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Assessment of Serum Biochemical Indices of Albino Rats Induced with *Naja nigricollis* Venom and Treated with *Parkia biglobosa* Stem Bark

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Abstract

Assessments of Serum Biochemical Indices of Albino Rats induced with *Naja nigricollis* venom treated with *Parkia biglobosa* stem bark were conducted and evaluated. A total of 42 healthy adult Albino rats of both sexes and average weight 153 -275g were used for the experiment. The venom was collected in low light condition at ambient temperature by using a short acting general anesthesia (Halothane) and kept at 4^{o}C. The rats were injected with venom at 0.04 mg followed by conventional anti venom (1 ml) and the plant extract were administered at different doses of 300, 500 and 700 mg/kg respectively. The animals were scarified, and 5ml of blood samples were collected from each rat within 24hrs and tested for ALP, ALT, AST, total protein and Albumin. The LD_{50} was determined using Lorke's method and found to be greater than 500mg/kg per oral. The results showed increased and decreased in ALT, ALP and AST while total protein and Albumin were normal. The stem bark extract of *Parkia biglobosaa* can be said to possess anti-snake venom activity which neutralize the toxic effects of *N. nigricollis* venom. The potent Snake venom neutralizing capacity of this plant extract is recommended for therapeutic purpose in case of snake bite.

Keywords: *Naja nigricollis venon,* Rats, Biochemical indices, *Parkia biglobosa,* and Stem bark

INTRODUCTION

The use of plant remedies to treat snake bite victims in rural areas and poor communities in developing countries is a common practice (Asuzu and Harvey, 2003). In Nigeria, nearly all plants are associated with some medicinal values. The use of plants especially in traditional medicine is currently well acknowledged and accepted in Nigerian health care practice (Hassan and Kamba, 2010). The snake, Naja *nigricollis* belongs to the most diverse and widespread genus of cobras. They are medium-sized, less than two meters, and black in coloration. Other species of this genus such as Mozambique spitting Cobra is red in coloration. Spitting Cobras are generally categorized as predators. They are well equipped to prey on a wide variety of small vertebrates (Bola, 2000). The plant, *Parkia biglobosa* is a multipurpose fodder tree that belongs to the family Mimosaceae (Tringali *et al.,* 2000). Popularly called the "African locust bean tree", ''*Dorawa* in Hausa and *Golo*'' in Dakarchi language is known to occur in a diversity of agro ecological zones from tropical rainforest where the rain is high to the arid zone where it is low. The plants extract constitute an extremely rich source of pharmacologically active compound and a number of extracts have been shown to act against snake venom (Goswami *et al.,* 2014).

Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity and reduced the risk of using them. It facilitates pharmacological studies and leads to the synthesis of pure and potent compounds with decreased toxicity (Hassan and Kamba, 2010). Snake bite is an important cause of mortality and morbidity in Nigeria that often results in puncture wound inflicted by the animal. Majority of snake species are said to be non-venomous rather than venomous (Kasturiratine *et al.,* 2010). Snake bite remains an important medical problem in both developing and developed countries. Snake bites pauses a major health risk in many developing countries, with the global Snake bites exceeding 5,000,000 per year (Kasturiratine *et al.,* 2010). Worldwide, about 30,000 to 40,000 people die annually of Snake bites of these, about 25,000 people die in India, mostly in rural areas, about 10,000 people in United States and rest of in other countries (Dravidamani *et al.,* 2008). Snake venom is badly needed to produce anti-venom required to treat potentially fatal snake bites (Dravidamani *et al.,* 2008).

Despite the great technological stride in modern medicine, approximately 75% of the African population still relies on traditional healing practices and medicinal plants for their healthcare needs (Mbi and Bilikha *et al.,* 1998). The biodiversity of the African flora provides medical practitioners with an impressive array of "natural pharmacy", from which plants are selected as remedies or as ingredients to prepare phytomedicines (herbal medicines) for human and animal disorders (Mbi and Bilikha, *et al.,* 1998; Oyewole, 2003 and Oyewole, 2004). Envenoming by snakes is responsible for several clinical complications of severe and local pathology. For example, *E. oscellatus* leads to inflammation such as swelling, blistering, necrosis and hemorrhages due to both metalloproteases and ecarin (an enzyme that activates prothrombin) (Bola, 2000).

On the other hand, envenoming by *Naja 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 (Chippaux *et al.,* 1998). Furthermore, the venom of the *Naja nigricollis* consists of phospholipase A2 (an anticoagulant enzyme which inhibits the prothrombinase complex by its binding to factor Xa) and cardiotoxin (Hassan *et al.,* 2010). Envenoming by *Naja nigricollis* can induce corneal ulceration and anterior uveitis (Bola, 2000). This research is aimed at assessing the anti- snake venom potentials of the stem bark extract of *Parkia biglobosa* plant against *Naja nigricollis* Venom with the view to ascertain its potential activity against the snake venom.

MATERIALS AND METHODS

Collection of Plant Material

Fresh stem bark of *Parkia biglobosa* was collected between 5/02/2016 to 10/02/2016 at the University Campus Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria. The plant stem barks was collected using cutlass and immediately put in sterilized plastic containers and were brought to the Department of Biological Science, Kebbi State University of Science and Technology, Aleiro for identification. The plant was identified by a Taxonomist and was assigned a Voucher No: (Jack) R.Br. V.M. 281. Specimen was preserved in the University Herbarium. The fresh stem bark of *Parkia biglobosa* was properly washed under tap water, rinsed with distilled water, and shade dried for seven days and pounded into fine powder using clean mortar and pestle. Five hundred grams (500g) of the *Parkia biglobosa* powdered was made and 300g was taken and put in a clean plastic container and kept at room temperature until required (Donatein *et al.,* 2005).

Extraction of Plant Material

Three hundred grams (300g) of powdered stem bark of *Parkia biglobosa* was soaked in 2700ml of Ethanol using 1000 ml conical flasks. The conical flasks containing the extract were covered with cotton wool and capped with aluminum foil and kept for 24hrs at room temperature. The aqueous extracts were filtered using (What man No. 1) filter paper in to three beakers of 1000 ml and allowed to evaporate on a water bath. Two hundred grams (200g) of *Parkia biglobosa* plant extract was collected in a sterile conical flask and kept at room temperature until required (Donatein *et al.,* 2005). One hundred and fifty(150g) was collected and divided into 30,50 and 70g each and dissolved in 100mls of distilled water to obtain 300, 500 and 700mls of the extract each respectively.

Collection of Snake and Snake venom

The Snake *(Naja nigricollis*) was captured and housed in wooden cage with the help of a local Snake charmer. The Snake was identified by a Zoologist from the Department of Zoology, Federal University Birnin Kebbi and was kept for one week and fed with fresh meat and water for one week. The venom was collected in low light condition at ambient temperature using a short acting general anesthesia (Halothane). The glands below the eyes of the snake were compressed and venom was released in to a plastic container. The venom was refrigerated in a sterilized plastic container at 4^{O}C (Goswami *et al.,* 2014). The conventional Antivenin with the following data: Manufactured by Bharat Serum and Vaccines Ltd, Batch Number: 01AS14054, Manufacture date: 10/2012, Expiring date: 11/2017 was purchased from Zeta Pharmacy Birnin Kebbi, Nigeria and was transported to Kebbi State University of Science and Technology, Aleiro, Nigeria and was preserved in a refrigerator prior to the experiment.

Experimental animals

A total of 42 healthy adult Wister albino rats of both sexes with an average weight of 153 -275g were obtained from a colony breed at the Biological Science garden,Usmanu Danfodiyo University Sokoto, Nigeria. The animals were brought and kept in General Biology Laboratory of Kebbi State University of Science and Technology Aleiro, and were housed with wooden cage at room temperature to acclimatize with the new environment for two (2) weeks. The rats were fed with feed produced by vital feeds Jos, and were allowed free access to tap water and the experiment was carried out for 21 days.

After two weeks of acclimatization, the rats were grouped for two (2) toxicity studies; acute and sub-acute toxicity studies respectively. Eighteen (18) rats were used for the acute toxicity study and twenty four (24) rats for the sub- acute toxicity study making a total of 42 Albino rats respectively. Group one (1) which is the control group received water and free feed only, all the rats in groups 2-6 were injected with 0.04 mg dose of the Snake venom at time intervals of 30 minutes under critical observation. After the exposure of the rats to the venom, they were then treated with conventional anti- venom and the plant extract dose. After ten (10) minutes interval, the rats in group (3) were treated with 1ml/kg of the conventional anti venom while the extract were orally administered for rats in groups 4, 5, and 6 using 5ml syringe at close concentrations of the plant extract of 300, 500 and 700 mg/kg respectively (Wannang *et al.*, 2005).

TOXICITY STUDIES

Acute toxicity study

The LD_{50} of the plant extract was determined using Lorke's method (1983) with modifications. The test was carried out in two phases and 18 rats were grouped in to 6 groups of 3 rats each. Phase 1: This phase contains 3 groups of 3 rats each per group. The three groups were administered orally with graded doses (100, 300 and 500 mg/kg) of the plant extract. Phase 2; contains three groups of three rats per group, which received graded doses (700, 900 and 1200 mg /kg) of the plant extract respectively. The number of deaths in each group within 24 hr was recorded and the final LD_{50} values were calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred).

Sub- acute toxicity study

Twenty four (24) rats were randomly selected and divided into four groups of six each. The first group served as control while the remaining three groups were given 300, 500 and 700 mg/kg of *P. biglobosa* single oral dose. The first day of dosing was taken as D0 where as the day of sacrifice was designated as D_{14}. This was carried out according to the method of (Aneja, 2010)

Collection of blood samples

About five (5ml) of blood samples from each rat in both the treated and control groups were collected on the 22nd day of the experiment. The rats were first euthanized individually with chloroform and then sacrificed for collection of blood. Two milliliters (2ml) of the blood was put into tubes containing disodium ethylene dramine tetra acetic acid (Na- EDTA) to obtain plasma and the remaining (3ml) into tubes without anti- coagulants (to obtain serum). The plasma and the serum were obtained from the blood samples by centrifuging at 2000 rpm for 10 minutes at room temperature Reitman and Frankel, (1957).

Biochemical analysis of blood samples

In this study, some commonly employed indices in assessing liver function were also assayed from both the control and study animals that received various doses (300,500 and 700mg/kg) of the aqueous extracts of the plant. The Biochemical parameters; Albumin, Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Total protein were analyzed as described by (Reitman and Frankel, 1957)

RELATIVE ORGAN WEIGT

On day 14th of the dosing period, all the animals were euthanized by exsanguinations under Chloroform anaesthesia. Different organs namely the heart, liver, lungs, spleen and kidneys were carefully dissected out and weighed in grams (absolute organ weight) as described by (Anaja, 210). The relative organ weight of each animal was then calculated using equation

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

The LD_{50} was greater than500mg/kg per oral (P.O) for both extracts.

Comparative Analysis was done using Rats Biochemical Reference range as described by John (1996).

RESULTS

The results of the Albino rats induced with *Naja nigricollis* 0.04mg venom and treated with Stem bark extract *of Parkia biglobosa* on Liver parameters (ALP, AST and ALT) administered different dose concentrations 300, 500 and 700 mg/kg of the plant extract was conducted. The results showed that, the rats in group 1 treated as control showed no mortality after 24hrs with average survival time 24.00 hrs and 100% resistance. However, the rats in group 2 treated with 0.04 mg/kg only venom showed 100% mortality with average survival 0.14 hrs and 0.58% resistance. Similarly, the rats in group 3 & 4 were given 0.04mg/kg venom and 1ml/kg anti-venom and 0.4mg/kg venom and 300mg/kg of the plant extract respectively. The results showed in group 3 showed mortality rate 2/5 and percentage mortality rate after 24 hrs 80% with average survival 21 hrs and percentage resistance 87.5% Table 1.

As in group 4, the rats were seen to expressed 3/5 mortality rate and 60% mortality rate after 24 hrs with average survival 10.23 hr and 43.25% resistance respectively. However, there results of the rats in groups 5 & 6 treated with different dose concentrations of 0.4mg/kg venom and 500mg/kg of the plant extract and 0.4mg/kg and 700mg/kg of the plant extract respectively. The results showed mortality rate 2/5 and 40 % mortality rate after 24 hrs with 12.10hrs survival and 50.42% resistance rate table 1.

The results of the physical observations showed within the 24hrs of the administered different dose concentrations of the stem bark extract of *Parkia biglobosa* plant with the experimental animals were recorded. The results of the control animals in group 1 showed normal activities throughout the period of research. The results of the animals observed in group 2 treated with venom only showed signs of respiratory arrest, inability to move, swellings venom injected site, and mortality before 14 minutes table 2 however, the results showed that, the animals in groups 3-5 showed same signs across the group as redness of the eyes, rough hair coat, loss of appetite and swellings in the sites of the injection of the venom, increase respiration rate, bulging of the eyes depression and death at the 24 hrs as the rest of the animals in groups 4-5 were observed to be weaker 30 to 24 hrs table 2. These signs were equally applied to animals from groups 2-5 the only exceptions was with the group 6 animals which showed normal feeding, drinking and movement within 15 minutes but there were no loss of appetite, difficulty in breathing, scratching of nose and swelling from the injected site and no mortality reported within 15-1hr. the only sign reported were low mortality rate (2) animals and general weakness of the body across the group 6 animals Table 2.

The results of the liver function parameters: TP, ALB, ALP, AST and ALT in rats administered with venom and orally with aqueous extracts stem bark of *Parkia biglobosa* at different concentrations 300, 500 and 700mg/kg and compared with the control value were presented in Table 3. The results of the analyses showed increased and decreased in ALT, ALP and AST while Total protein and Albumin remained normal. LD50 was determined as greater than 500mg/kg per oral. The phyto-chemical analysis of the plant stem bark extract was also conducted and the results revealed the presence of tannins, alkaloids and saponin, glycosides in large quantities (+++), while cardiac glycosides and saponin glycoside were moderately present (++) and steroids (+) was found in a trace amount respectively. The result however showed Anthraquinones, volatile oil, balsam and flavonoids were not detected in the samples analyzed Table 4.

Table 1: Resistance and Mortality Percentage (%) of the Rats Induced with *Naja nigricollis* Venom Anti-venin and Oral Administration of Stem Bark Extract of *Parkia biglobosa* within 24hrs

Group	Treatment	Dose of snake venom, plant extract and antivenom	No. of rats	Mortality rate	Mortality percentage (%) rate after 24hrs	Average survival time (h)	Resistance percentage (%) group
1	Control	-	5	0	0	24.00	100
2	Venom only	0.04mg/kg	5	5	100	0.14	0.58
3	Venom + Anti-venom	0.04mg/kg 1ml/kg	5	2	80	21	87.5
4	Venom + Extract	0.4mg/kg 300mg/kg	5	3	60	10.23	43.25
5	Venom + Extract	0.4mg/kg 500mg/kg	5	2	40	17.20	72.66
6	Venom + Extract	0.4mg/kg 700mg/kg	5	2	40	12.10	50.42

Table 2: Physical observation on the behavior of treated rats after environment and oral administration of the plant extracts

Group	Treatment	15 min	30min	1hour	24hours
1	Control	There is normal feeding, drinking and movement	-	-	No mortality observed throughout
2	Venom only	Respiration arrest, inability to move and swelling in the site venom of injection mortality before 14min is observed	-	-	-
3	Venom + Anti-venom	-	There is redness hair coat, loss of appetite and swelling in the site of venom injection	Increases respiration rate bulging of the eye depression	Mortality of two rate observed
4	Venom + Extract 300mg/kg	-	Scratching of nose, respiration arrest, loss of appetite and swelling of injection site	There is continuous scratching of nose no feeding and movement	Mortality of three rate is observed before 24hrs
5	Venom + Extract 500mg/kg	-	Loss of appetite difficult in breath scratching of nose and swelling of injection site	Bulging of the eye depression and no mortality observed	Mortality of two rate is observed while the rest very weak
6	Venom + Extract 700mg/kg	There is normal feeding, drinking and movement	_	_	Mortality of two rats is observed while the rest are very weak

Table 3: Result showed changes in the biochemical indices of treated rats

Group	Treatment & normal blood limit	TP (g/dl)	ALB (g/dl)	ALP	AST (µl)	ALT (µl)
1	Control	7.2	4.7	34.8	33.2	18.4
2	Venom only	4.1	2.2	22.6	29.8	6.1
3	Venom + Antiserum	7.1	4.3	20.6	10	21
4	Venom + Extract At 300mg/kg	5.9	2.9	27.5	32.1	7.2
5	Venom + Extract At 500mg/kg	6.3	3.8	30.0	34.9	40
6	Venom + Extract At 700mg/kg	7.3	4.4	19.9	10	12
	Normal value	5.6-7.6	3.8-4.8	16-50	16-50	17.5-30.2

Key: TP= Total Protein, ALB= Albumin, ALP= Alkaline Phosphatase, AST= Asparate amino-transferase and ALT= Alanine amino transferase.

Table 4: Phytochemical Composition of *Parkia biglobosa* of Stem Bark Extract

Phytochemical Constituent	Observation
Alkaloids	+++
Tannins	+++
Saponins	+++
Glycoside	+++
Saponin glycoside	++
Cardiac glycoside	++
Steroids	+
Balsam	N.D
Flavonoids	N.D
Volatile oil	N.D
Anthraquinones	N.D

Key: + = Trace amount, ++ = Moderate amount, +++ = Large amount and N.D = Not Detected

DISCUSSION

The alleviation of toxic symptoms and survival of experimentally protected laboratory animals (within 24hrs after being dosed with lethal venom) were used to infer the antivenin property of stem bark of *Parkia biglobosa*. The results of this study indicated that the stem bark extract of *Parkia biglobosa* exhibits an *invivo* detoxifying properties against the venom of *N. nigricollis*. The extract was observed to provide protection against the development of toxic signs and lesions due to envenomation. However, the findings in this research is in consistent with the findings of Anofi *et al*. (2012*),* which stated that the extract of *Securidaca longepedunculata* contains high amounts of saponin, in addition to other components, which are known to inactivate toxic proteins of the venom when compared with the *Parkia biglobosa* this is in agreement with the findings in this study which showed same high amount of saponin value.

The in *vivo activity* of the stem bark extract of *Parkia biglobosa* showed a progressive increased in average survival time of the envenom rats with an increased dose of the extract in a dose dependant manner. The progressive increase in the dosage (300-500 and 700mg/kg) of the extracts and anti-venom used in this present study were in accordance with that of the dose concentration of (300, 500 and 700mg/kg) in a similar study conducted by Ushannandini *et al*., (2006) to determine the anti-snake venom activity of different extracts of *S. longepedunculata* against *Russel viper* venom. The stem bark extract at different concentration showed faster rate in mortality at 300 mg/kg, but at 500-700mg/kg showed more delay in average survival time. However, this is in disagreement with the findings in a similar research conducted by Usubillaga *et al*. (2005) who used methanol extracts of *Uvaria chamae* stem bark at concentration of 300, 400, 500mg/kg body weight in male albino rats intraperitoneally and obtained up to 100% anti-venom activity in the animal models. These differences in results could have an effect due to different methods and or dosages used in the two researches compared.

However, the study showed extract of *Parkia biglobosa* stem bark of a concentration of 500mg/kg for 17.20hrs was effective in neutralizing lethal effects of *Naja nigricollis* venom in the experimental animals. This study is in line with the findings of Alam, *et al*. (1994) who confirmed that aqueous extract of *Boswellia dalzielli* stem bark has effect on the Snake venom at a concentration of 500 mg/kg with 0.1mg/kg for 17.30 hours as 15 albino rats were showed 73% survivals with significant ($p \leq 0.05$) anti-snake venom activity respectively. The liver enzymes aspartate and alanine aminotransferases (AST and ALT) are concerned with amino acid metabolism. Large amounts of AST are present in liver, kidney, cardiac muscle and skeletal muscle (Cheesbrough, 1991). However, ALT is known to be found principally in the liver, Serum AST and ALT levels were always found to increase in liver cell damage and greater the decreed of liver cell damage the higher the activities of both enzymes (Cheesbrough, 1991). Similarly, as described in others studies, the liver function parameters; (ALT, AST, ALP, ALB and TP) were also assayed from both the control and study rats after administered with various doses of the aqueous plant extracts of *Parkia biglobosa*.

Group 2 present the results of rats administered with venom only: The results obtained showed a wide difference in the Total protein, Albumin and ALT of the liver parameters of rats in group 2 compared with the same values of liver parameters obtained from those of rats in control Group 1. Although these biochemical parameters were observed to be within the limit value except ALP, which shows a greater decrease in value (6.1 U/L) when compared with the normal value of (17.5-30.2 U/L), this may suggest liver damage or disease, such as a blocked bile duct or venom was not able to impair the hepatic function except the liver ability to metabolize protein., This was in conformity with the work of Anofi *et al*. (2012) on the effects of stem bark of *Securidaca longepedunculata* extracts on blood profile of albino rat reported that decreased or increased ALP leads may be as a result of Liver damage or bile duct in ability to synthesize the protein. The result obtained from the administered with venom and anti serum in group 3, the effect of the anti venom was observed to be within the normal value except AST which showed decrease in value (10 U/L) compared with normal value (16-50 U/L). This suggest that there is therapeutic effect of the anti venom which could be responsible for the decreases in AST this was consistent with findings of Afolayan and Yakubu (2009). In their study on adverse effect of chemical compound of stem bark of plant extract on albino rats. This showed that, therapeutic effect of the anti venom is as an agent for restoring impaired protein metabolism in the liver of the Albino rats and this could be responsible for the decreases in AST values as indicated in the result.

However, the rats administered with 300mg/kg extract in group 4 showed differences in the Total protein, Albumin and ALT of the Hepatic function of the rats compared with the values obtained from the control in Group 1.

Although these biochemical parameters were observed to be within the limit value in except ALP, which was marked lower (6.1 U/L) compared with the normal value (17.5-30.2 U/L). This may suggest liver damage or disease. This agreed with the result of Anofi *et al*. (2012), in their study on effects of stem bark of *Securidaca longepedunculata* extracts on blood profile of albino rat which revealed damage in liver cell.

More so, the results of rats administered with 500mg/kg extract, showed the values obtained compared with the normal value were within the normal value except ALT which have increased in value (40 U/L) compared with the normal value (17.5-30.2 U/L). This result agreed with the researchs of Anofi *et al*. (2012), on effects of stem bark of *Securidaca longepedunculata* extracts on blood profile of albino rat which where they find that when ALT is released into the bloodstream the level of ALT will increase. This could be the an indication that the hepatic protein metabolism/ synthesis is greatly enhanced by the treatment or there is an inhibitory effect of hepatic protein metabolism due to the treatment

In Group 6; the results of rats administered with 700mg/kg extract showed decreased in AST and ALT (10 U/L) and (12 U/L) respectively, while the Total protein and Albumin were within normal range. The result of this decrease in the ALT and AST values compared with normal value suggested that there is total liver damage (Anofi *et al.,* 2012). According to Anofi *et al*. (2012) an increase or decrease in ALT and AST could result in total liver damage. This damage in the liver cell could be due to high concentration of the extract of the stem bark of *Parkia biglobosa* administered to the rats and dosage interval which were not in consistent with the dosage interval which should not be more than 50mg/kg.

CONCLUSION

Neutralization effect of the *Parkia biglobosa* stem bark extracts was checked for *Naja nigrocollis* venom. The *in vivo* activities was shown to determine the anti-venom potential of the plant extract using three (3) dose levels which showed to be effective against the snake venom. From the results, it can be deduce that, the stem bark of *Parkia biglobosa* showed a remarkable effect against the *Naja nigricollis* venom at 500mg/kg as the rats showed a survival period of 17.20 hrs after treatment.

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