



## Effect of *Balanite aegyptiaca* Seeds Oil on Some Haematological and Serum Biochemical Parameters in Broiler Chickens

Amina Bulama Yusuf<sup>1\*</sup>, Hafsat Lawal Omar<sup>2</sup>, Abubakar B. Yusuf<sup>3</sup> and Idriss Audu Ibrahim<sup>4</sup>

<sup>1</sup>Department Of Biochemistry, Faculty of Science, Federal University Gashua,  
631001 Gashua, Yobe State, Nigeria.

<sup>2</sup>Department of Animal Health Technology, Audu Bako College of Agriculture Dambatta,  
702104 Dambatta, Kano State, Nigeria.

<sup>3</sup>Department of Integrated Science, College of Education and Legal Studies Nguru,  
Yobe 630261 Nguru, State, Nigeria.

<sup>4</sup>Department of Biochemistry, College of Medical Sciences, Yobe State University Damaturu,  
600213 Damaturu Yobe State, Nigeria.

\*Corresponding author:

Amina Bulama Yusuf  
Department of Biochemistry,  
Faculty of Science,  
Federal University Gashua,  
P.M.B 1005, Yobe State,  
Nigeria.

Email: [aminabyusuf.bch@fugashua.edu.ng](mailto:aminabyusuf.bch@fugashua.edu.ng)

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### ABSTRACT

This research was conducted to find out what role of *Balanite aegyptiaca* seeds oil on haematological and biochemical parameters using broiler chickens. Preliminary qualitative phytochemicals tests were carried out on the *B. aegyptiaca* seeds oil in accordance with accepted practices for detecting secondary metabolites. Forty chicks were employed in the study, and they were split into four different groups, each group containing 10 chicks and brood on normal diet for two weeks, after which each group were administered varying concentration of *B. aegyptiaca* seeds oil (1 mL, 1.5 mL and 2 mL), while in the Control group no oil was administered. Blood samples were collected and tested at the University of Maiduguri Veterinary College laboratory for haematological (PCV, HB, RBC and WBC) and biochemical parameters (AST, ALT, ALP, ALB and TP) were evaluated two weeks after treatment. The result of phytochemical analysis of *B. aegyptiaca* seeds oil reveals the presence of alkaloids, steroids, flavonoids, saponins and cardiac glycosides while tannins terpenoids, sterols, anthraquinones were not detected. The result of haematological parameters showed a significant increase in the levels of RBC, PCV, HB as well as WBC of the test samples when compared with the control group. This indicates that the administration of *B. aegyptiaca* seeds oil significantly ( $p < 0.05$ ) increases the level of all haematological parameters. However, the two test groups (2 and 3) were not significantly ( $p < 0.05$ ) different. Biochemical testing showed that serum albumin total protein and liver enzymes were not affected by *B. aegyptiaca* oil treatment ( $P > 0.05$ ) (alkaline aminotransferase, aspartate aminotransferase, and alkaline phosphatase). No adverse effects on liver enzyme functions were seen after administration of *B. aegyptiaca* seed oil, as values were comparable ( $P > 0.05$ ) between the treated and untreated groups.

### INTRODUCTION

There are two possible families to which the tree species *Balanite aegyptiaca* (*B. aegyptiaca*) belongs: the zygophyllaceae and the Balanitaceae. Several regions in Africa and the Middle East are the original homes of this tree. The states of Borno and Adamawa in Nigeria are where the plants are most likely to be found growing in the wild. The English common name for this plant is "desert date" [1,2]. It has several names in Arabic (*lalob*, *Hidjihi*,

*Inteishit*, etc.). The Hausa word for it is *adowa* (sometimes spelled *aduwa*) [3]. The *Balanite aegyptiaca* plant is widespread over Africa's Sahel Savannah. It is widespread because it thrives in a broad range of conditions, from sandy to heavy clay soils and dry to sub humid climates [4]. It can withstand scarcity of water, grazing cattle, and fire without much damage. The fruit is bitter and yellow with a solitary seed [4]. After oil is recovered, the leftover seed cake is often utilized as animal feed in Africa [5,6]. The seeds of *Balanite aegyptiaca* have molluscicide effect on

*Biomphalaria pfeiffer* [7]. Historical record also shows that the oil extracted from the fruits/seeds of the plant is known to be used in folk medicine. The oil of the plant is used in treating different types of disorders and diseases such as malaria, mental disease, sore throat, syphilis, yellow fever, epilepsy, wounds, rheumatism and Anaemia etc [8]. When tested on albino rats, pure saponin isolated from the fruit mesocarp and aqueous extract were described as hypoglycaemic agents. It has also been found to prevent the spread of *Escherichia coli* in rats [9]. Streptozotocin-induced diabetic mice benefited from treatment with an aqueous extract of the mesocarp of *B. aegyptiaca* fruits [10]. It has been found that *B. aegyptiaca*, as evidenced by the contraction of wounds, possesses significant wound-healing ability. The data also shown that the plant has powerful antioxidant activity by preventing lipid peroxidation, neutralizing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, and shielding fibroblast cells from oxidative damage [11].

Wistar albino rats given oral dosages of (150 and 300 mg/kg) *B. aegyptiaca* leaf extract in ethanol and methanolic solvent revealed a diuretic effect. The usual treatment was furosemide. The results reveal that both ethanol and methanol extracts significantly increase the volume of urine and the excretion of electrolytes ( $P < 0.001$ ) compared to the control group [12]. Paracetamol-induced hepatotoxicity is reportedly mitigated by the hepatoprotective effects of *B. aegyptiaca* ethanolic extract [13]. The purpose of this research was to examine the effect of *B. aegyptiaca* seeds oil on the haematological and serum biochemical parameters of broiler chickens.

## MATERIALS AND METHODS

### Collection of Plant Material

*Balanite aegyptiaca* (*B. aegyptiaca*) fruits were obtained from *Balanite aegyptiaca* trees within the campus area of Federal University Gashua, located at Gashua town, Yobe State, Nigeria. The plant was identified and authenticated in the Department of Biological Science Federal University Gashua. The fruit was then cracked to remove the kernel (seeds).

### Experimental Birds

One (1) day old broiler chick weighing approximately 45 g were sourced from a reputable vendor in Gashua. They were kept on deep litter and fed with commercial starter mash (Vital feed pelletized super starter) and given clean tap water ad-libitum for two weeks. In addition, they were stabilized with glucose and vitalyte an anti-stress containing mineral and vitamins in their drinking water.

### Oil extraction

The seeds were selected weighed (200 g) and grinded to powder form. After pounding, the oil was extracted from the powdered seed using N-hexane with a Soxhlet extractor apparatus. During the extraction of *B. aegyptiaca* oil, the seed (powdered seed) was poured into a sack, then put into a Soxhlet tubule and then heated to a temperature of 70 °C which yielded a positive result in getting the extract; 100 mL of pure oil from 200 g of powder was obtained. About 50 mL was used for the experiment (Soxhlet extraction method).

### Phytochemical Screening

Preliminary qualitative phytochemicals tests were carried out on the *B. aegyptiaca* seeds oil using standard methods and procedures to identify the presence of secondary metabolites according to the methods described by [14].

## Experimental Procedure

The experiment was conducted at the poultry unit of Directorate of University farm of Federal University Gashua, Yobe State. All experimental procedure were in accordance with the guidelines for care and use of animals in research [15]. The chicks were brooded for 2 weeks (14 days) in deep litter pens. The birds were weighed and then randomly distributed into four (4) groups of ten (10) birds each. Feed and fresh water were supplied ad libitum throughout the experiment.

Group 1 received standard diet and water and serve as control group. Group 2, in addition to the standard diet and water they received 1ml of the n-Hexane extract of *B. aegyptiaca* seed (oil) intramuscularly (IM) as a single dose and served as Test 1. While group 3 and 4 received in addition to the standard diet they were administered 1.5 mL and 2 mL of *Balanite aegyptiaca* oil as a single dose and served as Test 2 and Test 3 respectively. The *B. aegyptiaca* oil was injected using a 2 mL syringe and 23 gauge needle ½” – 1” inch in length. They were then observed for one week.

### Blood Sample Collection and Analysis

At the end of two weeks, 2mls of blood samples were collected from all the birds aseptically with sterile syringe and needle (23gauge needle ½” – 1” inch in length) from the wing vein (brachial) of the birds into an Ethylene Diamine Tetra Acetic Acid (EDTA) container for haematological analysis of Red Blood Cell (RBC), White Blood Cell (WBC), Haemoglobin (Hb) and packed cell volume (PCV). Another 2mls of blood samples were collected into a plain container without anticoagulant for serum biochemical parameters.

The samples were analyzed to determine the activities of liver enzymes such as Aspartate amino transferase (ASAT), Alanine amino transferase (ALAT), alkaline phosphatase (ALP), serum albumin (ALB) and Total protein (TP). The haematological analysis was determined according to the method described by Jain [16]. The blood sample for serum biochemical analysis were allowed to clot before centrifuging at 1100-1300 rpm for 15mins in a microcentrifuge to obtain the serum which was stored at -20°C until use. Bilirubin and total protein were estimated by the method described by [17] and [18] respectively. While liver enzymes were estimated according to enzymatic method described by Schmidt and Schmidt [19] using a commercial kit Randox laboratories ltd UK.

### Statistical Analysis

The results are presented in mean  $\pm$  standard deviation of triplicate, values obtained were subjected to one-way analysis of variance and test means were compared using Duncan multiple range test [20] at 5% level of significance.

## RESULTS AND DISCUSSION

Alkaloids, steroids, flavonoids, saponins, and cardiac glycosides were all found in *B. aegyptiaca* seed oil, but tannins, terpenoids, sterols, and anthraquinones were not (see **Table 1**). These results are consistent with those of other studies that have found similar things [21-24]. Saponins, which are oily glycosides found in many plants [24], are notorious for their bitter flavor, foaming properties in aqueous solution, and ability to hemolyze red blood cells (RBC) when injected into the blood stream [25]. However, when ingested into/eaten, saponins are practically not toxic to

warm-blooded animals, though it is assumed that higher doses will cause gastroenteritis [26,27].

**Table 1.** Results of phytochemical screening of *B. aegyptiaca* seeds oil.

Phytochemical	<i>B. aegyptiaca</i> Seed Oil
Alkaloids	+
Saponins	+
Tannins	-
Steroids	+
Sterols	+
Cardiac glycosides	+
Flavonoids	+
Terpenoids	-
Anthraquinones	-

Key: + = Present, - = Absent

Although alkaloids can be harmful in large doses, they are often not deadly and have a great physiological activity, making them ideal for medical applications [28]. Reduced blood pressure, tumor cell death, analgesic effects, and increased circulation and respiration are just some of the key physiological effects of alkaloids at lower dosages [27]. Flavonoids are a kind of chemical molecule used to impart taste to foods like vegetables and spices [28]. Anti-inflammatory, anti-viral, anti-oxidant, anti-cancer, anti-microbial and anti-diarrheal actions are only some of the purported beneficial benefits of flavonoids [27].

Numerous important medical, pharmacological, and agricultural activities are attributed to steroids [29]. These include immunosuppressive, antibacterial, hepatoprotective, helminth-killing, tumor-inhibiting, activators of plant growth hormone, cytotoxic effects on sex hormones, and cardiotoxic effects. For many years, clinicians have relied on members of the glycosides family to treat heart failure and arterial arrhythmia due to this group's well-established direct effect on the heart, which serves to both sustain and enhance the pace of contraction [30].

These phytochemicals may be responsible for the stimulation of RBC production that was observed in the study. The result of haematological parameters (see **Table 2**) indicates a significant increase in the levels of RBC, PCV, HB as well as WBC of the test samples when compared with the control group. This indicates that the administration of *B. aegyptiaca* seeds oil significantly ( $p < 0.05$ ) increases the level of all haematological parameters evaluated. Further perusal of reveals that the Hb  $6.07 \pm 0.80$  for the control and  $7.13 \pm 0.77$ ,  $8.47 \pm 0.69$ ,  $6.53 \pm 0.38$  for T1, T2 and T3 respectively, which indicates that *B. aegyptiaca* has stimulating effect on the production of haemoglobin.

**Table 2.** The effect of *Balanite aegyptiaca* seeds oil on some haematological parameters.

Groups	HB (g/dl)	PCV (%)	RBC ( $\times 10^6 \text{mm}^3$ )	WBC ( $\times 10^6 \text{mm}^3$ )
Control	$6.07 \pm 0.80^a$	$18.20 \pm 2.39^a$	$2.81 \pm 0.39^a$	$6.42 \pm 0.10^a$
Test 1	$7.13 \pm 0.77^b$	$21.40 \pm 2.30^b$	$3.38 \pm 0.29^{bc}$	$8.17 \pm 0.15^b$
Test 2	$8.47 \pm 0.69^c$	$25.40 \pm 2.07^c$	$3.46 \pm 0.35^{bc}$	$8.93 \pm 0.46^c$
Test 3	$6.53 \pm 0.38^d$	$19.60 \pm 1.14^d$	$2.96 \pm 0.18^d$	$8.44 \pm 0.32^d$

Results are Mean  $\pm$ SD of triplication. Values with different superscript down the column are significantly different ( $p < 0.05$ ), values with the same superscript are not significantly ( $p > 0.05$ ) different.

The level of packed cell volume and RBC also significantly ( $p < 0.05$ ) high in the 3 test groups compared to the control group ( $18.20 \pm 2.39$ ), which may suggest the ability of the oil to cause an increase production of RBC which leads to the increase in the PCV level. *B. aegyptiaca* oil has been shown to contain high level of protein and essential lipids [31] and [2,32], which may have positive effect on the RBC production. It was also observed that

the Test 2 group has higher level of PCV and RBC which may indicate that the 1.5 mL of *B. aegyptiaca* oil administered is significantly ( $p < 0.05$ ) more stimulating (possibly optimum) in the production of RBC compared to the 1 mL and 2 mL administered in Test 1 and Test 3 groups respectively. This may be as a result of saponins in the oil, it has been reported by [25] in high concentration saponins has the ability to hemolyze RBC when injected into blood stream. White blood cells (WBCS) were found to be significantly higher in the control group ( $p < 0.05$ ). This seems to follow a general trend of greater ability of *B. aegyptiaca* oil to produce circulating blood cells. The phytochemicals that were detected in the oil could be responsible for the stimulation of RBC production that was observed in this present study.

**Table 3** indicates that administration of *B. aegyptiaca* oil has no significant ( $P > 0.05$ ) effect on Total protein, serum albumin and the liver enzymes (AST ALT and AP, in the evaluation of liver toxicity [33,34]. The deformations of hepatic structure liberate these enzymes into circulation thereby making their detection possible in the serum. High level of AST is an indication of liver damage. ALT catalyzes the conversion of alanine to glutamate and is also released in similar manner. ALT is more specific to the liver, is therefore a better candidate for the detection of liver injury [35]. However, serum ALP was important for the proper functioning of hepatic cells. Damage to the hepatocytes is reflected by an increase in blood levels of these enzymes [36].

**Table 3.** The effect of *Balanite Aegyptiaca* seeds oil on liver enzyme activities in broiler chickens.

Groups	AST (U/l)	ALT (U/l)	ALP (U/l)	ALB (g/dL)	TP (g/dL)
Control	$86.40 \pm 14.48^a$	$15.80 \pm 2.95^a$	$71.40 \pm 3.21^a$	$18.00 \pm 1.73^a$	$38.18 \pm 2.10^a$
Test 1	$88.60 \pm 8.99^b$	$18.80 \pm 3.56^b$	$69.20 \pm 2.78^b$	$18.60 \pm 1.51^b$	$40.00 \pm 8.48^{bc}$
Test 2	$84.20 \pm 10.64^c$	$21.00 \pm 3.74^c$	$62.60 \pm 15.45^c$	$17.80 \pm 0.45^c$	$40.00 \pm 3.00^{bc}$
Test 3	$84.60 \pm 16.30^d$	$20.20 \pm 5.89^d$	$50.60 \pm 16.99^d$	$16.80 \pm 0.45^d$	$36.60 \pm 3.59^d$

Results are Mean  $\pm$ SD of triplication. Values with different superscript down the column are significantly different ( $p < 0.05$ ), values with the same superscript are not significantly ( $p > 0.05$ ) different.

Leakage of the enzymes from liver cells into circulation is caused by reactive oxygen species (ROS) formation and lipid peroxidation of cell membranes. This results in loss of membrane integrity, changes in membrane potential, and an increase in membrane permeability [36] and thereby resulting increase in serum level. In this present study the *B. aegyptiaca* oil to chickens did not show any significant ( $p > 0.05$ ) toxicity on the serum marker enzymes as well as TP and ALB levels even at higher concentration of 2 mL that was administered to the chickens in Test 3 group. However, the inconsistency of results obtained in the various studies might be attributed to the mode of administration and quantity of the *B. aegyptiaca* seed oil used and it could be as a result of difference in the solvent use for extraction of the seed oil. Administration of *B. aegyptiaca* seed oil showed no deleterious effects on the functions enzymes liver as the values were similar ( $P > 0.05$ ) among the treated and untreated (control) groups (see **Table 3**). The result of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase means that broiler chickens could tolerate the addition of up to 2 mL of the *B. aegyptiaca* seeds oil without any deleterious effects on liver functions.

The findings are in accordance with the work of [37]. The findings are also in agreement with the work of [23,38] and [13] which showed that various part of *B. aegyptiaca* possess hepatoprotective effect on liver damage which was principally as a result of the phytochemicals present in the plant particularly flavonoids. Flavonoids have been known for its antioxidant and

hepatoprotective effect. It is suggested that saponins in the oil plays an important role in as an antioxidant for the prevention of oxidative hepatic damage [39]. Furthermore, flavonoids and saponins in the n-hexane oil of *Balanite aegyptiaca* may be able to stabilize ROS by reacting and subsequently oxidizing them to more stable metabolites.

## CONCLUSION

*Balanite aegyptiaca* seed oil has significantly stimulated the production of both erythrocytic series there by demonstrating a positive ability to stimulate hematopoiesis in the bone marrow. It also has the ability to enhance the body immune system because of its ability to stimulate the leucocytes production in the blood. It could be that the bone marrow is stimulated in a general way as to produce and release hemopoietic cells into circulation. In this case the seed oil of *B. aegyptiaca* could be seen as a nutrient because it stimulates RBCs production and immune stimulator. Findings of this present study supports the reported therapeutic use of herbs in folklore medicine for liver and other liver related ailments. Further studies are required to determine the quantities of the active phytochemicals in the seed oil, studies on isolation, characterization, and evaluation of the possible mode of action of these active components is also required.

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