Original Research Article

Anti-venom Activity of *Mucuna prurien* Leaves Extract Against

ABSTRACT

Aim: The study was done to investigate the anti-venom activity of *Mucuna prurien* leaves extract against cobra snake (*Naja Hannah)* venom.

Study Design: The mice were randomly grouped into six groups (A, B, C, D, E, and F) of five rats each. Group A served as the normal control (no induction), and the mice in the group were given normal saline (1ml/kg/body weight).Group B served as the test control (snake venom was induced but no treatment administered), Group C served as the standard control (snake venom was induced and treated with antivenin, a standard drug), Group D, E and F were all induced with the cobra snake venom and treated with ethanolic extracts of the leaves of *Mucuna prurien* for 14days.

Methodology: The induction with cobra snake venom was done with 0.075mg/kg b.w of venom and thereafter the treatment with *Mucuna prurien* extract for Group D, E and F were done with 40 mg/ kg, 60 mg/ kg and 80 mg/ kg respectively intraperitoneally in the mice. Serum blood of the animals was used to assay for total cholesterol, bilirubin, AST, ALT, GSH and catalase levels after 14days.

Result: The injection of crude venom of cobra snake (*Naja hannah)* caused an increase in cholesterol, AST, ALT, bilirubin, catalase and glutathione in envenomated mice which significantly reduced ($p<0.05$) compared to all the controls after 14 days of treatment with the extract.

Conclusion: The results suggests that 80 mg/ kg of the plant extract is more effective than the standard drug, therefore *Mucuna prurien* leaves has a greater anti-venom potential for curing snake bite, than antivenin.

Keywords: Anti-venom, *Mucuna prurien*, Antivenin, Cobra snake, Haemorrhage

1.0 INTRODUCTION

Each year in the world a lot of people receive venomous bites by snake and about 40, 000 of them die (Chippaux, 1998). *Echis carinatus* and *Naja hannah* are the major causes of snakebite deaths in Plateau State, Nigeria. Snake venoms of Viperidae and Elapidae are known to consist of a complex mixtures of toxins and enzymes which are responsible for haemorrhage, myonecrosis, neurotoxicity and alteration of blood coagulation (Markland, 1998; Warrell,1989).

The only effective treatment of the modern medicine for serious snakebites is the use of the antidote (antivenin), derived from antibodies, produced in horse's blood serum after injecting the animal with snake venom. In humans, antivenin is administered either through the veins or injected into muscle and acts by neutralizing the snake venom which has entered the body. Because antivenin is obtained from horses, snakebite victims sensitive to horse products must be very carefully treated. The danger is that they could develop an adverse reaction or even an anaylactic shock. Moreover the efficacy of the serums is dependent on the rapidity with which the specificity treatment is started and it is only specific for the venom used for the immunization. These represent an important limitation of this kind of therapy.

The genus M*ucuna*, belonging to the Fabaceae family, sub-family Papilionaceae, includes approximately 150 species of annual and perennial legumes. Among the various under-utilized wild legumes, the velvet bean *Mucuna prurien* ("Cowitch" and "cowhage" are the common English names) is widespread in tropical and sub-tropical regions of the world. It is considered a viable source of dietary proteins (Janardhanan *et al*., 2003; Pugalenthi *et al*., 2005) due to its high protein concentration (23–35%) in addition its digestibility, which is comparable to that of other pulses such as soybean, rice bean, and lima bean (Gurumoorthi *et al*., 2003). It is therefore regarded as a good source of food.

Mucuna prurien is a popular Indian medicinal plant, which has long been used in traditional Ayurvedic Indian medicine, for diseases including Parkinsonism (Sathyanarayanan *et al*., 2007). The beans have also been employed as a powerful aphrodisiac (Amin, 1996) and have been used to treat nervous disorders and arthritis (Jeyaweera, 1981). The bean, if applied as a paste on scorpion stings, is thought to absorb the poison (Jeyaweera, 1981). The non-protein amino acid-derived L-dopa (3, 4-dihydroxy phenylalanine) found in this underutilized legume leaves resists attack from insects, and thus controls biological infestation during storage. According to D'Mello (1995), all anti-nutritional compounds confer insect and disease resistance to plants. Further, L-dopa has been extracted from the leaves to provide commercial drugs for the treatment of Parkinson's disease. L-Dopa is a potent neurotransmitter precursor that is believed, in part, to be responsible for the toxicity of the *Mucuna* leaves (Lorenzetti *et al.,*1998). Antiepileptic and anti-neoplastic activity of ethanolic extract from *Mucuna prurien* had been reported (Gupta *et al*., 1997).

An ethanolic extract of *Mucuna prurien* leaves has demonstrated significant in vitro anti-oxidant activity, and there are also indications that ethanolic extracts of *Mucuna puriens* may be a potential source of natural anti-oxidants and anti-microbial agents (Rajeshwar *et al.,* 2005). All parts of *Mucuna prurien* possess valuable medicinal properties and it has been investigated in various contexts, for its anti-diabetic, aphrodisiac, anti-neoplastic, anti-epileptic, and anti-microbial activities (Sathyanarayanan *et al.,* 2007). Its anti-venom activities have been investigated by (Guerranti *et al*.(2002) and its anti-helminthic activity has been demonstrated by Jalalpure (2007). *Mucuna prurien* has also been shown to be neuroprotective (Misra and Wagner, 2007), and has demonstrated analgesic and anti-inflammatory activity (Hishika *et al*., 1981). The primary objective of the present study is to confirm the bioactivity of *Mucuna prurien* leaves extract against cobra snake (*Naja Hannah)* venom.

2.0 METHODOLOGY

2.1 Collection and Preparation of the Plant Sample

The fresh leaves of *Mucuna prurien* were collected from Kogi State, Nigeria. It was identified by Dr. S. M. Ayodele (Dept. of Botany, Kogi State University). The leaves were dried under room temperature for 5days. The dried sample was ground powdery form, using the warring commercial blender.

2.2 Extraction of *Mucuna prurien* leaves

The plant material (500g) was defatted with 400ml hexane ($C_6 H_{14}$) by using a Soxhlet apparatus for 5h. The defatted powder plant material was air-dried. The air-dried defatted powdered plant material was then extracted with 400 ml ethanol (C_2H_5OH) by using a Soxhlet apparatus for 8h. The residue was dried over night and extracted with

250 ml water (H_2O) by using a shaking water bath at 70^0C for 2h. The extraction with water was repeated three time. The water filtrates were mixed together. The ethanol and water extract were filtered and evaporated by using a rotary evaporator and freeze dryer to give the crude-dried extract. The dried extracts were stored at -20^0C until used.

Calculation:

$$\text{Percentage yield } (\%) = \frac{\text{weight of the plant extract}}{\text{Weight of the dried plant used}} \times 100$$

2.3 Proximate Analysis af *Mucuna prurien*

The moisture content, ash content, carbohydrate content, crude fibre and crude protein were determined using methods as described by AOAC (1990).

2.4 Phytochemical Screening

The ethanolic extract of *Mucuna prurien* leaves were screened for the presence of phytochemical compound as described by Treatise and Evans (1989) and Sofowora (1993).

2.5 Animals

Experimental mice were purchased from the animal house of NIPRID. The animals were housed in steel cages and kept at room temperature. The mice had no history of drug consumption that is; they had not been used for any investigation. The mice were put on standard mice pellet (feed) and pure drinking water and allowed to get acclimatized for 21 days before the start of the experiment.

2.6 Induction of Anti-Snake Venom

The cobra snake venom native preparations, from *Mucuna prurien*, were given intraperitioneally (i.p) to the mice at a dose which were proportional to the weight of the animals. The volumes of preparation were identical and the same amounts were injected.

2.7 Experimental Design

White male albino mice of wister strain of body weight ranging between 15-30g were used for the research study. The mice were randomly grouped into six groups (A, B, C, D, E, and F) of five rats each. Group A served as the normal control (no induction), and the mice in the group were given normal saline (1ml/kg/body weight).Group B served as the test control (snake venom was induced but no treatment administered), Group C served as the standard control (snake venom was induced and treated with antivenin, a standard drug), Group D, E and F were all induced with the cobra snake venom and treated with ethanolic extracts of the leaves of *Mucuna prurien* (Table 1).

Table 1: Experimental Design

Group	Name	Treatment for 14 days
A	Normal Control	No induction, No treatment
B	Test Control	Induced with 0.075mg/kg b.w of venom, but no treatment
C	Standard Control	Induced with 0.075mg/kg b.w of venom and treated with antivenin (a standard drug).
D	Group A	Induced with 0.075mg/kg b.w of venom and treated with 40mg/kg extract
E	Group B	Induced with 0.075mg/kg b.w of venom and treated with 60mg/kg extract

F	Group C	Induced with 0.075mg/kg b.w of venom and treated with 80mg/kg extract

2.8 Determination of Serum Cholesterol Level

Total cholesterol was determined by the enzymatic endpoint method as described by Trinder (1969). In this method, cholesterol was determined after enzymatic hydrolysis and oxidation in a series of reaction. The indicator quinoneimine used in this method was formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

2.9 Determination of Serum Bilirubin Level (Vander Bergh's Reaction)

The total bilirubin in serum or plasma is determined using the method of Jendrassik and Gróf (1938) by coupling with diazotized sulfanilic acid after the addition of caffeine, sodium benzoate and sodium acetate. A blue azobilirubin is formed in alkaline Fehling solution II. This blue compound can also be determined selectively in the presence of yellow by products (green mixed coloration) by photometry at 578 nm.

2.10 Determination of Transaminases (ALT and AST)

Alanine Aminotransferase was determined in a method as described by Reitman and Frankel (1957). In this method, ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine in a reaction. Aspartate Aminotransferase was determined in a method as described by Reitman and Frankel

(1957). In this method, AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine in a reaction.

2.11 Determination of Serum Glutathione level

The principle was based on the reduction of 5,5 dithiobis (2-nitrobenzoic acid) (DTNB) with reduced glutathione (GSH) to produce a yellow compound. The reduced chromogen was directly proportional to GSH concentration and its absorbance was measured at 405 nm (Tietz, 1990).

2.12 Determination of Serum Catalase Level

Catalase was determined by the method described by Cohen *et al.* (1970). In this method, catalase catalysed the conversion of hydrogen peroxide to oxygen and water in a reaction.

2.13 Statistical analysis

Values are expressed as mean + S.E.M randomized complete block design analysis of variance was used for statistical analysis. P values less than 0.05 was considered significant.

3.0 RESULTS

3.1 Percentage Yield of Extract

The extract was thick and greenish in colour, with an ethanolic extraction of *Mucuna prurien* leaves which indicated the yield of 6.73%.

3.2 Phytochemical Screening

The preliminary phytochemicals test reveals that the major phytochemical constituents in *Mucuna prurien leaves* are alkaloids, flavonoids, tannins, saponins, steroids, teroenoids, cardiac glycosides and anthraquinones (Table 2).

Table 2: Phytochemical composition of *Mucuna prurien leaves*

Phytochemicals	**Presence in *Mucuna prurien leaves***
Alkaloids	+ +
Flavonoids	+ +
Tannins	+
Saponins	+ +
Steroids	+ +
Terpenoids	+ +
Cardiac glycosides	+
Anthraquinones	+

3.3 Proximate Analysis

The proximate analysis of the leaves in Table 3 has a moisture content of 11.37%, crude fiber 31.91%, crude fat 2.97%, carbohydrate 45.65%, Ash content 3.00%.

Table 3: Proximate composition of *Mucuna prurien leaves*

PARAMETER	**COMPOSITION**
Moisture content	11.37 %
Crude protein	31.91%
Crude fat	2.97 %
Carbohydrate	45.65 %

Ash	3.00 %

3.4 Effect of Venom Induction and Extract on Biochemical Parameters

The results revealed in Table 4a and 4b that, the injection of crude venom of cobra snake (*Naja hannah)* caused an increase in cholesterol, AST, ALT, bilirubin, catalase and glutathione in envenomated mice compared to the normal control mice. There was no significant difference in the levels of cholesterol, AST, ALT, bilirubin, catalase and glutathione of the treated groups when compared to the test control group.

Table 4a: Result obtained on the effect of extract on Cholesterol, AST and ALT at day one after venom induction

Treatment	Cholesterol (mg/dl)	AST (U/l)	ALT (U/l)
Normal control	55.94 ± 0.07	28.93 ± 0.14	22.60 ±0.49
Test control	127.41 ±0.13[a]	74.34± 2.24[a]	36.64 ±0.64[a]
Standard control	125.91 ± 0.06[a]	74.96 ± 0.18[a]	34.96 ±0.35[a]
Group A 40mg/kg	127.48 ±0.05[a]	75.67 ± 0.21[a]	36.20 ±0.42[a]
Group B 60mg/kg	128.30 ± 0.04[a]	73.61 ± 0.14[a]	37.10 ±0.21[a]
Group C 80mg/kg	128.11 ± 0.03[a]	73.93 ± 0.07[a]	37.04 ±0.14[a]

Values are expressed as means ± SEM. [a] indicate values that are significantly different when compared to the normal control at ($p < 0.05$).

Table 4b: Result obtained on the effect of extract on Bilirubin, Catalase and Glutathione at day one after venom induction

Treatment	Bilirubin (mg/dl)	Catalase (mg/dl)	Glutathione (U/L)
Normal control	0.42 ± 0.04	17.19 ±0.05	4.69 ± 0.51
Test control	0.95 ± 0.07[a]	25.45 ±0.93[a]	8.47 ± 1.21[a]
Standard control	0.90 ± 0.02[a]	26.06 ±0.06[a]	8.21 ± 0.68[a]
Group A 40mg/kg	0.94 ± 0.05[a]	25.82 ±0.74[a]	8.37 ± 0.74[a]
Group B 60mg/kg	0.91 ± 0.03[a]	27 48 ±0.62[a]	7.95 ± 0.55[a]
Group C 80mg/kg	0.95 ± 0.02[a]	25.59 ±0.50[a]	8.11 ± 0.48[a]

Values are expressed as means ± SEM. [a] indicate values that are significantly different when compared to the normal control at ($p < 0.05$).

The levels of cholesterol, AST, ALT, bilirubin, catalase and glutathione were significantly reduced when compared to the test control after 14 days of treatment with the extract (Table 5a and 5b).

Table 5a: Result obtained on the effect of extract on Cholesterol, AST and ALT at day fourteen

Treatment	Cholesterol (mg/dl)	AST (U/l)	ALT (U/l)
Normal Control	50.00 ± 0.07	25.54 ± 0.07	17.46 ± 0.20
Test control	113.45 ±0.17	62.25 ± 1.24	38.88 ± 0.44
Standard control	60.00± 0.06[ab]	30.37± 0.19[ab]	19.71 ± 0.28[ab]
Group A 40mg/kg	96.67 ± 0.05[abc]	40.45 ± 0.21[abc]	26.24 ± 0.35[abc]
Group B 60mg/kg	83.33 ± 0.04[abc]	36.39 ± 0.14[abc]	21.46 ± 0.21[ab]
Group C 80mg/kg	55.67 ± 0.03[abc]	28.82 ± 0.09[abc]	18.41 ± 0.17[b]

Values are expressed as means ± SEM. [a] indicate values that are significantly different when compared to the normal control at ($p < 0.05$), [b] indicate values that are significantly different when compared to the test control at ($p < 0.05$) and [c] indicate values that are significantly different when compared to the standard control at ($p < 0.05$).

Table 5b: Result obtained on the effect of extract on Bilirubin, Catalase and Glutathione at day fourteen

Treatment	Bilirubin (mg/dl)	Catalase (mg/dl)	Glutathione (U/L)
Normal Control	0.34 ± 0.02	14.16 ± 0.32	2.59 ± 0.31
Test control	0.98 ± 0.07	20.45 ± 1.30	6.47 ± 1.21
Standard control	0.47 ± 0.03[ab]	13.06 ± 0.56[b]	1.83 ± 0.33[ab]
Group A 40mg/kg	0.76 ± 0.04[abc]	16.67 ± 0.95[abc]	4.32 ± 0.49[abc]
Group B 60mg/kg	0.54 ± 0.03[abc]	15.89 ± 0.84[bc]	3.97 ± 0.22[abc]
Group C 80mg/kg	0.41 ± 0.01[abc]	12.95 ± 0.72[abc]	1.82 ± 0.12[ab]

Values are expressed as means ± SEM. [a] indicate values that are significantly different when compared to the normal control at ($p < 0.05$), [b] indicate values that are significantly different when compared to the test control at ($p < 0.05$) and [c] indicate values that are significantly different when compared to the standard control at ($p < 0.05$).

4.0 DISCUSSION

Nevertheless, in *in vivo* studies tannins may interact with plasma proteins from the blood circulation. The anti-venom activity observed in the mice treated with the extract may be attributed to the presence of any of these compounds alkanoids, tannins, flavonoids, steroids and terpenoid (Rajendran *et. al*, 2010). The proximate analysis of the leaves in Table 3 has a moisture content of 11.37%, crude fiber 31.91%, crude fat 2.97%, carbohydrate 45.65%, Ash content 3.00%. The Ash content is known to enhance digestibility, slow down the release of glucose into the blood stream and reduces blood cholesterol level.

The present study revealed in Table 4a and 4b that, the injection of crude venom of cobra snake (*Naja hannah)* caused an increase in cholesterol, AST, ALT, bilirubin, catalase and glutathione in envenomated mice which significantly reduced after 14 days of treatment with the extract as shown in Table 5a and 5b. These findings are in agreement with other investigators who reported that the reduction in cholesterol, AST, ALT, bilirubin and catalase in envenomated mice was observed in laboratory animals treated with the extracts of *mucuna prurien* leaves Abdul-Nabi *et al*. (1997). It might be assumed that, the increased levels of these serum constituents could be due to disturbance in renal functions as well as haemorrhages in some internal organs when challenged with a snake venom. 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 and Stocker 1999; Meier and Theakston; 1986). Also, the reduction in serum cholesterol, AST, ALT, catalase, glutathione albumin and total bilirubin levels in the envenomated mice could be attributed to the anti-venom potentials of the extract of mucuna pruriens administered.

Furthermore, acute renal damage together with glomerular, tubular and vascular lesions following various snake bites have been reported (Sitprija *et al*., 1982; Sani and Purandare, 1972; Aung-Khin, 1978) with additional, increased vascular permeability and hemorrhages in various vital organs. Another factor is the increase vascular permeability due to toxic action of the venom which could contribute to the low level of protein from plasma and tissue (Olajide *et al,* 1999).

In Table 4a, elevation of ALT and AST in the mice administered with venom as observed in the serum have serious implication on health of the animals. Such elevations are found in cases of both liver damage and myocardial infarction (Gray and Howorth, 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, 1974). From the experiment it was observed that some of the mice died after 30 minute of induction with the snake venom except the (Normal control) and the ones treated with different dose of the venom/extract (Groups A-C) which survived till the end of the experiment. There was significant difference between the time of death in the extract treated group and those treated with venom only showing that the plant extract had effect on the activity of the venom. Thus, it was obvious that *Mucuna prurien* leaves did show greater anti-venom activity, the extract of *Mucuna prurien* showed a better anti-snake activity compared with antivenin. This can be attributed to the fact that tannins are able to non-specifically bind to *Naja hannah* venom proteins and precipitate them, thus provoking the anti-lethal effects.

CONCLUSION

In conclusion, this investigation revealed that the ethanolic extract of *Mucuna prurien leaves* has the following phytochemical components of saponin, terpenoid, flavonoids, steroids, Alkaloids, Anthraquinones. It was observed that 80 mg/ kg of the plant extract is more effective than the standard drug, therefore *Mucuna prurien* leaves has a greater medicinal plant for curing snake bite, than anti-venin.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

CONSENT

Not applicable

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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