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Evaluation of the toxicological status of *Balanite aegyptiaca* seed oil

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Abstract
A total of fourteen rats were fed with diets containing either 10 % groundnut oil (control group) or 10 % *Balanites aegyptiaca* seed oil (experimental group) for six weeks. At the end of the experiment, the animals were sacrificed and blood samples and some organs of the rats in both groups were collected for analysis. The albino rats appeared to suffer no toxicological effect and weekly monitoring of the rats showed good physical appearance. The rats in the experimental groups displayed fairly similar body weight gain when compared with those from the control group. There was no significant difference between the haematological and histopathological results obtained for both the experimental and control groups except for the liver of two of the rats in the experimental that showed some lesion. There might be need for refining of the seed oil before it can be safe for animal/human consumption.
Key words: *Balanites aegyptiaca*; feed; haematological analysis; seed oil

1. Introduction

Balanites aegyptiaca is a perennial tropical plant that belongs to the family *Balanitecea*. They may be grown for both its fibre and oil. In Africa and some developing countries, it is used for food preparations and herbal medicine. Extracts from several parts of this tree have been intensively used in Africa and India for various ethno botanical purposes. It is called desert date (English), Adowa (Yoruba, Nigeria), adua or Aduwa (Hausa, Nigeria), tanni (Fulfulde, Nigeria), cungo (Kanuri, Nigeria) and heglig (Arabic). The plant attains a height of more than 6 m. It has a multiplicity of uses and almost every part of the plant is useful including, leaves, thorns, back of root and fruit. *B. aegyptiaca* has been used over thousands of years. The seed oil of *Balanites aegyptiaca* has been used in Nigeria as ingredient and substitute to groundnut oil as food supplement and also in traditional medicine. The fleshy pulp of the fruit is eaten fresh or dried. It contains 64 – 72 % carbohydrates, plus crude protein, steroidal saponins, vitamin C, ethanol and other minerals (Abu Al-Futuh, 1983). 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 corticosteriods, contraceptives and sex hormones (Farid *et al.*, 2002; FAO, 1985).

Balanites seed kernel is considered as an extremely useful edible product. It contains good quality oil and high protein content (Mohamed *et al.*, 2002; Abu Al- Futuh, 1983). The debittered kernel is used as snacks by humans. The extracted oil is used for many uses and the remaining cake is used as animal feed; both fruits and kernel are widely used in many countries during the dry season and drought periods including Nigeria (Lockett *et al.*, 2000). The objective of this study is to assess the toxicity effect, if any, of total replacement of groundnut oil with *B. aegyptiaca* seed oil in albino rat feed. This is in continuation of previous work on seed oils/cake and their nutritional /industrial applications (Ajayi *et al*., 2004; 2012a and 2012b).

1.1 Material and methods

1.1.1 Seed material

B. aegyptiaca seeds used for this study were purchased from Tundun wada market in Zaria, Kaduna State of Nigeria. The seeds were identified in the botanical garden of the University of Ibadan. The seeds were screened to remove the defectives ones and soaked in a large bowl of water overnight to remove the glycoside pulp from seed coats. The seeds were sun dried and then crushed by a metal hammer to obtain its kernel.

1.1.2 Sample preparation

The seed kernel of dried *Balanite aegyptiaca* was grounded using mortar and pestle to increase extent of extraction of the oil. Oil was extracted from the seeds by solvent method using soxhlet extractor and n-hexane (b.pt 67-68 ^{o}C) as the solvent. The solvent was distilled off from the mixture by distillation and the oil was recovered for further analysis.

1.1.3 Proximate analysis of compounded feeds

The moisture content of the compounded feeds was determined by drying a representative 2 g sample in an oven with air circulation at 105 ^{o}C, Nitrogen content was estimated by kjedhal method; crude protein was calculated by multiplying the evaluated N by 6.25 while crude fat and ash were analyzed according to AOAC (1990). Carbohydrate was content was determined by difference [100- (protein + ash + crude fat + crude fibre + moisture content)].

1.1.4 Feed compoundment

Feed was formulated to meet the entire nutrient requirement for young rats. The feed was prepared according to the formula and procedure used by Toyomizu *et al.* (2003) with little modification. The basic ingredient used were: maize (40 %), soybeans (18.21 %), bone (3.3 %), salt (0.79 %), groundnut cake (4.2 %), palm kernel cake (7.08 %), wheat (7.08 %), corn bran (7.08 %), extracted oil (10 %), and oyster shell (2.26 %). The above were used to compound feed for experimental group (B), while in the control group (A), the desert date oil was replaced with groundnut oil. Feed intakes were recorded daily while the body weights were recorded weekly.

1.1.5 Animal, diets and feeding

Fourteen albino rats (weighing between 60-80 g) were used for this experiment. They were obtained from the animal house of Veterinary Department, University of Ibadan, Nigeria. The animals were divided into two groups (A and B) of seven rats per group and were fed for a period of 6 weeks before sacrifice. They were allowed to acclimatize for two weeks before commencement of the experiment. During the 6 weeks of the experiment, the rats were fed the compounded diets with unrestricted access to water. At the end of feeding period of six weeks, the rats were fasted for twelve hours overnight and blood collected by heart puncture under diethyl ether anesthesia for serum chemistry. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were determined by the method of Reitman and Frankel (1957) using commercial kits (Randox laboratories Co Atrium, UK). Serum creatinine, urea, total protein, albumin, and globulins and albumin/globulin ratio (A/G) were also determined (Chawla, 1999).

1.1.6 Haematological analysis

Haematological analyses were carried out in about 3 ml of rat blood collected into EDTA bottles through ocular puncture. The packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC), white blood cell (WBC), differential WBC counts, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were determined and calculated respectively using the standard technique as described by Jain (1986).

1.1.7Tissue pathology

Histological analyses of the heart, liver, kidney, lungs, intestine, spleen and brain samples were carried out. Small portions of these tissues already harvested and stored in formalin were fixed and put through series of dehydration in graded concentration of xylene. They were embedded in wax, sectioned at 5 µ and transferred to clean glass slides. The thin sections were stained with haemotoxylin and eosin (H and E) dyes for examination under the light microscope for histological changes following the method outlined by Jain (1986).

1.1.8 Statistical analysis

Results were expressed as mean standard error. Organ weights, biochemical and hematological determinations were analyzed by student's t-test. A probability level of $p < 0.05$ was considered significant.

1.2 Result and discussion

1.2.1 Proximate analysis

The result of the proximate composition of the control and experimental diets are: moisture content (7.23±0.06 % and 6.66±0.15 %); ash content (8.650±0.11 % and 9.17±0.04 %); crude protein (25.32±0.13 % and 25.03±0.12 %); crude fibre (10.61±0.06 % and 10.61±0.03 %); crude fat (12.32±0.02 % and 11.39±0.02 %) and total carbohydrate (46.43±0.17 % and 47.74±0.07 %) respectively. The protein contents of both diets are reasonably high; this suggests *B. aegyptiaca* seed oil as useful supplement in compounding animal feed. It was observed that there was an increase in the carbohydrate and ash contents of the experimental diet when compared to that of the control feed. Also, a decrease was noticed in the moisture content and crude fat while the crude fibre and crude protein content remained the same. Interestingly, moisture, carbohydrate, crude fat and ash contents were not significantly different in both diets labeled groups A and B.

1.2.2 Body weight and feed consumption

The weight and food consumption of rats treated with crude *B. aegyptiaca* seed oil are presented in Table 2. There were no significant ($p>0.05$) changes in the body weight and food consumption between control and experimental groups. The effect of food consumption on body weight gain has already been studied (Coleman *et al.*, 1997; Hubert *et al.*, 2000 and Moriyama *et al.*, 2006). In our findings, no treatment related changes were observed in food consumption and final body weight of the animals, which may imply that consumption of the diet mixed with the oil, had no negative effect on rat appetite.

1.2.3 Physical appearance

The physical appearance of the rats was normal throughout the six weeks of the experiment (Table 3). The eyes and the mouth of the animals from each group appeared normal throughout the period of the study.

1.2.4 Organ weight

The weight of the seven organs collected for tissue pathology did not differ significantly from each other in both groups (Fig. 1). An average kidney weight of 0.82±0.15 g was noted for control rats while 0.83±0.11 g was noted for the experimental rats. Ajayi and Aghanu (2012) had similar report for defatted residue of *G. mangostana*.

1.2.5 Haematological analysis

Presented on Table 4 are the haematological parameters of both the test and control rats. There was significant difference in the platelet count, absolute eosinophyl and absolute monocyte. All the other haematological parameters did not differ significantly from each other in the two groups thus indicating that the diet compounded with *B. aegyptiaca* seed oil had no adverse effect on the blood of the rats under study since it compared favourably with the indices obtained for the diet compounded with groundnut oil. The haematological values obtained from this study are similar to what was reported in the toxicity study of defatted residue in rats (Ajayi and Aghanu, 2012).

1.2.6 Histological analysis

There were no pathological changes in all the heart and kidney of the rats from both groups at histology level. *B. aegyptiaca* seed oil appeared not to have adverse effect on the rats; it actually supported their gradual growth. However, there was a focal and moderate vacuolar degeneration in three-quater of the liver of one of the rats in the experimental group (Fig. 3) and several in one-quarter of another rat still in the experimental group (Fig. 4).

1.2.7 Serum biochemical parameters

Fig. 2 shows the result of serum biochemical parameters in rats treated with crude *B. aegyptiaca* seed oil for six weeks. The result showed no significant ($p>0.05$) changes in serum ALT and AST activities, total protein, albumin and A/G ratio between the control and treated groups. The absence of significant changes in ALT and other related indicators such as AST, total protein, albumin and A/G ratio may suggest that the hepatotoxic effect of the oil on the rats was mild. High levels of AST and ALT are usually present within hepatocytes and plasma levels rise as hepatocytes membrane integrity is disturbed during hepatocellular cell injury (Kew, 2000 and Dobbs, 2003). The changes in serum urea and creatinine were statistically insignificant ($p> 0.05$). These parameters are indicators of kidney injury and are elevated in renal toxicity (Chawla, 1999). The insignificant rise in serum urea and creatinine is suggestive of normal functional kidney.

1.2.8 Serum Chemistry

The two groups gave non- significantly different mean values for each of measured parameters except total glycerol (Table 5). The significance was determined at the level of $P < 0.05$. Activities of total glycerol in the control group was significantly higher than that of the experimental group while there was absence of significant changes in other parameter like total cholesterol, HDL, and LDL respectively.

Conclusion

The overall result revealed that it is probable that *B. aegyptiaca* seed oil could successfully be used to totally replace groundnut oil in rat diet with other supplements after refining it. This will reduce the competition on groundnut oil.

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Table 1. Result of proximate composition of control and experimental feed

Parameter (%)	Control	Experimental
Moisture content	7.23 ± 0.06[a]	6.66 ± 0.15[b]
Crude protein	25.32 ± 0.13[a]	25.03 ± 0.12[b]
Crude fat	12.32 ± 0.02[a]	11.39 ± 0.02[a]
Crude fibre	10.61 ± 0.06[a]	10.61 ± 0.03[a]
Ash content	8.65 ± 0.11[a]	9.17 ± 0.04[b]
Carbohydrate content	46.43 ± 0.17[a]	47.74 ± 0.07[b]

*Values are expressed as mean ± SD; Means in the same row having the same letter are not significantly different (P <0.05)

Table 2. Average weight increase of rats and feed consumptions (g)

Week	Control group		Experimental group	
	Animals	Feeds	Animals	Feeds
0	71.43 ± 4.39	0	77.14 ± 3.02	0
1	93.57 ± 9.88	49.39 ± 13.26	102.43 ± 9.38	52.57 ± 13.36
2	111.86 ± 9.70	66.00 ± 10.23	115.00 ± 6.45	82.57 ± 14.36
3	109.29 ± 8.38	74.29 ± 7.86	113.57 ± 7.48	76.14 ± 12.94
4	122.14 ± 11.13	70.00 ±5.00	124.71 ± 7.45	79.00 ±4.86
5	126.43 ± 13.45	66.43 ±11.80	127.85 ± 10.74	69.71 ± 10.75
6	133.33 ± 13.29	62.14 ± 9.94	141.43 ± 11.07	68.57 ± 9.45

*Values are expressed as mean ± SD

Table 3. Weekly physical appearance of rats in the control and experimental groups

Week	Eye		Hair		Mouth	
	Control	Experimental	Control	Experimental	Control	Experimental
1	+++	+++	+++	+++	+++	+++
2	+++	+++	+++	+++	+++	+++
3	+++	+++	+++	+++	+++	+++
4	+++	+++	+++	+++	+++	+++
5	+++	+++	+++	+++	+++	+++
6	+++	+++	+++	+++	+++	+++

+++: Normal

Table 4. Result of Haematological analysis

Parameter	Control group	Experimental group
PCV	40.50 ± 2.07[a]	38.67 ± 3.08[a]
Hb	13.62 ± 0.68[a]	11.92 ± 2.91[a]
RBC	6.79 ± 0.30[a]	6.40 ± 0.64[a]
WBC	7325.00 ± 1865.41[a]	7900.00 ± 1467.99[a]
MCV	59.61 ± 0.82[a]	60.58 ± 1.54[a]
MCHC (%)	33.63 ± 0.52[a]	30.75 ± 6.68[a]
MCH	20.04 ± 0.16[a]	18.63 ± 4.03[a]
Lymphocyte (%)	67.83 ± 5.42[a]	66.33 ± 6.53[a]
Neutrophyl (%)	27.50 ± 4.97[a]	28.33 ± 6.53[a]
Eosinophyl (%)	2.67 ± 0.82[a]	3.00 ± 1.26[a]
Monocyte (%)	2.00 ± 1.67[a]	2.33 ± 1.03[a]
Absolute Lymphocyte	4070.00 ± 325.15[a]	3980.00 ± 391.92[a]
Absolute Neutrophyl	1650.00 ± 298.19[a]	1700.00 ± 391.92[a]
Absolute Eosinophyl	160.00 ± 48.99[a]	180.00 ± 75.89[a]
Absolute Monocyte	120.00 ± 100.40[a]	140.00 ±61.97[a]
Platelet	136000.00 ± 54611.35[a]	150333.33 ± 36037.02[a]

Values are expressed as mean±SD
Values in the same row with different superscripts are significantly different at P< 0.05

Table 5. Result of biochemical analysis

Parameter	Control group	Experimental group
Total protein	7.77 ± 0.25[a]	8.20 ± 0.36[a]
Albumin	4.87 ± 0.21[a]	5.13 ± 2.08[a]
Globulin	2.90 ± 0.20[a]	3.07 ± 0.15[a]
Albumin/Globulin	1.67 ± 0.15[a]	1.60 ± 0.00[a]
AST	46.67 ± 4.04[a]	45.33 ± 5.69[a]
ALT	31.00 ± 1.00[a]	31.00 ± 2.65[a]
Urea	15.00 ± 1.00[a]	14.00 ± 1.00[a]
Creatinine	0.83 ± 0.15[a]	0.80 ± 0.20[a]

Values are expressed as mean±SD; Values in the same row with different superscripts are significantly different at P< 0.05

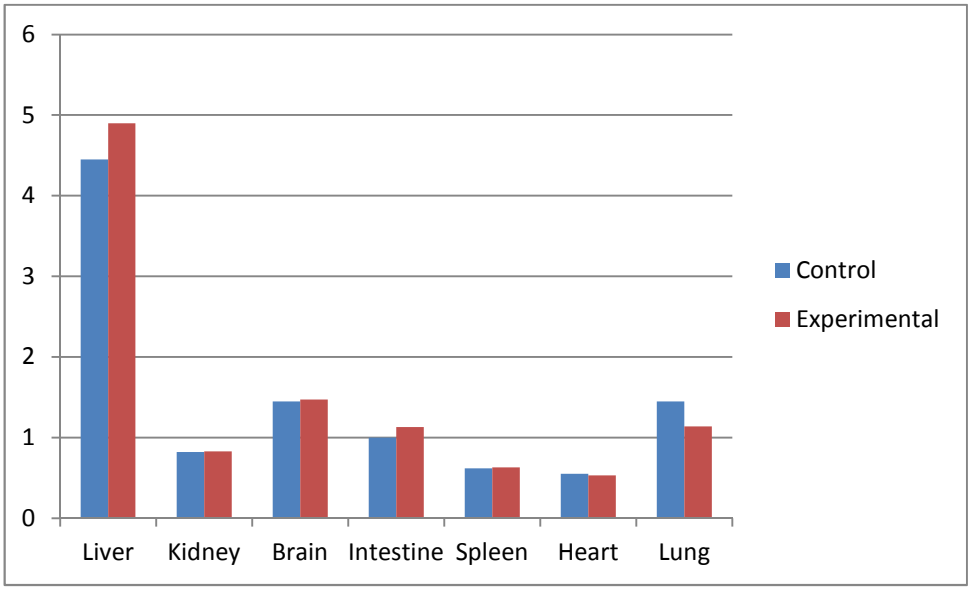

Figure 1. Graph showing the average weight of organs of rats in both control and experimental groups

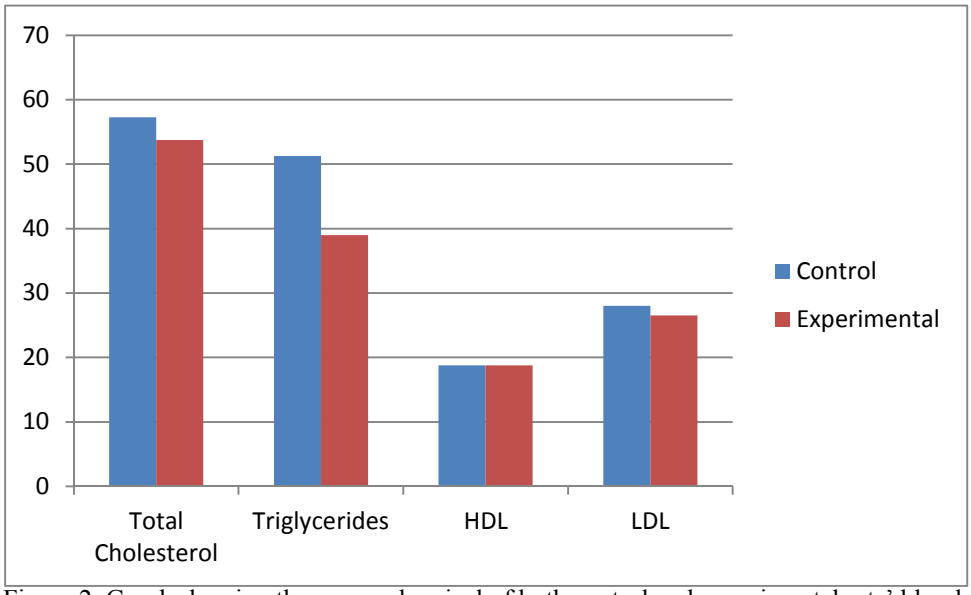

Figure 2. Graph showing the serum chemical of both control and experimental rats' blood

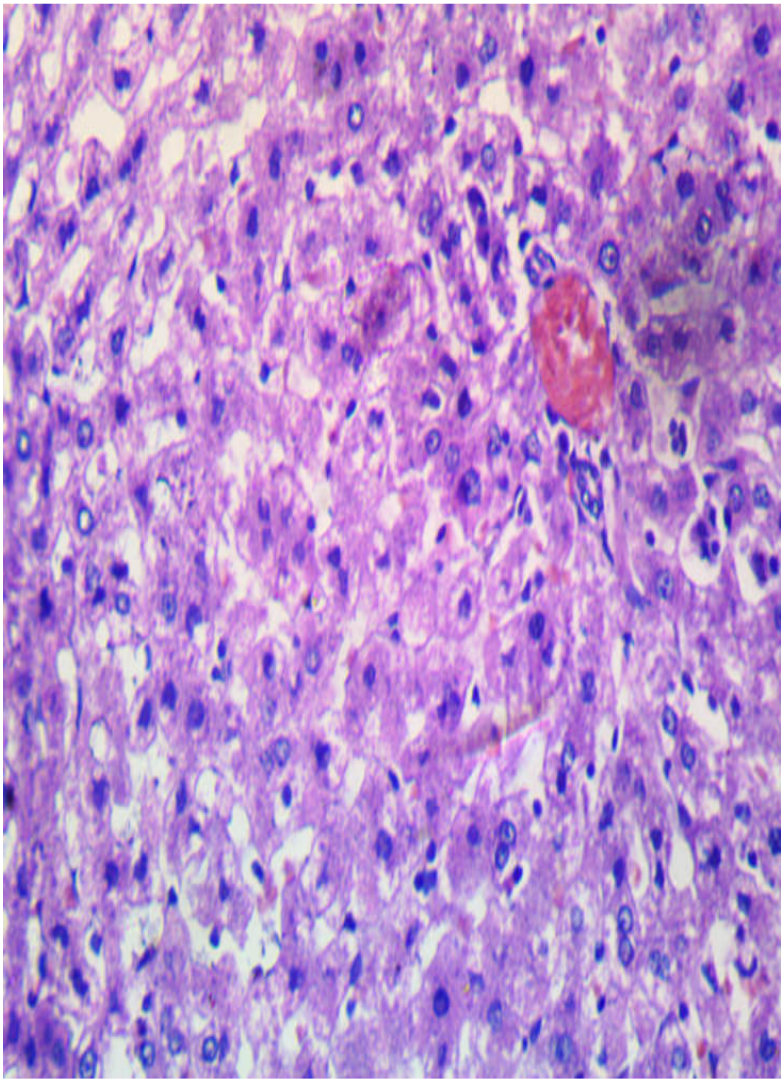

Figure 3. Photograph of liver of rat given 10% *Balanite aegyptiaca* seed oil in normal rat feed showing moderate vacuolar degeneration in three-quarter of the liver of one the rats in experimental groups. H and E x 650

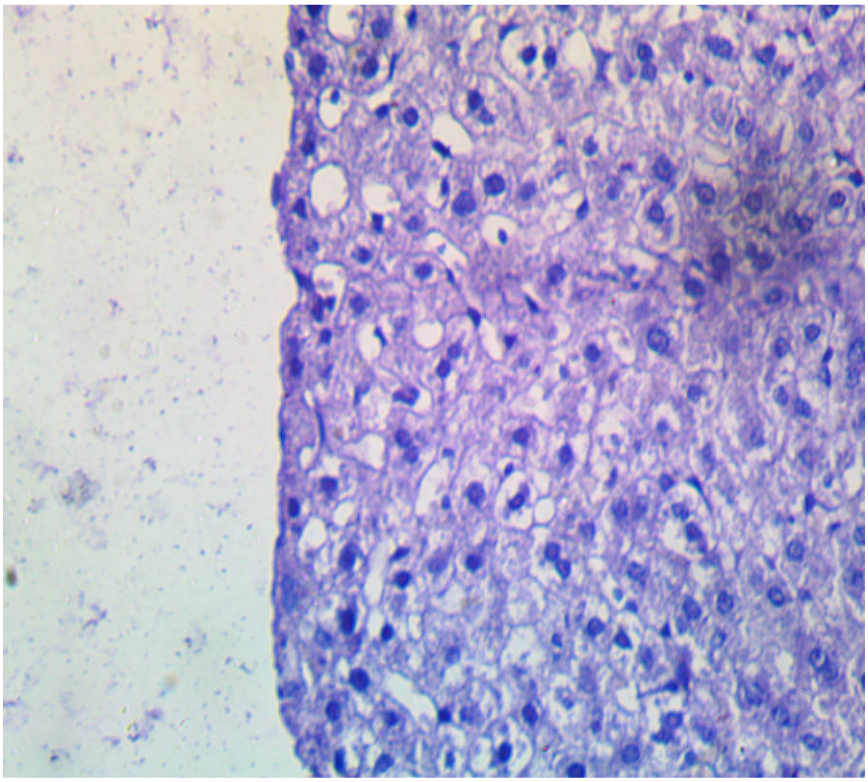

Figure 4. Photograph of liver of rat given 10% Balanite aegyptiaca oil in normal rat feed showing focal and several vacuolar degeneration of hepatocytes. H and E x 650