medium and high dosed groups and were administered with 150 mg/kg, 300 mg/kg and 450 mg/kg body weight of the extract respectively via oro-gastric intubation for a period of 21 days. The control and treated groups were studied in parallel with administration done between 08.00hr-09.00hr daily. They were allowed free access to feed and clean water after the administration and the animals were weighed on daily basis.

2.4 Sample collection

Prior to cervical dislocation, the rats were deeply anaesthetized via inhalation of 4% (v/v) isoflurane to eliminate perception of pain. Blood sample was collected via

cardiac puncture and was centrifuged. For determination of the blood urea and creatinine, 3 ml of the plasma was obtained from each sample. The rats were disinfected with 70% ethanol, pinned on dissecting board and the abdominal skin was incised to expose the abdominal cavity. The liver and the kidneys were excised, collected and fixed in 4% paraformaldehyde (PFA).

2.5 Tissue processing and staining

Tissue processing was performed according to standard paraffin embedded procedure. Liver and kidneys were fixed in 4% paraformaldehyde (PFA) solution for 24 hours. The tissues were then removed and placed into automatic tissue processor (Leica, TP 1020) for tissue dehydration, clearing and infiltration. Tissues embedding was performed using paraffin embedding machine (Leica, EG1160) and tissue blocks were removed from the mould and kept at 4°C.

Paraffin-embedded samples were sectioned at 8 µm using rotary microtome (LP 1950). Tissue sections were gently transferred into a tissue floating water bath set at 40°C. Serial multiple sections of the tissues were collected and mounted onto a microscope slides (Thermo Fisher Scientific) and slides were labelled accordingly. The slides were dried at 40°C on hot plate. The haematoxylin and eosin (H & E) (Haematoxylin (HHS16); Eosin (HT110116) Sigma- Aldrich, St. Louis, Missouri, USA) stain was used to assess the general morphology of the liver and kidney following the manufacturer's protocol.

2.6 Microscopic Examination

Examination of the sections was performed using a bright-field light microscope (Olympus BX51, Tokyo, Japan) and images were captured through CCD digital camera attached to the microscope. Five slides were randomly selected from each sample and three images were obtained from each slide. The captured slides were assessed for any histopathological conditions.

2.7 Statistical Analysis

Numerical data obtained from the study were analysed statistically using Prism 7 (GraphPad, Software, San Diego, California, USA). The difference in the significance level between the control groups and treated groups was determined using analysis of variance (ANOVA) and the probability values of (P) $\leq$ 0.05 were considered to be statistically significant. The results were expressed as mean ± SEM.

3. Result

3.1 Effect of the aqueous extract of *Balanite. aegyptiaca* on the mean body weight

There was an increase in the body weight in the control group but it was not statistically significant. However, a significant increase in the body weight was observed in the treated groups when compared with the control group and this reflected to be highly significant (P< 0.05) in the groups administered with 150 mg/kg and 450 mg/kg body weight of the extract Table 1.

Table I: Effect of the aqueous extract of *Balanite. aegyptiaca* on the mean body weight.

Dose of Extract (mg/kg)	Initial body weight (gm)	Final body weight (gm)	Body weight difference (gm)	% weight change (%)
0.0	180.71±5.07	186.57±6.24	6.86±0.17	3.79
150.0	181.71±5.59	192.71±6.14*	11.00±0.55	6.05
300.0	184.00±4.75	190.86±4.85	6.86±0.10	3.72
450.0	180.86±5.00	193.43±5.94*	12.56±0.94	6.94

**Statistically significant, N*=7

3.2 Altered activities of the liver enzymes caused by the aqueous extract of *Balanite aegyptiaca*

The effect of the various doses of the aqueous extract of *Balanite agyptiaca* stem-bark on some biochemical components of serum (urea and creatinine) and liver enzymes are shown in table 2.

The activities of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP) were markedly affected following treatment of the animals with different doses of the extract. The result showed an increase in the level of ALT in all the treated groups when compared with the control group. However, it appeared to be highly significant (P<0.03) in the group administered with 450 mg/kg body weight of the extract (Table 2).

The level of the liver AST was also observed to increase in the treated groups which appeared to be dose dependent. The increase was statistically significant (P<0.05) in the groups administered with 300mg/kg and 450 mg/kg of the extract. The result also showed an increase in the level of AST in the group administered with 150 mg/kg, but was not statistically significant (P< 0.07) (Table 2). The effect of the extract on the ALP level also showed an increase in the activities of the enzymes in all the treated groups and it was found to be statistically significant (P < 0.05) as compared with the control group.

Table 2: Effect of the aqueous extract of *Balanite aegyptiaca* on the liver enzymes

Dose of Extract (mg/kg)	ALT (UL^{-1})	AST (UL^{-1})	ALP (UL^{-1})
0.0	16.00±0.49	34.42±1.17	22.86C3.03
150.0	19.28±0.42	39.14±2.00	70.86±6.33**
300.0	24.71±0.99*	42.00±2.47**	90.85±2.93**
450.0	31.86±1.30**	50.28±3.07**	132.86±11.54**

**Statistically significant, ** highly statistically significant, N=7*

3.3 Altered activities of Glucose-6-phosphate dehydrogenase and Lactate dehydrogenase caused by the stem-bark extract of *Balanite aegyptiaca*

Analysis of the serum urea and creatinine showed an increase in the level mainly in the 300 mg/kg and 450 mg/kg treated groups and this was found to be statistically significant (P<0.05) as compared with the control group. Both the urea and creatinine concentrations increased in the group administered 150 mg/kg of the extract but it showed to be statistically insignificant (P<0.1) (Table 3).

Lactate dehydrogenase (LDH) activity was observed to decrease in groups that were administered 150 mg/kg and 300 mg/kg body weight of the extract when compared with the control, though this decrease was not statistically significant. An increase in the level of LDH was observed in the group that were administered with 450 mg/kg body weight of the extract compared with the control group, and this appeared to be statistically significant (P<0.05) (Table 3).

Again the effect of the extract on the activity level of Glucose-6-phosphate dehydrogenase (G-6-PDH) was also quantified in this study. It was showed that, G-6-PDH activity decrease in all the groups, but appeared to be statistically significant (P<0.05) only in the group that were treated with 450 mg/kg body weight of the extract. The decrease in the G-6-PDH level in the groups administered with 150 mg/kg and 300 mg/kg body weight of the extract was statistically insignificant (Table 3).

Table 3: Effect of the aqueous extract of *Balanite aegyptiaca* on the liver enzymes and serum components

Dose of Extract (mg/kg)	Urea (mmol/L)	Creatinine (mmol/L)	LDH (U/L)	G-6-PDH (U/L)
0.0	3.60±0.16	58.14±4.69	936.0±21.5	6174.0±52.0
150.0	3.94±0.14	74.14±6.60	900±17.3	5978.0±39.0
300.0	4.57±0.17*	83.28±3.88*	894.5±32.5	6075.0±40.0
450.0	5.03±0.33*	105.29±10.0*	997.6±35.8*	5888.0±42.0*

**Statistically significant, ** highly statistically significant, N=7*

3.4 Histopathological assessment of the effect of aqueous extract of *Balanite agyptiaca* stem-bark on liver and kidney

Cross sections of the liver and kidney stained with H & E showed some morphological changes in the treated groups as compared with the control group (Fig 1A & Fig 2A).

Analysis of the liver sections administered with 150 mg/kg body weight showed a mild congestion and few infiltrations with the central vein partially occluded. Similarly, the kidney sections in this group showed thickening of glomerular wall and necrosis was observed (Fig 1B & Fig 2B). Moreover, morphological changes such as vascular congestion and infiltration were observed in the group administered with 300 mg/kg body weight of the extract. In addition, necrosis of the hepatocytes and the parenchymal distortion were observed in the liver section of this group.

It is apparent that, the histomorphological appearance of the kidney treated with 300 mg/kg body weight of the extract was distorted. Severe congestion of the blood vessel due to cellular infiltration, the Bowman's capsule and vascular wall were observed to be thickened. Also there is shrinkage of glomerulus (Fig 1C & Fig 2C).

The liver sections of the group treated with 450 mg/kg body weight of the extract showed cellular infiltration, congestions, dilatation of the central vein. Similarly, the kidney sections of the same group showed different morphological changes such as infiltration, mild congestion of the vessel, necrosis and as well thickening of the Bowman's capsule (Fig 1D & Fig 2D).

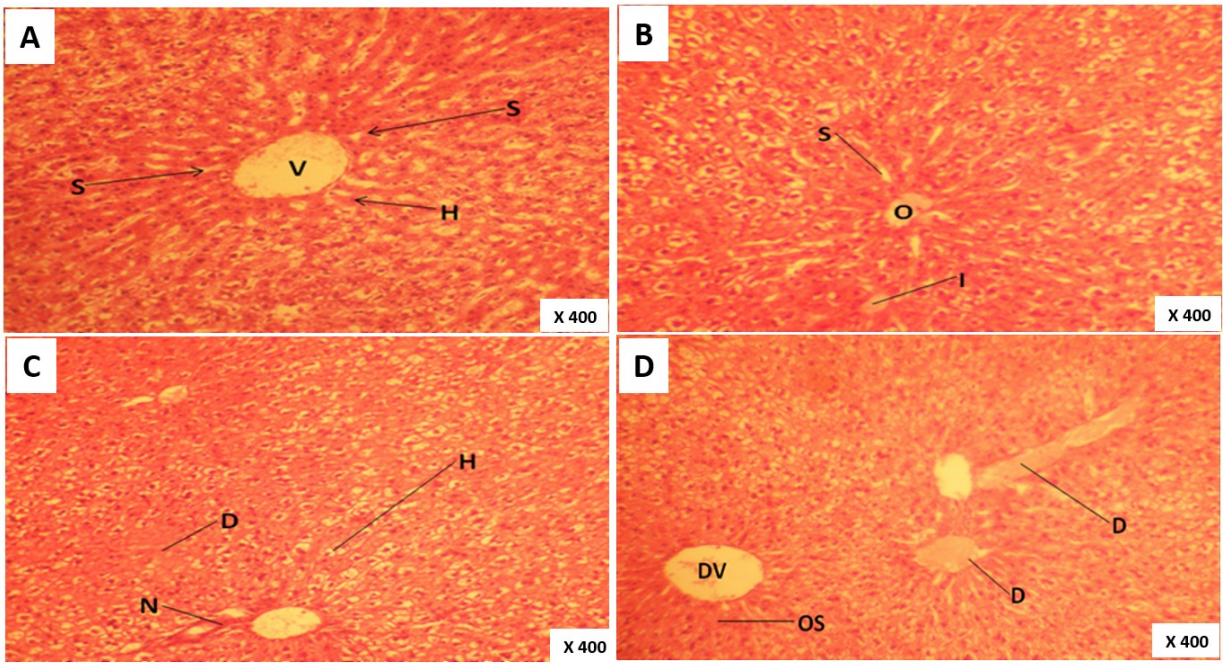

Figure 1: Haematoxylin and Eosin staining of the liver sections.

Histomorphological assessment of the liver section from the control groups **(A)** showed normal arrangement of central vein (V), sinusoids (S) and hepatocytes (H). Liver sections from group two **(B)** showed occlusion of central vein (O), infiltration, (I) and congestion along the sinusoids. Assessment of the liver section of group three **(C)** showed constriction of central vein, mild necrosis and parenchyma distortion, (D). The liver sections from group four **(D)** showed dilatation of central vein (DV), infiltration, (I) oedema (D) and congestion along the sinusoids (OS).

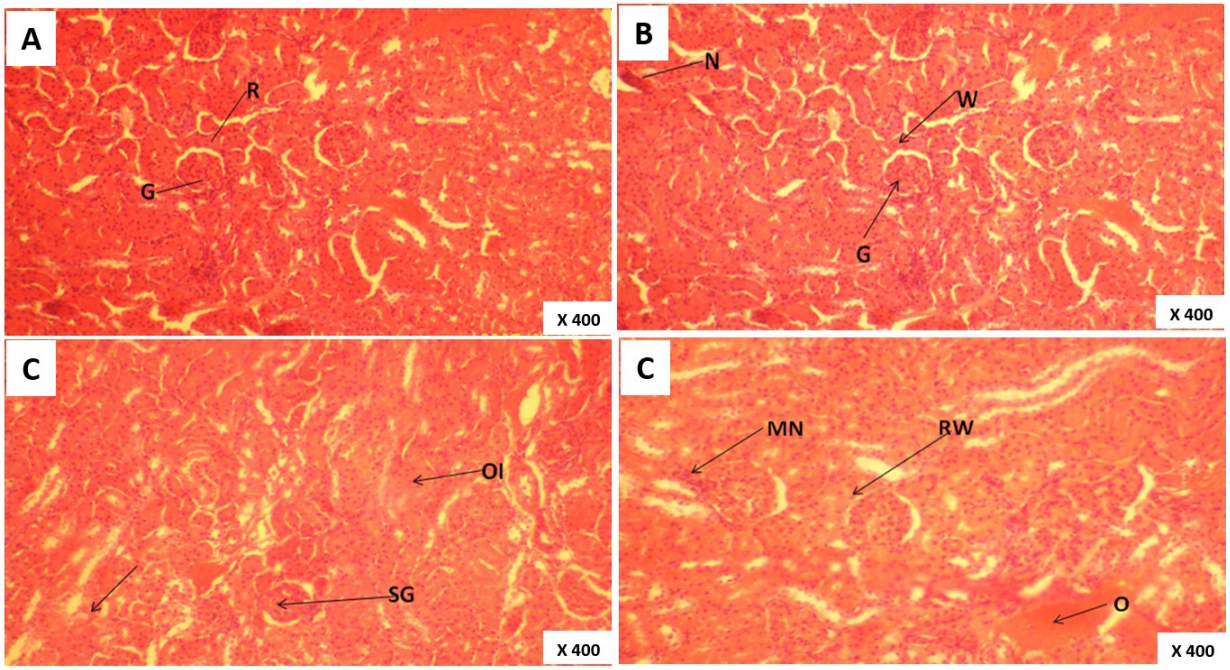

Figure 2. Haematoxylin and Eosin staining of the kidney sections

Histomorphological assessment of the kidney sections from the control groups **(A)** showed normal arrangement of glomerulus (G) and renal corpuscle (R). Kidney sections from group two **(B)** showed thickening of the glomerular wall (W), necrosis (N) and an intact glumerulus (G). Assessment of the kidney sections from group three **(C)** showed occlusion of the interstitial space (OI), glomerular shrinkage (SG) and blood vessel congestion (Arrow). Kidney sections from group four **(D)** showed thickening of glomerular wall (RW), oedema (O) and mild necrosis (MN).

The integrity of the brush border of the glomerular tubules was accessed using the Periodic Acid Schiff (PAS). In all the treated groups, the brush border of the proximal tubules was eroded and this was observed to be severe in the group administered 450 mg/kg body weight of the extract (Fig 3).

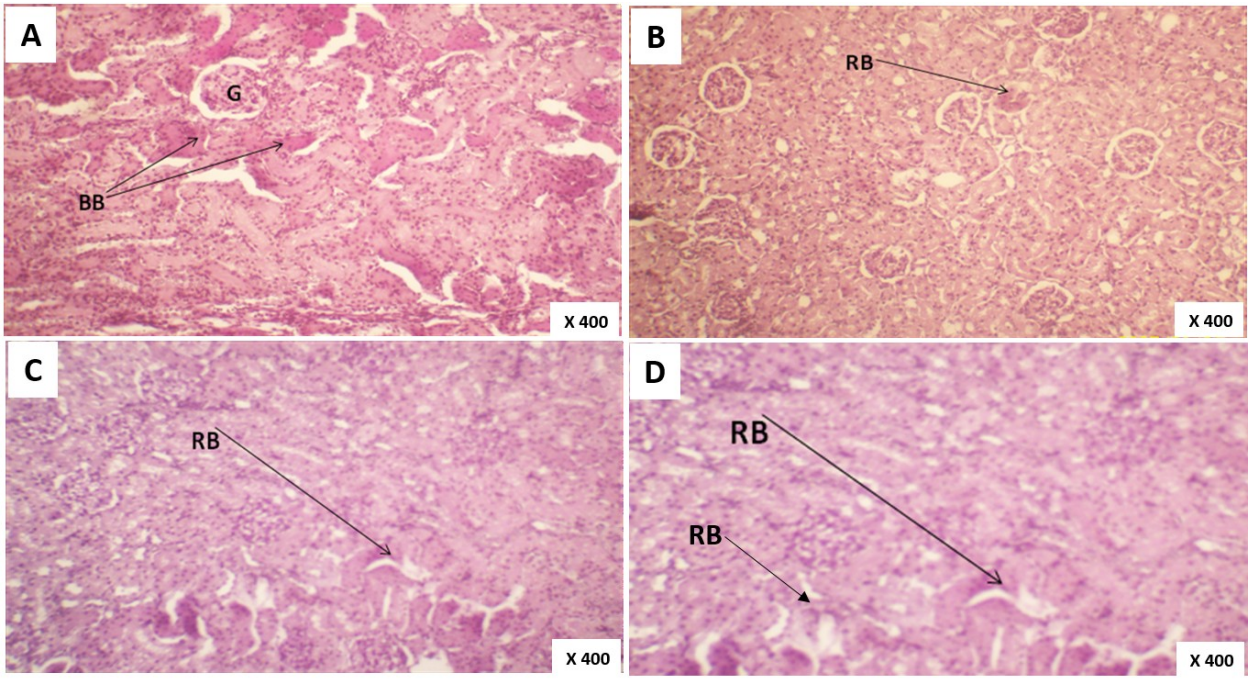

Figure 3. Periodic Acid Schiff (PAS) staining of the kidney sections.

Assessment of the kidney sections from control groups showed normal arrangement of glomerulus (G) and brush border of the Proximal Tubules **(A)**. Morphological assessment of the kidney sections showed eroded brush border of the proximal tubules in both group two **(B)** and three **(C)**. Kidney section from group four showed a complete eroded brush border of the proximal tubules (arrows) **(D).**

4. Discussion

The administration of the *Balanite. aegyptiaca* stem-bark to animals (rats) at different doses (150 mg/kg, 300 mg/kg and 450 mg/kg) orally was observed to induce an increased in both the serum urea and creatinine concentrations. It is known that, urea is the main product of protein metabolism and the urea cycle takes place in the liver, where ammonia released is converted into urea and is excreted by the kidney through urine [16].

The elevated serum urea level observed in this study may have resulted from kidney injury due to exposure to the extract. It is a known that, variety of renal diseases with different permutation of glomerular, tubular, interstitial, or vascular damage can cause an increase in the serum urea concentration [17]. Several studies that evaluated the toxicity and efficacy of medicinal plants have observed increased level of serum urea [18].

Increase in the creatinine level in this study may also be attributed to the impairment of the kidney function due to the exposure to the extract. The creatinine is a waste product formed in the muscle by creatine metabolism [19]. Findings from this studies showed increased level of serum creatinine and similar studies showed increased level of serum creatinine at different doses of the plant extract [17, 18].

Moreover, assessing the activity of the marker enzymes in tissues plays a significant role in disease investigation and in the assessment of plant extract for safety or toxicity risk. In this study, the enzymes considered are useful marker enzymes of liver cytolysis or damage to plasma membrane of liver [20].

The indicators of liver functions were all increased significantly after extract administration. The ALT appeared to be highly significant especially at 450 mg/kg body weight indicating possible liver injury. An elevated ALT level is a sensitive clinical indicator of hepatocellular injury [21]. A similar finding was observed following administration of aqueous extract of *Balanite aegyptiaca* stem-bark in rats [22].

An increase in the level of ALT is usually accompanied by the elevation in the level of AST which plays a role in the conversion of amino acid to keto acid. Primary and secondary hepatic injury causes elevation of both enzymes. Both ALT and AST are excellent markers of liver damage caused by exposure to toxic substance [23]. AST is not specific for liver only but also located in other organs like kidney, heart, brain and skeletal muscles [24]. ALT is the more liver specific enzyme for diagnostic use. When the integrity of the hepatocellular membrane is compromised, there is extrusion of the enzyme in to the

plasma [25], hence the significant increased level of ALT in all the groups treated with the extracts. This increase is an indication of hepatocelluar damage caused by the extract [26].

The ALP is a marker enzyme for plasma membrane of the organ of study and it is often used to assess the integrity of plasma membrane [27]. Increases in the level of ALP in this study may be an indicator of either liver, kidney or bone disease, as they are main source of ALP [27]. The current findings showed an increased level of the ALP and this is also in line with other studies that reported an increased level of ALP following administration of the *Balanite aegyptiaca* [22].

Different types of cells in the body contain LDH and some of the organs relatively rich in in this enzyme are the heart, liver, kidney and muscle [28]. When cells die or tissue breakdown results in elevation of the LDH levels and serve as a major indicator of hemolysis [29]. The significant increase in the level of LDH observed in group administered 450 mg/kg might be as result of cell death (necrosis) observed in the liver and as well in the kidney tissues. Several studies have reported similar findings [30, 31].

It is known that, inhibition of G-6-PDH activity decreases Nicotinamide Adenine Dinucleotide Phosphate (NADPH), a co-enzyme that is essential for the protection against and a repair of oxidative damage [32]. Alteration of G-6-PDH will also alter the supply of energy to the cells, therefore the decrease in the level of G-6-PDH observed in all the experimental animals indicate an insufficient supply of energy to the organ and might also suggest lack of protection against oxidative damage, hence the histopathological lesions.

The histopathological changes observed in liver and kidney also appeared to be dose dependent. The presence of the pathological lesions may not be amazing since liver is the main organ for biotransformation and the kidney is the primary organ of excretion in the body, hence the two organs may have been exposed to the toxic or active substance(s) present in the extract. This is in line with a similar work where major lesions were observed in liver following administration of *Balanite aegyptiacia* in rats [22]. Findings from this study also agreed with the theory of drugs toxicity as all compound are toxic at high doses [33]. Liver and kidneys are most susceptible to damage by high doses extracts since metabolism of substances mostly takes place in the liver while excretion occurs through the kidneys.

5. Conclusion

From this study, it shows that, aqueous extract of *Balanite aegyptiaca* stem-bark at the dosages used and the duration possesses hepatotoxic properties due to the change in the level of activities of the liver enzymes and the histopathological lesion observed on the liver tissue. Moreover, the extract brought about changes in the biochemical parameter (urea and creatinine) in addition to lesions to kidney. In view of this, it is suggested that, the stem-bark of *Balanite aegyptiaca* should be used with caution and at lower dosage.

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