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Anti-venom activity of ethanolic extract of *bridelia ferruginea* leaves against *naja nigricollis* venom

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The *Bridelia ferruginea* plant as anti-snake venom activity against the venom of a clinically important snake, *Naja nigricollis* was investigated. The *Bridelia ferruginea* leaves were dried and extracted with ethanol and bioactive compounds such as tannins alkaloids,saponnin,flavonoids,steroids,terpenoids were determined by standard method. The effect of the extract on some selected enzymes activity in the serum/liver of albino rats induced with the snake venom was studied and the histopathology. The result showed that *Bridelia ferruginea* leaf extract has little anti-snake venom activity. Possibly, the plant extract could be more effective in vivo if the experiment model is modified to stimulate actual life experience.

Keywords: *Bridelia ferruginea,* anti-snake venom; *Naja nigricolis;* Histopathology; Enzyme activity.

INTRODUCTION

Envenoming resulting from snake bites is a particular important public health problem in rural areas of tropical and sub tropical countries situated in Africa, Asia, Oceania and Latin America [Chippaux JP 1998]. The usage of medical plants, in the treatments of various ailments is known to us since ancient times. There are various medicinal plants which have been used against snake envenomation in folk and traditional medicines. *Bridelia ferruginea* belongs to the Euphorbiaceae family. It is an indigenous medicinal plant in Nigeria which is used extensively in herbal medicine in Nigeria. *Bridelia ferruginea* is used for the treatment or several clinical complications such as physical, mental and social ailments (Keay et al., 1989].

Traditional healers are the first line defense against illnesses. The success of these healers is vaguely understood, partly due to the unknown material medical and occult mystical nature of their practice, but direct testimony from victims confirms success of their treatment. Biomedicine ignores their practice but they serve more snake bite accident victims than modern practitioners [Chippaux JP 1998]. Traditional healers attest to the usage of *Bridelia ferruginea* leaves in the treatment of snake bite. However, no scientific reports were found for its anti-snake venom potential.Thus, this work aim to investigate the ability of this plant to confer some level of resistance against snake venom especially *Naja nigricolis.*

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EXPERIMENTALS

Collection of the Plant Material:

Fresh leaves of *Bridelia ferruginea* were collected with assistance of a traditional healer from Oganaji, Anyigba, Kogi State, Nigeria. Authentication and the taxonomic identification were confirmed by botanist Dr. Ekwuno, Professor of Botany, Kogi State University, Nigeria.

TABLE 1. qualitatie phytochemical analyses (*Bridelia ferruginea*)

S/No	Bioactive Constituent	Chemical Test	Colour Intensity
1	Alkaloids	Picric acid test	++
2	Flavonoids	Ammonia test	+
3	Tannins	Fecl3	+
4	Saponins	Frothing test	++
5	Steroids and Terpenoids	Salkowski test	+++
6	Resins	Precipitate test	+

Preparation of Extract

The shade dried coarsely powdered leaves (1kg) of *Bridelia ferruginea* were extracted exhaustively with ethanol. After completion of extraction, the extract was freed of solvent by distillation, concentrated and then stored in desiccators until further use.

Chemical Analysis of Extract

Different chemical tests were carried out for the ethanolic extract of *Bridelia ferruginea* to identify the presence of various chemical constituents like alkaloids, tannins, resins, saponins, flavonoids, steroids and terpenoids [Warrel DA, Ormerold LD 1926].

Venom

The Lyophilized snake venom, *Naja nigricolis* was obtained from Ahmadu Bello University, Zaria, Nigeria and was preserved at 4℃. Before use, the venom was dissolved in saline, centrifuged at 2000rpm for 10mins and the supernatant used for anti-venom studies. Venom concentration was expressed in terms of dry weight.

INVIVO ACTIVITY

Animals:

Healthy adult Wister rats weighing 200-250g were used for the study. They were housed in polypropylene cages, maintained under standard conditions (12h light and 12h dark cycle; 25± 30℃; 35-60%). The animals were fed with standard rat pellet diet and water ad libitum.

Treatment: Experimental animal were treated as follows

Group 1 - Group administer with water
Group 2 - Group administer with venom
Group 3- Group administer with extract and venom

Neutralization potential of ethanolic extract of *Bridelia ferruginea* on lethal venom effect:

The toxicity of venom was assessed in Wister rats (200-250g) by I.P. administration of different concentrations of venom dissolved in 0.2ml of physiological saline to different groups (n=10). The median lethal dose (LD100) of venom was determined 24h later by the method of Theakston and Reid. The neutralizing potency of the leaf extract was assessed by I.P. administration of different doses (125,250 and 500 mg/kg body weight) of the plant extract.

ENZYME ASSAY

The quantitative invitro determinations of aspartate aminotransferases Creatine kinase and alanine amino transferase in blood serum including the total protein were carried out at room temperature according to the method described [Reitman SB, Frankel S (1957);Gornall et al., (1949)].Necessary precautions for enzyme activities measurement were taken and blanks were prepared for each sample. These enzymes are commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury to determine liver health [Rosalki SB (1974].

HISTOPATHOLOGY

A piece of the tissue from the most indicative area of the organ of each rat was incised and fixed for 48hrs in 10% formal saline solution. Thereafter, the tissues were processed manually for dehydration, cleaning and impregnation using alcohol for dehydration, xylene for cleaning the alcohol and paraffin for impregnation.The tissues were then embedded in paraffin wax using rubber cassette, then trimmed and sectioned. Sections of the tissues were obtained using the Hertz rotary microtome (Cambridge model) and tiny sections were obtained by cutting with two microns (2m).Staining of the tissue sections was done using heamatoxylin and Eosin technique described by [Warrel DA, Ormerold LD (1926)].

RESULTS

Leaf extract of *Bridelia ferruginea* was investigated for its potential anti-snake venom activity. The obtained extract was subjected to phytochemical screening for its constituents by standard methods and the result was tabulated in table 1. Preliminary phytochemical studies

Table 2: Biochemical/Enzymes assay in serum from the rat

Group	Creatine kinase	Total protein	ALT	AST
1	51.35±2.52	49.65±0.74	31.65±2.76	20.65±0.35
2	130.00±3.06	42.01±2.08	39.67±3.50	32.18±0.46
3	9.06±3.43	42.95±14.73	42.13±2.66	22.04±8.36

Table 3: Biochemical/Enzyme assay in liver homogenate of rat

Group	Total protein	ALT	AST
1	48.65±0.74	100.46±1.13	48.44±0.47
2	36.33±0.32	100.01±8.51	28.85±0.50

Table 4: Histopathology results

Cage	Dose mg/kg		Histological scoring skin	
	Inflammatio of ciliate infiltrate	distortion of skin appendages	distortion of epidermis	effect on dermis
group 1	-	-	-	-
group 2	+++	++	+	-
group 3	++	+	+	-

key: + = presence of features
++ = marked presence OF feature
± = intermediate features= normal
group 1… administered with distilled water only
group 2… administered with snake venom only
group 3… administered with plant extract and snake venom

revealed the presence of alkaloids, tannins, resins,saponins, flavonoids, steroids and terpenoids.

The results of the serum/liver enzyme assay are shown in tables 2&3 and the histopathology shown in table 4 reveal maximum inflammation of ciliate infiltrate and distortion of skin appendages and dermis of the rats induced with venom alone but lesser effect on those administered with the extract.Total plasma protein was significantly reduced after venom injection in rats that took snake venom and same decrease is also observed with the rats that were administered with venom/extracts.

This was also seen in the AST, ALT activity of the serum and homogenized liver sample (table 3). The creatine kinase activity of the serum was markedly increased in serum after i.p injection of the venom and also remained high in the rats that took venom/extracts as compared to control.

DISSCUSSION

Snakebite is a major health hazard that leads to high mortality rate especially in India. Vipera rusell and Naja kaouthia are the common snakes found throughout India and a large number of deaths occur due to envenomation by these snakes. Antisnake venom remains the specific antidote for snake venom poisoning. This antisnake venom is usually derived from horse sera. They contain horse immunoglobulins, which frequently caused complement mediated side effects, and other proteins that cause serum sickness and occasionally, anaphylactic shock. Although, use of plants against the effects of snakes bite has been given since last 20 years [Santosh et al., (2004)]. Many Indian medical plants are recommended for the treatment of snakebite [Alam MI, Gomes A (2003)]. In this study we examined the antivenom potential of *Bridelia ferruginea* plant extracts.

Several works dealing with the effects of snake venom in blood cells, marrow cells and in cells from other organs of animals, like muscle, liver, kidney and skin, showed varying results, depending on the experimental concentrations, exposure time site of injection, and type of toxin [Maria et al., (2003].The liver is a major producer for most of serum proteins and its total level in the blood is a main liver function test. It is established that liver is the main source of plasma albumin. Decrease in plasma albumin is mainly due to the diminishing of its synthesis in hepatic cells, accomplished by looses of amount of

albumin into the urine and gastrointestinal tract due to damage kidney and intestinal mucosa [West JB (1985]. It is worth mentioning that bone marrow is the main site of immunoglobulin production. Bone marrow plasma cells are derived from plasma plastic cells that have been generated in the peripheral lymphoid organs following antigen stimulation and have migrated to the bone marrow. These cells find in the bone marrow environment the survival and activation signals that allow them to generate mature plasma cells to produce high amounts of Igs [Roldan et al., (1992]. Moreover, cytokines such as IL-6 and IL-10 control the production of Igs by non-dividing mature plasma cells [Roldan et al., (1992].

The present study revealed Table 2 that, the injection of crude venom of *Naja nigricolis* causes a reduction in serum total proteins, albumin in envenomated rat at 2 hours post-injection of crude venom. These findings are in agreement with other investigators who reported that the reduction in serum total proteins, albumin, globulin and uric acid in envenomated rats was observed in laboratory animals injected with viper snake venoms [Abdul-Nabi et al., 1997; Fahim, 1998; Al-Jammaz et al., 1999; Al-Jammaz et al., (1998]. It might be assumed that, the reduced levels of these serum constituents could be due to disturbance in renal functions as well as haemorrhages in some internal organs. In addition, the increasing in vascular permeability and haemorrhages in vital organs due to the toxic action of various snake venoms were described by [Meier J, Stocker K (1999); Meier J, Theakston RDQ (1986)].Also, the reduction in serum total protein, albumin and total bilirubin levels in the envenomated rats could be attributed to the disturbance in protein synthesis in the hapatocytes due to cellular damage, together with hemorrhages in vital organs, which have led to protein lose. Such disturbances have been reported by various other investigators with snake venom [Tilbury et al., 1987; Abdul-Nabi, 1993; Abdul-Nabi et al., 1997; Al-Jammaz et al., 1999). Furthermore, acute renal damage together with glomerular, tubular and vascular lesions following various snake bites have been reported [Sitprija et al., 1982; Sani SM, Purandare NM 1972; Aung-Khin, 1978].
In addition, increased vascular permeability and hemorrhages in various other vital organs. In general and in the kidneys in particular, as has been observed by [Mohamed et al., 1981] in the majority of snake envenomation, further aggravates the reduction in serum proteins. It is also reported that the disturbance of retinal function of venom and the hemorrhage, usually associated with snake bit at the major factors responsible for the observed hypoproteinemia. Another factor is the increase vascular permeability due to toxic action of the venom which could contribute to the law of protein from plasma and tissue [Olajide et a.,l (1999].

Elevation of ALT and decrease of AST in the rat administered with venom/extract both in the serum and liver homogenate as observed have serious implication on health. Such elevations are found in cases of both liver damage and myocardial infarction [Gray C, Howorth PJN (1982)]. The elevation of AST and ALT makes the liver a target of suspicion as this is usual in cases of hepatotoxicity caused by toxic agent [Rosalki SB (1974]. The little anti-venom activity observed by the rat administered with venom/extract may be attributed to the presence of any of these compounds alkanoids,tannins, flavonoids,steroids and terpenoid present in the plant extract[Rajendran et al., 2010].

From the experiment it was also observed that all the rats died after 2 hours except control (group 1) and the one administered with venom/extract (group 3) who died 8 hours later. There was significant difference between the time of death in treated venom/extract and treated with venom (only) groups showing remarkably that the plant extract has effect on the activity of venom. Thus it was obvious that *Bridelia ferruginea* leaves did show little anti-venom activity.Possibly, the plant extract could be more effective against snake venom activity in vivo if the experiment model is modified to stimulate actual life experience.

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