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Effect of Aqueous Leaves Extract of *Balanite Aegyptiaca* on Some Biochemical and Haematological Parameters in Wistar Rats.

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Abstract

There is exponential interest in phytotherapy and the demands for information on the effect and implications of the various plant material used in the management of diseases because it is scanty particularly in most developing countries. This study is aimed at determining the effect of aqueous leaves extract of *Balanite aegyptiaca* on some biochemical and haematological parameters in Wistar rats. A total of twenty-eight (28) Wistar rats were used in the study (twelve was use for acute toxicity studies, with very high concentrations of Aqueous extract of *Balanite aegyptiaca* no mortality was recorded). Sixteen (16) Wistar rats were divided into four (4) groups. Group I served as control, group II to IV were administered orally with 300, 400 and 500mg/kg aqueous leaves extract of *Balanite aegyptiaca* daily for 21days. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), total bilirubin (TB), direct bilirubin (DB), albumin, total protein, electrolytes (K^+ , Na^+ , Cl^- , Ca^{2+}), urea, creatinine and some haematological parameters (WBC, RBC, HCT, MCV, MCH, MCHC), were analysed using standard laboratory Techniques. The results of the acute toxicity studies indicate that this extract is well tolerated at doses as high as 5000mg/kg body weight. The result of the study showed that, the total protein, creatinine, haematocrit and haemoglobin showed a significant increase ($p<0.05$) with increased dose when compared with the control. However, no significant differences in other parameters ($p>0.05$) was observed when compared with the control. Based on the result of this study, it may be considered that aqueous leaves extract of *Balanites aegyptiaca*

administered for up to 21 days may not be hepatotoxic, does not alter electrolyte and some haematological parameters. Its ability to increase erythrocyte may make it useful in management of mild anaemia. Aqueous leaves extract may be nephrotoxic with increased creatinine levels following an increase in the dose and prolong usage.

Key words: Aqueous extract, *Balanite aegyptiaca* leaves, creatinine, Enzyme activity, Electrolyte, Haematocrit, Haemoglobin, Kidney, Liver.

Introduction

For the period of around thousands of years, plants were utilized as the basis of the traditional medicine systems in Nigeria, India, China, Indonesia etc. (Hammer, 1999). They were also utilized as the source for potent and powerful drugs (Srivastava *et al.*, 1996). Even the developing countries have initiated the adaptation of the traditional medical practice to be an integral part of their culture. Plants still remains the primary source of supply of many important drugs and minerals in the orthodox medicine today. Plants are being exploited for its active compound which is responsible for their biological function (Clark, 1996). In Nigeria, both herbal traditional and orthodox medicines are practiced, though; the



conventionally trained medical doctors are contemplating the former. Despite the fact that traditional approach to health care delivery system is popularly practiced and in spite of the availability of the medicinal herbs in our local markets, there are very few if any, in government health centres. Thus, it is believed that there are problems due to lack of adequate information of the subject, the crudity of the medicinal plant and technique involved in the traditional medicine (Aliyu, 2006). Medicinal plants play a significant role in providing primary health care services to rural people, they serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine (Sofowora, 1993).

One among the promising plant is the *Balanites aegyptiaca* (L.) Delile which belongs to the family of *Balanitaceae*. It is found to be an evergreen xerophytic tree of tremendous medicinal importance and was found to be distributed throughout the dried parts of Nigeria (Yadav and Panghal, 2010). It is commonly known as the Desert date, 'Aduwa' in Hausa, 'enyi-ndi- mmuo' in Igbo, and 'Tanni' in Fulani (Ugwah *et al.*, 2016). It's a desert plant that is widely distributed in the arid zone of Nigeria. It is also found in other African countries like Senegal and Sudan, as well as in India (Chothani and Vaghasiya, 2011; Pandey, 2005). In India, Nigeria and Asia, it was reported to be used in a variety of folk medicines. Various parts of the plants have been used in the Ayurvedic and other folk medicines in order to treat different ailments which includes syphilis, jaundice, liver and spleen problems, epilepsy and yellow fever. The plant also possesses several biological activities such as insecticidal, anthelmintic, antifeedant, molluscicidal and contraceptive activities (Yadav and Panghal, 2010). They were also used in the treatment of illnesses such as laxative, diarrhoea, haemorrhoid and stomach aches (Ojo *et al.*, 2006). It also contains steroids (saponins, sapogenins, diosgenins) used as raw materials for industrial production of contraceptive pills, corticoids, anabolisants and other sexual hormones (Kavita and Asha, 2014). All parts of

the tree have a medicinal use including fruits, seeds, barks and roots. The most important is steroidal saponins, which yield diosgenin, a source of steroidal drugs, such as corticosteroids, contraceptives and sex hormones (Farid *et al.*, 2002). Despite the extensive use of the different parts of this plant for consumption and management of various diseases by local herbalist, there are paucity of information with regards it effect in the body. This study is aimed at determining the effect of aqueous leaves extract of *balaniteaegyptiaca* on some biochemical and haematological parameters in Wistar rats.

Material and Methods

Experimental Animals: Twenty-eight (28) adult Wistar rats weighing between 180-240g were purchased from the animal house of Department of Physiology, Bayero University Kano, Nigeria and were allowed to acclimatize for two weeks before the commencement of experiment. The animals were grouped into five groups including control of 4 rats each and they were fed pelletized growers' feed (Vital Feed, Jos, Nigeria) and allowed access to water *ad libitum* throughout the experimental period. The principles of laboratory animal care guidelines were adopted (Axel and Paul, 1994).

Preparation of Extract

The plant material was washed under running tap water to remove the surface pollutants and the whole plants leaves were allowed to air dried under shade. It was shade-dried and made into a coarse powder which will pass through a 40-mesh sieve to get a uniform particle size and then used for aqueous extraction. A weighed quantity of the powder containing 100g was dissolved into 500ml of distilled water and allowed to stand for 48-72hrs. It was filtered through Whatman filter paper No. 1 and distilled using Rotary evaporator at 60°C until aqueous free solid powder (lyophilisation) is obtained. The resulting extracts was subsequently labelled as aqueous extracts and preserved at 5°C in airtight bottles until further use, according to the method described by Gberikon *et al.* (2015).

Experimental design

A total of 28 Wistar rats were used for the study. The rats were divided into groups (I-IV) and each group contains four rats. Extracts were administered orally, Group I was normal untreated rats (Animal control), while group II-IV were administered 300mg/kg, 400mg/kg and 500mg/kg of *aqueous leaves extract of Balanite aegyptiaca* respectively. The extracts were reconstituted in distilled water, and administered orally on daily basis by gastric intubation for 21 days. At the end of 21 days, the animals were anaesthetized using chloroform and bled by cardiac puncture 24 hours after the last treatment.

Determination of LD₅₀

The lethal dose (LD₅₀) was determined by method of Lorkes (1983). The LD₅₀ study dose was done in two phases as follows: First Phase requires nine Wistar rats. The nine animals were divided into three groups of three animals each. Each group of animals were administered different doses (10,100 and 1000 mg/kg) of *Balanite aegyptiaca* leaves. The animals were placed under observation for 24 hours to monitor their behaviour (Alertness, Feeding, weakness etc) as well as if mortality will occur. Second phase involves the use of three animals, which was distributed into three groups of one animal each. The animals were administered higher doses (1600, 2900 and 5000 mg/kg) of *Balanite aegyptiaca* leaves and was observed for 24 hours for behaviour as well as mortality (Table 2). Then the LD₅₀ was calculated by the formula below:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D₀ = Higher dose that gave no mortality,

D₁₀₀ = Lower dose that produced mortality

Biochemical and Haematological Analysis

The blood samples were collected by cardiac puncture in ethylene diamine tetra acetic acid (EDTA) for haematological analysis and plain specimen bottles which is allowed to clot and

the serum separated by centrifugation at 3000rpm for 10 minutes and then subjected to biochemical parameters analysis. EDTA sample was used for full blood count using haematology autoanalyser (Nortek Genesis Tm HA6000, China), while the serum was used for the analyses of urea by Randox Urease-Berthelot method as described by Weatherburn (1967), creatinine using Jaffes method as described by Pardue *et al.* (1987), sodium ion, potassium ion, chloride ion, and calcium ion using ion-selective electrode (AC9900 Automatic Electrolyte Analyzer, China), AST and ALT activities were determined by the methods of Reitman and Frankel (1957), ALP by Roy (1970) and Demetrious (1974) method, bilirubin levels were determined using Jendrassik and Grof (1938) method, Total protein and Albumin were determined using Grant (1987) method.

Statistical Analysis

The statistical analysis was carried out using SPSS 20 software package. The results obtained are presented as mean \pm standard deviation (M \pm SD). A one-way analysis of variance (ANOVA) was used for the data analysis. Significant differences between groups were detected using Bonferroni test at (p \leq 0.05).

Results

The results obtained from the current study are presented in tables 1, 2, 3 and 4 respectively. Table 1 below shows the mean value of the activities of AST, ALT, ALP and Bilirubin (Total bilirubin and Direct Bilirubin) in the treatment and control rats. The result show there is no significant difference between the treatment groups (II-IV) and control- Total protein significantly increased in the treatment groups compared to the control (p< 0.05). However, there is no significance difference in serum albumin between the treatment groups compared to the control as shown in table 2. Depicted from table 3, urea and creatinine significantly increased in the treatment groups compared to the control (p< 0.05). However, there no significance difference in potassium, sodium, chloride, and calcium in both groups (p >0.05). HGB and HCT significantly increased



in the treatment groups compared to the control (p < 0.05), but no significant difference between the groups in WBC, RBC, MCV, MCH and MCHC (p>0.05) (Table 4).

Table 1: Effect of oral administration of Aqueous extract of *Balanites aegyptiaca* leaf on liver enzymes and bilirubin in Wistar rats fed with different doses

Animals	Dose (mg/kg)	AST (IU/l)	ALT(IU/l)	ALP(IU/l)	BILIRUBIN ($\mu\text{mol/l}$)	
					TB	DB
Group I (Control) n=4	0	193.75 \pm 28.76	74.5 \pm 22.28	7.75 \pm 2.29	3.00 \pm 0.82	1.75 \pm 0.50
Group II n=4	300	237.25 \pm 23.51	79.00 \pm 8.45	11.00 \pm 2.16	3.25 \pm 0.96	1.50 \pm 0.58
Group III n=4	400	190.00 \pm 28.76	79.25 \pm 13.82	10.00 \pm 2.96	4.00 \pm 1.41	2.00 \pm 0.82
Group IV n=4	500	187.25 \pm 40.90	101.25 \pm 27.62	12.50 \pm 1.29	4.75 \pm 1.26	2.25 \pm 0.50

Results are presented as mean \pm standard deviation; 'n' signifies the number of Rats; 'TB' Total Bilirubin; 'DB' Direct Bilirubin

Table 2: Effect of the extract on Total protein and Albumin in Wistar rats fed with different doses

Animals	Dose(mg/kg)	T/P(g/l)	ALB(g/l)
Group I (Control) n=4	0	62.25 \pm 4.27 ^a	35.25 \pm 1.5
Group II n=4	300	63.25 \pm 1.71	36.5 \pm 1.29
Group III n=4	400	68.25 \pm 4.11 ^a	34.00 \pm 0.82
Group IV n=4	500	69.75 \pm 4.11 ^a	35.00 \pm 1.16

Results are presented as mean \pm standard deviation; 'n' signifies the number of Wistar Rats. T/P' Total Protein; 'ALB' Albumin; Values bearing superscript 'a' in the same column are statistically significant at 5% (i.e. p < 0.05) level of significance.

Table 3: Effect of the extract on Some Electrolytes, urea and creatinine in Wistar rats fed with different doses

Animals	Dose (mg/kg)	K ⁺ (mmol/l)	Na ⁺ (mmol/l)	Cl ⁻ (mmol/l)	Ca ²⁺ (mmol/l)	UREA (mmol/l)	CRTN (µmol/l)
Group I (Control) n=4	0	5.53±0.55	137.75±2.87	104.00±2.31	2.30±0.06	3.78±1.70 ^a	40.50±7.42 ^a
Group II n=4	300	5.88±0.65	138.25±3.30	104.05±2.65	2.27±0.07	5.05±1.12	54.50±9.04
Group III n=4	400	5.50±0.58	137.00±2.45	103.25±3.50	2.31±0.09	6.58±2.33 ^a	56.50±5.07
Group IV n=4	500	5.65±0.10	135.00±2.94	102.75±2.99	2.24±0.08	5.28±1.01	61.75±10.37 ^a

Results are presented as mean ± standard deviation; ‘n’ signifies the number of Rats. ‘K⁺, Potassium;’ Na⁺ ‘Sodium;’ Cl⁻ Chloride; ‘Ca²⁺’ Calcium; ‘CRTN’ Creatinine; Values bearing superscript ‘a’ in the same column are statistically significant at 5% (i.e. p<0.05) level of significance.

Table 4: Effect of extract on Some Haematological parameter in Wistar rats fed with different doses

Animals	Dose (mg/kg)	WBC (10 ⁹ /L)	HGB (g/dL)	RBC (10 ¹² /L)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Group I (control) n = 4	0	2.08±0.76	9.48±1.08 ^a	5.14±0.57	23.03±5.00 ^a	44.50±4.67	18.45±2.19	42.25±8.75
Group II n = 4	300	2.24±0.18	9.76±0.45 ^a	4.90±0.50	23.41±0.51	43.02±1.72	19.32±1.38	45.00±5.10
Group III n = 4	400	1.50±1.14	10.45±2.70 ^a	3.74±1.49	25.69±2.24 ^a	41.80±2.16	18.73±1.84	45.38±6.47
Group IV n = 4	500	3.55±1.83	11.34±0.82 ^a	5.12±2.10	27.37±1.23 ^a	41.28±4.27	21.38±3.48	53.03±13.2

Results are presented as mean ± standard deviation; ‘n’ signifies the number of rats. ‘WBC’ White Blood count; ‘HGB’ Haemoglobin; ‘RBC’ Red Blood Cell; ‘HCT’ Haematocrit or Pack Cell Volume; ‘MCV’ Mean Corpuscular Volume; ‘MCH’ Mean Corpuscular Haemoglobin; ‘MCHC’ Mean Corpuscular Haemoglobin Concentration. Values bearing superscript; ‘a’ in the same column are statistically significant at 5% (p <0.05).

Discussion

The oral acute toxicity study of aqueous extract of *Balanites aegyptiaca* leaves carried out among Wistar rats at a dose of 5000 mg/kg body weight showed no major toxic effects. This suggests that the extract of *Balanites aegyptiaca* may be relatively safe with LD₅₀ greater than 5000 mg/kg. This is because any compound or pharmaceutical with an oral LD₅₀ greater than 1000 mg/kg is considered safe or to be of low toxicity (Ugwah *et al.*, 2016). The administration of aqueous extract of *Balanites aegyptiaca* left for 21 days at 300, 400 and

500mg/kg orally showed no significant differences in AST, ALT and ALP. High level of AST and ALT are usually present within hepatocytes and plasma levels increase when hepatocytes membrane integrity is disturbed during hepatocellular injury (Dobbs *et al.*, 2003; Kew, 2000). The absence of significant changes in AST, ALT and ALP may suggest that there is no hepatotoxic effect in the rat administered aqueous *Balanites Aegyptiaca* leaves extracts. According to Paracelsus (the Father of toxicology) all things are poison and nothing is without poison, only the dose makes a thing not



poison (Paracelsus, 2001). The administration of aqueous extract of *Balanites aegyptiaca* leaves in Wistar rats showed a significant increase in the Total protein and no significant changes in the Albumin. This elevation in total protein may be due to the rich protein components of the leaves. This is similar to the report given by Samuel *et al.* (1997). The potassium, sodium, chloride, and calcium showed no significant change when compared with control ($p > 0.05$). Creatinine and urea showed a significant difference when compared with control ($p < 0.05$) and the values of creatinine increases with increase in the dose of aqueous extract of *Balanite aegyptiaca*. The elevation in the serum creatinine and urea concentration may reflect a reduction in the glomerular filtration rate (GFR) and subsequently nephrotoxicity (Manpreet and Antoine, 2012). Therefore, *Balanite aegyptiaca* leaves which contain the Arginine, Glycine and Methionine may be responsible for the formation of creatine in the body (David and Christopher, 2001).

This study shown that HGB and HCT in group II, III and IV showed a significant difference when compared with control ($p < 0.05$). Haemoglobin reacts with oxygen carried in the erythrocyte to form oxyhaemoglobin during respiration (Chineke *et al.*, 2006) The significant change in HGB and HCT without changes in MCV, MCH MCHC may be as a result of the low dose in mg/kg used in the study. The level of HGB and HCT tends to increase with dose. This shows that the aqueous extract of *Balanite aegyptiaca* may be used managing anaemic patients (Aster, 2004). The increase could be due to some phytochemical constituents which may be present in the plant.

Conclusion

The result of this study indicated that the daily oral administration of the aqueous leaves extracts of *Balanites aegyptiaca* for up to 21 days did not show any alteration in liver enzymes, electrolyte and some haematological parameters. Therefore, it didn't have hepatotoxicity, but could have nephrotoxic effects on increase dose and prolong usage due

to the increase in urea and creatinine concentration. It also showed that, *Balanites aegyptiaca* has the ability to increase erythrocyte, which may be useful in the management of mild anaemia.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Consent

It is not applicable.

Authors Contribution

All authors contributed significantly to the writing of the manuscript. All authors read and approved the final manuscript.

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