The Anti Snake Venom Effects of the Aqueous Extracts of *Boswellia delzielli* Stem Bark on the Parameters of Hepatic Functions and Energy Metabolism of *Naja nigricollis* (Spitting Cobra) Envenomed Albino Rats

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4Department of Human physiology, College of Medical Sciences,Gombe State University,P.M.B 127 Gombe, Nigeria.

**ABSTRACT**

The study was carried out to determine the antisnake venom effects of the oral administration of the aqueous extract of *Boswellia dalzielli* stem bark on the parameters of hepatic and energy metabolism of *Naja nigricollis* (spitting cobra) envenomed albino rats. During the research fifteen (15) male albino rats weighing between 220-250g were used. They were grouped into five groups each consisting of three (3) rats. Group 1 was pre treated with the aqueous extract of the stem bark of the plant at a concentration of 400mg/kg for twenty four (24) hours before the administration of 0.1mg/kg of the snake venom. Group 2 was administered 400mg/kg of the extract orally for every six hours after being injected with 0.1mg/kg of the snake venom. Group 3 was not injected with the snake venom but was administered 400mg/kg of the aqueous extract orally for every six hours until the end of the research. This group served as the extract control. Group 4 was injected with 0.1mg/kg of the snake venom intraperitoneally and not treated until it was sacrificed by cervical dislocation after five hours. Group 5 was not given any treatment but was fed normally for the period of the study and parameters from this group served as baseline data. The research lasted for 48 hours after which the following analyses were carried out after sacrificing the animals by cervical dislocation; hepatic function test, total protein, glucose and serum albumin. The results obtained showed elevated levels of all these parameters in the venom control group *p* ≤ 0.05 compared to the other groups. Therefore, it was concluded that the plant possessed anti snake venom activity since there was no any death recorded among the rats treated with the extract throughout the period of this research.

Key words: *Boswellia dalzielli*, envenomation, snakebite, snake venom, antivenin

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**INTRODUCTION**

Snake bite remains a public health problem in many countries, including Nigeria. Even though it is difficult to be precise about the actual number of cases, it is estimated that the true incidence of snake envenomation could exceed 5 million per year [5]. About 100,000 of these developed severe squal. The global differences in the epidemiological data reflect variations in the health reporting accuracy as well as the diversity of economic and ecological conditions [5]. Hospital records fall far short of the actual number owing to dependence on traditional healers and practitioners of witchcrafts, especially in developing countries like Nigeria, Ghana, Congo etc. It has been reported that in most developing countries, up to 80% of individuals bitten by snakes first consult traditional practitioners before visiting a medical center [14]. Due to these kinds of delay, several victims die during transit to the hospital.

Snake venom is one of the most amazing and unique adaptations of snakes in animal planet. Venoms are mainly toxic modified saliva consisting of a complex mixture of chemicals called enzymes found in snake poisons throughout the world known to man [15]. Broadly, there are two types of toxins namely: neurotoxins, which attack the central nervous system and hemotoxins, which target the circulatory system. Snakes with neurotoxic venom include cobras, mambas, sea snakes, kraits and coral snakes. Snakes with hemotoxic venom include cobras, mambas, sea snakes, kraits and coral snakes. Snakes with hemotoxic venom include rattle snakes, copperhead and cotton mouths [3].

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It is a common belief by most people that all snakes are venomous but this is far from the reality due to the fact that, of the 2,700 species of snakes known to humans, only about 300 are venomous [14]. Envenomation by snakes is responsible for several clinical complications of severe and local pathology. For example, *E. ocellatus* leads to inflammation such as swelling, blistering, necrosis and hemorrhages due to both metalloproteases and ecarin (an enzyme that activates prothrombin) [13]. On the other hand, envenomation by *Naja n. nigricollis* (the venom which is being studied in this research) induced clinical complications different from that caused by *E. ocellatus*. These include local necrosis, hemorrhage, complement depletion and respiratory arrest or paralysis [5]. Furthermore, the venom of the *Naja n. nigricollis* consists of phospholipase A₂ (an anticoagulant enzyme which inhibits the prothrombinase complex by its binding to factor Xa) and cardiotoxin [14]. Envenomation by *Naja n. nigricollis* can induce corneal ulceration and anterior uveitis [18].

The treatment of snake bite is as variable as the bite and its symptoms [11]. Although an intravenous administration of antivenin, prepared from IgG of venom-immunized horses or sheep, is an effective treatment for systemic envenomation since 1894, the clinical consensus is that antivenin is of limited effectiveness against the effects of local envenomation that develop rapidly after a bite [4]. Such effects include severe pain, edema, localized hemorrhage and necrosis [21] which often results in permanent scarring and deformity. The ineffectiveness of antivenin in treating local envenomation has been attributed to the rapid activity of the toxins and the inability of antivenin IgG to cross the blood/tissue barrier [12]. Research to develop a treatment for local envenomation is therefore of clinical priority and has focused on the application of natural or synthetic inhibitors of snake venom potent molecules [11].

The use of plant remedies to treat snake bite victims in rural areas and poor communities in developing countries is a common practice [16]. The natives whose majority are rural farmers come in contact with snakes during their farming engagements. Due to high cost of hospital treatment and unavailability of antivenins, most often, the rural people find it more convenient to consult native doctors who are acclaimed for curing snake bite patients. There are many anecdotal evidences which indicate that plant remedies used by native doctors are effective in curing snake bites, and there appears to be a high rate of survival among snake bite patients in advanced clinical stages of venom toxicity [14].

The plant *Boswellia dalzielli* (Frankincense tree) called ‘ararrhabi’ or ‘Hano’ in Hausa belonging to the family Burseraceae, a tree growing to 13 meters high of the wooded Savanna, with characteristically pale papery bark, peeling and ragged, locally abundant from northern Nigeria to Northern Ivory Coast has been reported to be useful in the treatment of arthritis, rheumatism, asthma, and syphilis in combination with *Hibiscus Sabdariffa*, and the roots and stem bark extracts are used as antidotes to venomous stings, bites e.t.c. [20]. The plant has been reported to contain alkaloids, flavonoids, tannins, saponins, balsam, cardiac glycosides, terpenes, resins and phenol [9].

It appears that there is no work done to assess the anti snake venom activity of this plant despite many anecdotal evidences from traditional medicine practitioners on its efficacy in the treatment of snake bites locally [8]. Hence this research is focused towards assessing the anti snake venom activity of the aqueous stem bark extract of *Boswellia dalzielli* on the venom of *Naja nigricollis* hawk-shawii. The antivenins taken from the horses are used to treat humans suffering from snake envenomation. When injected into the human blood stream, the antibodies attack the venom, neutralizing its effect. But the usage of snake venoms antisera has its own limitations due to its high cost and lack of availability. It is, therefore, difficult for the rural patients to access antisera. Furthermore, due to the difficulty of its storage and short shelf life, its usage is restricted. Snake venom anti serum (AVS) has administration problem in the sense that the exact dosage is not clear. Also administration of AVS is often associated with hypersensitivity reactions, both early and late, which require further medical attention [4]. Hence the need for other alternative sources for curing snakebites.

**Aim and objectives of the research**

This research is aimed at assessing the anti snake venom potentials of the stem bark extract of the plant *Boswellia dalzielli*. The research involves determination of parameters of hepatic functions and energy metabolism of rats infected with the venom of *Naja nigricollis* and/or treated with the aqueous extracts of the stem bark of *Boswellia dalzielli*.

**MATERIALS AND METHODS**

**Plant**

The plant sample was collected from Maitunku Hill Bambam, Dadiya District of Gombe State using matchet. The plant was identified by Mr. Daniel M. Mshelbwala, Federal School of Forestry and Horticulture, Department of Forestry, Jos, and was further authenticated by Prof. S.S. Sanusi, Department of Biological Sciences, University of Maiduguri, Borno State. The stem bark of the plant was air dried in
the laboratory, Department of Biochemistry, University of JOS. It was then ground into powder using pestle and mortar.

**Extraction of the aqueous extract of the plant**
Twenty grams (20 g) of the stem bark powder was soaked in 200mL of distilled water and was allowed to stand for 48 h after which it was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator maintained at 40°C.

**Snake venom**
The venom from *Naja nigricollis hawkshawii* (spitting cobra) was purchased from sigma chemical Co. St. Louise U.S. (Courtesy of Prof. G.I. Adoga) and used for the experiment. The snake venom was administered by intraperitoneal injection in a dose rate of 0.1mg/kg. This dose was determined after a pilot study was conducted on four groups containing three rats each using different concentrations of the venom, 0.04mg/kg, 0.06mg/kg,0.08 and 0.1mg/kg. The 0.1mg/kg is the most effective dose which killed the rats after six (6) hours of its administration, and is the concentration used in this research.

**Experimental animals**
Thirty (30) healthy male albino rats were purchased from the animal farm, University of Jos, Plateau State. The rats weighed between 220g-250g. They were maintained on commercial preparations of growers' marsh containing 54% carbohydrate, 10% proteins, 19% fat, 20% fiber, 2% minerals and 1% vitamin premix. The rats were divided into five (5) groups of three (3) each, on the basis of their weights, the groups were treated as follows:

Group 1: The rats in this group were first given 400mg/1kg of the stem bark extract of the plant for a period of twenty four (24) hours before they were injected with 0.1mg/kg of the venom intraperitoneally.

Group 2: The rats in this group were injected with 0.1mg/kg of the spitting cobra venom and treated with 400mg/kg of the stem bark extract of the plant orally every six (6) hours until recovery or death.

Group 3: The rats in this group were not poisoned but were administered 400mg / kg of the aqueous extract of the stem bark of the plant orally every six (6) hours until the end of the research.

Group 4: The rats in this group were injected with 0.1mg/kg of the purified spitting cobra venom intraperitoneally and not treated and this group was sacrificed by cervical dislocation after five hours because their maximum period of survival is six (6) hours as determined from the pilot study carried out.

Group 5: (Normal control) The rats in this group were neither injected with the venom nor given treatment for the period of the studies, and the parameters from this group served as baseline data.

**METHODOLOGIES**

**Homogenization of rats' liver**
The liver of the rats in each group was isolated, and 1g of the liver was homogenized in 10ml of Tris-Hcl buffer, pH 7.4 which was prepared by weighing 79.0g of Trezma Hcl in a volumetric flask and making it up to 1litre with distilled water. The homogenization was carried out using a homogenizer. The homogenate was centrifuged using a refrigerated centrifuge maintained at 4°C and a speed of 12000xg for 30 minutes. The supernatant was carefully separated into a clean test tube, and it serves as the homogenate for the assay of alkaline phosphatase activity.

**Determination of liver alkaline phosphatase (ALP) activity by colorimetric method (Retman and Frankel, 1957).**
The underlining principle is as follows:

\[ \text{P-nitrophenylphosphate} + \text{H}_2\text{O} \rightarrow \text{ALP phosphosphate} + \text{P-nitrophenol}. \]

The concentration of P-nitrophenol formed is measured calorimetrically at 405nm and is proportional to alkaline phosphatase activity.

A reagent kit containing Diethanolamine buffer (1mmol/L, PH 9.8), Mgcl\(_2\) (0.5mmol/L), P-nitrophenol phosphate (10mmol/L) was used.

**Procedure:** 0.01ml of the test sample was put into a clean test tube, followed by 0.5ml of the reagent and mixed. Then the initial absorbance was read at 405nm against air. Start timer simultaneously and read absorbance again after 1, 2 and 3 minutes at 37°C.

**Calculation:**

\[ \text{ALP activity (U/L)} = 2760 \times \text{Absorbance (average) of test at 405}. \]

The value above is taken as the ALP activity of the sample.

**Determination of aspartate amino transaminase (AST) by colorimetric method (Retman and Frankel, 1957)**

**Reaction principle:** The underlining principle is as follows:

\[ \text{L-Oxoglutarate} + \text{L-aspartate aspartate} \rightarrow \text{ASAT Glutamate} + \text{oxaloacetate}. \]
Aspartate amino transaminase activity is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl hydrazine. A reagent kit containing phosphate buffer (10 mmol/L, pH 7.4), L-aspartate (100 mmol/L), L-oxoglutarate (2mmol/L), 2,4-dinitrophenyl hydrazine (2 mmol/L) and sodium hydroxide (NaOH) 0.4 M was used. Sixteen grams (16 g) of NaOH was dissolved and made up to 1 L in distilled water.

**Procedure:**
To each of the two clean test tubes marked 'test' and 'blank', 0.5 ml of substrate buffer solution was placed and incubated at 37°C for 5 min, then 0.2 ml of the test sample was added to the test tube labeled 'test' and further incubated at 37°C for 30 min. To all the test tubes, 0.5 ml of dinitrophenyl hydrazine was added. 0.2 ml aliquot of serum was then added to the test tube labeled 'blank'. The mixture was shaken and allowed to stand at room temperature for 20 min after which 5 ml of 0.5 M NaOH was added to each of the tubes and allowed to stand for 5 min. The absorbance was measured at 540 nm after zeroing the instrument with distilled water.

**Determination of serum alanine amino transaminase (ALT) by colorimetric method (Reitman and Frankel, 1957)**

**Principle**
L-oxoglutarate + L-alanine → ALT → L-glutamate + pyruvate

Alanine amino transaminase activity is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine. Kit used contained Phosphate buffer (10 mmol/L, pH 7.4), L-alanine (200 mmol/L), L-oxoglutarate (2.0 mmol/L), 2,4-dinitrophenyl hydrazine (2 mmol/L) and NaOH (4 M).

**Procedure:**
To 2 test tubes marked 'test' and 'Blank', 0.5 ml of buffer substrate solution was placed and incubated at 37°C for 5 min. Then 0.1 ml of the serum was added to the test and incubated for 30 min. To all the test tubes, 0.5 ml of 2,4-dinitrophenyl hydrazine was added. The mixture was shaken and allowed to stand at room temperature for 30 min, and then 5 ml of NaOH was added and allowed to stand for 5 min. The absorbance was measured using a spectrophotometer at 540 nm after zeroing the instrument with distilled water at the same wave length.

**Determination of serum total billirubin (TBIL) and direct billirubin (DBIL) by (Van den Berg diazo Reaction).**

The underlying principle is as follows:
Sulfanilic acid reacts with sodium nitrite in the presence of diazotized sulfanilic acid to form azobillirubin. In the absence of dimethylsulfoxide, only the direct billirubin reacts to give azobillirubin.

A reagents kit containing Sulfanilic acid (28.9 mmol/L), Hydrochloric acid (165 mmol/L), Dimethyl sulfoxide (7mmol/L), total billirubin standard (10mg/dL) and total billirubin activator was used for the determination of total billirubin. Also a reagents kit containing Sulfanilic acid (28.9mmol/L), Hydrochloric acid (165 mmol/L), Direct billirubin standard (7.7mg/dL) and Direct billirubin activator was used for the determination of direct billirubin.

**Procedure:**
Serum/plasma free of haemolysis is used for the analysis. 1000 µL of total and direct billirubin reagents were added respectively to the test tubes labeled sample blank, test, and standard. This is followed by the addition of 50µL of the serum sample to both test tubes labeled test and sample blank respectively for direct and total billirubin, 20µL of both direct and total billirubin activator was added to the test tubes labeled standard and test respectively. 50µL of the standard for both total and direct billirubin was added to the test tube labeled standard. They were mixed well and incubated for exactly 5 minutes. The absorbance of the sample against respective sample blanks was measured at 546nm.

**Calculation.**

Total billirubin = \( \frac{O.D \text{ of test} - O.D \text{ of sample Blank}}{O.D \text{ of artificial STD}} \times 10 \)

Direct billirubin = \( \frac{O.D \text{ of test} - O.D \text{ of sample Blank}}{O.D \text{ of artificial standard}} \times 7.7 \)

**Estimation of total protein in serum (Reinhold Modification of Welchelbaum Biuret formulation, 1961).**

The underlying principle is as follows.
Alkaline copper solution reacts with peptide bonds in the protein molecule producing a violet color which is directly proportional to the amount of protein present in the sample.

The reagents used for this analysis are prepared as follows.

**Stock biuret solution:** 45g of sodium potassium tartrate (Rochelle salt) was dissolved in 400ml of 0.2M NaOH. 15g of copper sulphate (CuSO₄·5H₂O) was added with constant stirring when in solution followed by the addition of 5g of KI and finally diluted to one (1) liter with 0.2M NaOH.
Working biuret solution: 200ml of stock solution was diluted to one liter with 0.2M NaOH containing 5g KI per liter.

Sodium hydroxide solution: 8g of NaOH was dissolved in distilled water and made up to one liter with distilled water.

Standard protein solution: Multisera was used as standard protein solution.

Procedure: To three clean test tubes marked 'test', 'standard' and 'blank' 2ml of Biuret reagent was added to all, then 0.02ml of serum was added to tube marked 'test', 0.02ml of standard was added to tube marked 'standard'. Then 0.02ml of distilled water was added to all the three test tubes. Then the above was incubated at 37°C for 10 minutes and the absorbance was read at 550nm.

Measurement of serum albumin (by bromocresol green method).

The underlying principle is as follows. The measurement of serum albumin is based on its quantitative binding to the indicator 3, 3', 5, 5'-tetrabromo-m-cresol sulphophthalein (Bromocresol Green, BCG). The albumin –BCG- complex absorbs maximally at 578nm, the absorbance being directly proportional to the concentration of albumin in the sample.

A reagent kit containing Succinate buffer (75mmol/L; pH 4.2), Bromocresol green (0.15 mmol/L),Standard(Human serum albumin 45g/L), Tris buffer (100mmol/L, pH 7.3) was used.

Procedure: To three test tubes labeled test, standard and blank. 0.01ml of distilled water was added to the tube labeled “blank”, 0.01ml of standard solution was added to the test tube labeled “standard”, and 0.01ml of serum sample was added to the test tube labeled “test”. Then 3.0ml of BCG reagent was added to all the test tubes, it was mixed and incubated for 5 minutes at 25°C. The absorbance of the test and standard was read against the reagent blank at 630nm using a spectrophotometer.


The underlying principle is as follows. Glucose oxidase catalyses the oxidation of glucose in the presence of atmospheric oxygen to give gluconic acid and hydrogen peroxide. Peroxidase contained in the reagent acts on hydrogen peroxide to yield water and oxygen. The oxygen then couples with an oxygen acceptor,4-aminophenazone to give a pink color. The color so formed is proportional to the concentration of glucose present in the sample.

The reagents used in this test are prepared as follows.

Phenol reagent: 1g of phenol and 9g of sodium chloride were dissolved in 1000ml of distilled water.

Enzyme Reagent: 12.88g of sodium acetate (trihydrate), 0.32 ml acetic acid, 15 ml of ferricozyme, 301.0 mg of 4-aminophenazone and 15mg of peroxidase were all dissolved in distilled water and made up to 100ml.

Glucose standard (Stock): Dissolve 1.802g in 100ml saturated benzoic acid i.e. 2% benzoic acid. Store at 4°C.

Working standard: The stock solution was diluted to 1:10 ratio with saturated benzoic acid. This is equivalent to 180mg/dL.

Procedure: 2.5ml of phenol reagent was placed into three different test tubes labeled test, standard and blank. Thereafter, 0.05ml of serum was added to the test tube labeled “test” and 0.05ml of standard glucose was added to the test tube labeled “standard”. 0.05ml of distilled water was added to the test tube labeled “blank” respectively. Then 2.5ml of enzyme reagent was added to each of the test tubes and mixed thoroughly. The tubes were then covered with plastic caps and then incubated at 37°C for 20 minutes then the optical density of the test and standard tubes were read against the blank at 510nm.

Statistical analysis.

The biochemical parameters (data) generated from the study was entered into an Excel spreadsheet (MS Excel 2007). Descriptive statistics for each parameter were calculated and Analysis of Variance (ANOVA) was used to compare the means of all the parameters both within and between all the groups against group 3 (venom control) and group 8 (normal control) respectively, using Epi Info version 3.5 computer program [7]. The level of statistical significance was considered at a p-value less than or equal to 0.05 (5%).

RESULTS

Anti snake venom properties

A pilot study was conducted in order to ascertain the dosage of the snake venom to be administered to the rats. In these studies three different concentrations of snake venom, 0.04, 0.06mg/kg, 0.08mg/kg and 0.1mg/kg were administered to four groups of three rats each respectively.

On administration of the snake venom in all the groups the following were observed.

1.0 There was localized edema and swelling at the site of injection.
There was increased heart beat and breathing rate.

Paralysis was observed after 1 hour in the groups given 0.08 mg/kg body weight of the venom. This was followed by death which was noticed as follows.

- The first death was recorded in the groups given 0.1mg/kg body weight of the venom, the first rat died after 6 hours of the administration of the venom, and the other two rats in that group died also, two hours later.

- The rats in the other groups survived for a while but all died within 12 hours of administration of the snake venom.

Therefore, the most effective dosage of the venom (0.1mg/kg) was injected into the rats and they were treated with the aqueous extracts of the stem bark of *Boswellia dalzielli*, and no death was recorded in all the rats treated with the extract during the 48 hours of the research, and the rats in these groups were still active before they were sacrificed.

**Table 1**: Comparison of the indices of hepatic functions between all the other groups and the venom control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALP (IU/L)</th>
<th>DBIL (µmol/L)</th>
<th>TBL (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Pretreated)</td>
<td>31.33±0.58*</td>
<td>98.97±0.15*</td>
<td>138.07±0.86*</td>
<td>0.83±0.01*</td>
<td>2.12±0.04*</td>
</tr>
<tr>
<td>2 (Treated)</td>
<td>32.37±0.55*</td>
<td>135.80±0.72*</td>
<td>118.23±0.52*</td>
<td>0.86±0.06*</td>
<td>3.43±0.09*</td>
</tr>
<tr>
<td>3 (Extract control)</td>
<td>35.00±1.00*</td>
<td>103.00±2.00*</td>
<td>98.96±0.11*</td>
<td>1.07±0.03*</td>
<td>3.34±0.20*</td>
</tr>
<tr>
<td>4 (Venom control)</td>
<td>75.33±2.52</td>
<td>166.33±1.53</td>
<td>155.60±2.31</td>
<td>5.68±0.05</td>
<td>7.93±0.05</td>
</tr>
<tr>
<td>5 (Normal control)</td>
<td>28.33±1.15</td>
<td>120.33±0.58</td>
<td>147.87±0.81</td>
<td>2.73±0.01</td>
<td>4.06±1.03</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± standard deviation of three replicates. Values indicated with asterisk are statistically different from the venom control at (p ≤ 0.05.)

**Table 2**: Comparison of the indices of hepatic functions between all the extract treatment groups and the normal control.

<table>
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<th>Groups</th>
<th>ALT (IU/L)</th>
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**Table 3**: Comparison of the indices of energy metabolism between all the groups and the venom control group.

<table>
<thead>
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<th>Groups</th>
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<th>TP (mg/dL)</th>
<th>ALB (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Pretreated)</td>
<td>6.82±0.04*</td>
<td>62.04±0.76*</td>
<td>32.92±0.26*</td>
</tr>
<tr>
<td>2 (Treated)</td>
<td>6.42±0.10*</td>
<td>68.03±0.17*</td>
<td>25.98±0.75*</td>
</tr>
<tr>
<td>3 (Extract control)</td>
<td>3.57±0.30*</td>
<td>61.08±0.67*</td>
<td>27.40±0.09*</td>
</tr>
<tr>
<td>4 (Venom control)</td>
<td>9.25±0.37</td>
<td>110.09±6.39</td>
<td>55.27±0.85</td>
</tr>
<tr>
<td>5 (Normal control)</td>
<td>3.43±0.57</td>
<td>62.04±0.76*</td>
<td>28.73±0.75*</td>
</tr>
</tbody>
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<tr>
<td>1 (Pretreated)</td>
<td>6.82±0.04*</td>
<td>46.63±1.23*</td>
<td>32.92±0.26*</td>
</tr>
<tr>
<td>2 (Treated)</td>
<td>6.42±0.10*</td>
<td>68.03±0.17*</td>
<td>25.98±0.75*</td>
</tr>
<tr>
<td>3 (Extract control)</td>
<td>3.57±0.30*</td>
<td>61.08±0.67*</td>
<td>27.40±0.09*</td>
</tr>
<tr>
<td>4 (Normal control)</td>
<td>3.43±0.57</td>
<td>62.04±0.76*</td>
<td>28.73±0.75*</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± standard deviation of three replicates. Values indicated with asterisk are statistically different from the normal control at (p ≤ 0.05.)

**DISCUSSION**

The signs and symptoms observed by the administration of the *Naja nigricollis* venom in this research of the anti-snake venom properties of the plant *Boswellia dalzielli* which are: localized edema and swelling at the site of injection, increased heart beat, paralysis, and subsequently death were confirmation of earlier findings on the researches carried out on this venom [10].
Higher dosage of the venom (0.1mg/kg) was used in this research as compared to other works carried out with the same venom, and also it took a longer period of time (6 hours) for the venom to elicit its effect i.e. for the first death to be recorded as compared to 30 minutes with 0.04mg/kg body weight of the same venom [11]. This can be attributed to the fact that the venom used in this research might have been kept for a long period of time before the research, and it might have lost some of its activities.

Comparison between all groups and group 3 (venom control) showed statistically higher significant serum parameters in hepatic and energy metabolism in the venom control as compared to other groups. The increased in serum levels of ASAT, ALAT, ALP, Direct bilirubin and Total bilirubin in the venom control as compared to other groups may be due to the lesions caused by the venom from different tissues including liver, kidney, heart, and even skeletal muscle. These are known to be diagnostic of destruction due to heart disease, liver cirrhosis, and erythrocytes caused by the administration of the venom [2]. Similar work carried out using the venom of Vitis arietans and Bitis gabanica, gave the same increased in these parameters [19].

There was also an increase in the level of protein and albumin in the venom control group compared to others and this is also in line with earlier findings suggesting that these parameters were altered as a result of direct effects of the venom on the muscle tissues and the blood circulatory system [17; 6].

There was a marked increased in the rate of metabolic activities by the venom control group compared to all other groups. This is evident by the increase level of serum glucose, protein and albumin which is, probably, due to the stressful situation following envenomation and also the associated release of catecholamine and glucocorticoids which possibly accounted for the marked hyperglycemia [2]. The marked hyperglycemia can also be clarified by phenomenon such as: central and peripheral adrenergic mechanism, B-receptor activation, release of tissue and medullary catecholamine, inhibition of glucose uptake of skeletal muscle, inhibition of insulin release, stimulation of glucagon secretion, glycogenolysis and/or retarded glucose utilization by peripheral tissues caused by the administration of the venom. These were all in agreement with earlier findings [6;2;1].

In general there was an increased in serum parameters in the venom control compared to all other groups, this could be due to increased metabolic activity by the rats as a result of trying to mobilize energy in order to cope with the toxic effects of the venom.

Comparison between all extract treatment groups and group 8 (normal control) showed a significantly higher (P<0.05) serum parameters in the treatment groups in the case of ALT compared to normal control. AST showed significant decrease in the treatment groups compared to the normal control with the only exception in the extract control group. ALP, DBIL, TBIL all showed significant decreased in all the groups compared to the normal control (all these are indicated by asterisk in table 2.0). Glucose showed increase level in the post treated and treated groups with the extract control not showing significant difference compared to the normal control, Total protein and albumin showed both significant increased and decreased among the treatment groups compared to the normal control (these can be seen in Table 4.0). These are, therefore, indications of the effectiveness of this plant extract in gradually trying to stabilize the increased level of all the serum parameters caused by the venom of Naja nigricollis as evident from the data in table 1.0 and table 3.0 to the same level with the normal control. Therefore, the stem bark extracts of Boswellia dalzielli can be said to possess an anti snake venom activity.

CONCLUSION
In conclusion, it is very obvious from this research work that the claims made by traditional medicine practitioners of the use of the stem bark extracts of Boswellia dalzielli, in the treatment of arrow poisons and snake bites is true. This is due to the fact that the plant extracts showed significant effects in reducing the levels of these biochemical parameters as compared to the venom control and also the plant extract was able to prolonged the life-span of the rats that were injected with the venom and treated with the aqueous extracts of the stem bark of the plant for up to 48 hours of this research without any casualty as opposed to those that were injected with the venom and not treated in which the first casualty was recorded after 6 hours and all the rats died within 12 hours. Therefore, if properly purified and formulated the stem bark extract of the plant, Boswellia dalzielli, can be used as a cheap and reliable antivenin for the treatment of snakebites among rural dwellers, peasant farmers and the world over.

REFERENCES
Goje et al


Citation of this article: