# *Original Research Article*

## Anti-venom Activity of *Mucuna pruriens* Leaves Extract Against Cobra Snake (*Naja hannah)* Venom


## ABSTRACT

**Aim:** The study was done to investigate the anti-venom activity of *Mucuna pruriens* 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 *M. pruriens* for 14days.

**Methodology:** The induction with cobra snake venom was done with 0.075mg/kg b.w of venom and thereafter the treatment with *M. pruriens* 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 *M. pruriens* leaves has a greater anti-venom potential for curing snake bite, than antivenin.

**Keywords:** Anti-venom, *Mucuna pruriens*, 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 anaphylactic 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 pruriens* ("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.

*M. pruriens* 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 *M. pruriens* had been reported (Gupta *et al*., 1997).

An ethanolic extract of *M. pruriens* 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 *M. pruriens* 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). *M. pruriens* has also been shown to be neuroprotective (Misra and Wagner, 2007), and has demonstrated analgesic and anti-inflammatory activity (Hishika *et al*., 1981). *M. pruriens* has been reported to be a potential natural source of antioxidants with greater importance as a therapeutic agent in preventing or slowing oxidative stress related degenerative diseases (Sonpetkar *et al.*, 2012). In Nigeria, where the *M. pruriens* seeds are locally prescribed as an oral prophylactic for snake bite, it is claimed that when two seeds are swallowed, they protect the individual for a year against snake bites (Scire *et al.* 2011). Therefore, the primary objective of the present study is to confirm the bioactivity of *M. pruriens* leaves extract against cobra snake (*Naja hannah)* venom.

## 2.0 METHODOLOGY

### 2.1 Collection and Preparation of the Plant Sample

The fresh leaves of *M. pruriens* 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 *M. pruriens* 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 $70^0$C 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 -$20^0$C 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 *M. pruriens*

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 *M. pruriens* 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 was procured in a sample bottle from National Veterinary Research Institute, Vom, Plateau State, Nigeria. The cobra snake venom native preparations 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 *M. pruriens* (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** | Venom + 40mg/kg | Induced with 0.075mg/kg b.w of venom and treated with 40mg/kg extract |
| **E** | Venom + 60mg/kg | Induced with 0.075mg/kg b.w of venom and treated with 60mg/kg extract |
| **F** | Venom + 80mg/kg | 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 (ALT) 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 (AST) 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 *M. pruriens* leaves which indicated the yield of 6.73%.

## 3.2 Phytochemical Screening

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

Table 2: Phytochemical composition of *M. pruriens leaves*

| **Phytochemicals** | **Presence in *M. pruriens leaves*** |
|---|---|
| Alkaloids | + + |
| Flavonoids | + + |
| Tannins | + |
| Saponins | + + |
| Steroids | + + |
| Terpenoids | + + |
| Cardiac glycosides | + |
| Anthraquinones | + |

(+) indicates weak presence; (++) indicates strong presence

### 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 *M. pruriens 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] |

| | | | |
|---|---|---|---|
| **Venom + 40mg/kg** | 127.48 ±0.05[a] | 75.67 ± 0.21[a] | 36.20 ±0.42[a] |
| **Venom + 60mg/kg** | 128.30 ± 0.04[a] | 73.61 ± 0.14[a] | 37.10 ±0.21[a] |
| **Venom + 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] |
| **Venom + 40mg/kg** | 0.94 ± 0.05[a] | 25.82 ±0.74[a] | 8.37 ± 0.74[a] |
| **Venom + 60mg/kg** | 0.91 ± 0.03[a] | 27 48 ±0.62[a] | 7.95 ± 0.55[a] |
| **Venom + 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] |

| | | | |
|---|---|---|---|
| **Venom + 40mg/kg** | 96.67 ± 0.05$^{abc}$ | 40.45 ± 0.21$^{abc}$ | 26.24 ± 0.35$^{abc}$ |
| **Venom + 60mg/kg** | 83.33 ± 0.04$^{abc}$ | 36.39 ± 0.14$^{abc}$ | 21.46 ± 0.21$^{ab}$ |
| **Venom + 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$^{ab}$ |
| **Venom + 40mg/kg** | 0.76 ± 0.04$^{abc}$ | 16.67 ± 0.95$^{abc}$ | 4.32 ± 0.49$^{abc}$ |
| **Venom + 60mg/kg** | 0.54 ± 0.03$^{abc}$ | 15.89 ± 0.84$^{bc}$ | 3.97 ± 0.22$^{abc}$ |
| **Venom + 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

The phytochemical constituents in *M. pruriens leaves* are alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, cardiac glycosides and anthraquinones (Table 2). The anti-venom activity observed in the mice treated with *M. pruriens* 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 *M. pruriens* 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 *M. 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). An earlier study by Fung *et al.* (2010) reported that injection of *M. pruriens* seed extract conferred protection against *C. rhodostoma* venom, as the pretreatment attenuated (albeit only partially) the cardio-respiratory depressant effects of *C. rhodostoma* venoms in anesthetized rats.

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 D-F) 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 *M. pruriens* leaves did show greater anti-venom activity, the extract of *M. pruriens* 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. However, Fung *et al.* (2012) reported that the protective effect of

*M. pruriens* extract pretreatment against cobra venom involved a direct action of *M. pruriens* extract on the heart, and, as such, immunological neutralization is not the only mechanism of protective effect of the *M. pruriens* extract pretreatment. Also, Tan *et al.* (2009) had reported the protective effect of *M. pruriens* seed against snake venom poisoning, which corroborates the results of this study that *M. pruriens* plant has great potential for use in the treatment of common cobra bites from the *Naja* subspecies.

## CONCLUSION

In conclusion, this investigation revealed that the ethanolic extract of *M. pruriens 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 *M. pruriens* 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.

## REFERENCES


AOAC. Official Methods of analysis. 15th Edn. Association of Official Agricultural Chemists, Washington, DC. 1990.

Abdul-Nabi IM, Raafat A, El-Shany H. Biological effect of intraperitoneal injection of rats with the venom of the snake *Echis carnetus*. Egypt J. Zool. 1997;29:195-205.

Amin KMY, Khan MN, Zillur-Rehman S, Khan NA. Sexual function *Mucuna pruriens* roving effect of *Mucuna pruriens* in sexually normal male rats. Fitoterapia. 1996; 67, 53-56.

Aguiyi JC, Uguru MO, Johnson PB, Obi CI. Effect of *Mucuna pruriens* leaves extract on smooth and skeletal muscle preparation. Fitoterapia.1997; 4: 366-70.

Aung-Khin M. Histological and ultrastructural changes of the kidney in renal failure after viper envenomation. Toxicon. 1978;16: 71-75.

Chippaux JP. Snake Bite: Appraisal of the Global Situation. Bulletin WHO 1998;76:515-524.

Chippaux JP, Goyffon M. Venoms, antivenoms and immunotherapy. Toxicon;36:823-46.

316 Cohen G, Dembiee D, Marcus J. (1970). Measuremment of catalase activity in tissues
317 extracts. Anal. Biochem. 1998;34: 30-38.

318 D'Mello JPF. Leguminous leaf meals in non-ruminant nutrition. In: Tropical Legumes in
319 Animal Nutrition. J.P.F. D'Mello and C. Devendra, Eds. CAB International, Wallingford,
320 UK. 1995.

321 Fung SY, Tan NH, Sim SM. Protective effects of *Mucuna pruriens* seed extract
322 pretreatment against cardiovascular and respiratory depressant effects of *Calloselasma*
323 *rhodostoma* (Malayan pit viper) venom in rats. Trop Biomed. 2010;27(3): 366–372.

324 Fung SY, Tan NH, Sim SM, Aguiyi JC. Effect of *Mucuna pruriens* Seed Extract
325 Pretreatment on the Responses of Spontaneously Beating Rat Atria and Aortic Ring to
326 *Naja sputatrix* (Javan Spitting Cobra) Venom. Evid Based Complement Alternat Med.
327 2012;486390: 6.

328 Gray C, Howorth PJN. Clinical Chemical Pathology. 9th ed. The ELBS, Edward Arnold
329 Publishers Ltd., 1982.

330 Gupta M, Mazumder UK, Chakraborti S, Bhattacharya S, Rath N, Bhawal SR.
331 Antiepileptic and anticancer activity of some indigenous plants. Indian J. Physiol. Allied
332 Sci. 1997;51, 53-56.

333 Guerranti R, Aguiyi JC, Errico E, Pagani R, Marinello E. Effects of *Mucuna pruriens*
334 extract on activation of prothrombin by Echis carinatus venom. J Ethnopharmacol.
335 2001;75, 175-180.

Gurumoorthi P, Pugalenthi M, Janardhanan K. Nutritional potential of five accessions of a south Indian tribal pulse *Mucuna pruriens* var. utilis; II. Investigation on total free phenolics, tannins, trypsin and chymotripsin inhibitors, phytohaemagglutinins, and in vitro protein digestibility. Trop. Subtrop. Agroecosys. 2003;1,153-158.

Hishika R, Shastry S, Shinde S, Guptal SS. Preliminary, phytochemical and anti-inflammatory activity of seeds of *Mucuna pruriens*. Indian J. pharmacol. 1981;13 (1), 97-98.

Jalalpure SS, Alagawadi KR, Mahajanashell CS. In vitro antihelmintic property of various seed oils against Pheritima posthuma. Ind. Pharm. Sci. 2007;69, 158-160.

Janardhanan K, Gurumoorthi P, Pugalenthi M. Nutritional potential of five accessions of a South Indian tribal pulse, *Mucuna pruriens* var. utilis. Part I. The effect of processing methods on the contents of L-Dopa, phytic acid, and oligosaccharides. J Trop and Subtrop Agro-ecosys. 2003;1, 141-152.

Jendrassik L, Grof P. Colorimetric Method of Determination of bilirubin. Biochem Z. 1938; 297:81-82

Jeyaweera, DMA. Madicinal plants used in Ceylon. Colombo, Sri Lanka; National Science Council of Sri Lanka, 1981.

Lorenzetti E, MacIsaac S, Arnason JT, Awang DVC, Buckles D. The phytochemistry, toxicology and food potential of velvet bean (M*ucuna* adans spp. Fabaceae). In D. Buckles, O. Osiname, M. Galiba, & G. Galiano, Cover crops of West Africa; contributing to sustainable agriculture. IDRC, Ottawa, Canada & IITA, Ibadan, Nigeria, 1998.

Maria DA, Vassa RC, Ruiz IRG. Haematopoietic effects induced in mice by the snake venom toxin jarahagin. Toxicon, 1998;42: 579-585.

Markland FS. Snake Venoms, Drugs. 1997;54 (Suppl.3):1-10.

Markland F. Snake venoms and the hemostatic system. Toxicon. 1998;36, 1749–1800.

Meier J, Stocker K. Effect of venoms on homeostasis. Toxicol. 1999;21 (3): 1711-1820.

Meier J, Theakston RDG. Approximate LD50 determinations of snake venoms using eight to ten experimental animals. Toxicon 1986;24, 395 – 401.

Misra L, Wagner H. Extraction of bioactive principles from *Mucuna pruriens* seeds. Indian J. Biochem. Biophys. 2007;44, 56-60.

Olajide OA, Makinde JM, Awe SO. Effect of aqeous extract of mucuna prurien stem bark on corragenan induced oedema and granuloma tissue formation in rats and mice. J. Ethnopharmacol. 1999;66(1): 113-177

Pugalenthi M, Vadivel V, Siddhuraju P. Alternative Food/Feed Perspectives of an Underutilized Legume *Mucuna pruriens* var. Utilis—A Review. Plant Foods Hum. Nutr. 2005;60: 201–218.

Rajendran K, Annie S, Maneesh MR, Vijaya B. Invitro and In vivo anti snake venom (Daboia russelli) studies on various leaf extracts of Acalpha indica Linn. Int J Phytomed. 2010;2: 217-220

375 Rajeshwar, Y., Kumar, S.G.P., Gupta, M., Mazumder, K.U., (2005). Studies on in vitro
376 antioxidant activities of mhetanol extract of *Mucuna pruriens* (Fabaceae) seeds. Euro
377 Bull Drug Res., 13, 31-39.

378 Reitman S, Frankel S. A colourimetric method for the determination of serum glutamate-
379 oxaloacetate and pyruvate transaminases. Am J Clin Pathol. 1957;28: 56-63.

380 Roldan E, Rodriguez,C, Naves G, Parra C, Brieva JA. VLA-4fibronectin interaction is
381 required for the terminal differentiation of human bone marrow capable of spontaneous
382 and high rate immunogolobulin secretion. J Exp Med. 1992;175: 1739-1747.

383 Rosalki SB. Enzyme profiles as indicators of susceptibility to environmental toxic
384 agents. J Proc Soc Med. 1974;67:633 – 636.

385 Sani SM, Purandare NM. Autopsy study of cases of snake bite with special reference to
386 renal lesions. J. Postgrad Med. 1972;18: 181-188.

387 Santosh R, Fattepur, Shivaji P, Gawade. Preliminary screening of herbal plant extracts
388 for antivenom activity against common sea snake (Enhydrina schistose) Poisoning.
389 Pharmacog, Magazing. 2004;16: 56-60.

390 Scirè A, Tanfani F, Bertoli E, Furlani E, Nadozie HO, Cerutti H, Cortelazzo A, Bini L,
391 Guerranti R. The belonging of gpMuc, a glycoprotein from *Mucuna pruriens* seeds, to
392 the Kunitz-type trypsin inhibitor family explains its direct anti-snake venom activity.
393 Phytomed. 2011;18(10):887-95.

394 Siddhuraju P, Becker K, Makkar HPS. Studies on the nutritional composition and
395 antinutritional factors of three different seed material of an under-utilised tropical
396 legume, *Mucuna pruriens* var. utilis . J. Agric. Food Chem. 2000;48, 6048-6060.

397 Sitprija V, Suvanpha R, Pochanugool C, Chusil S, Tungsanga K. (1982). Acute
398 intestinal nephritis snakebite. Am J Trop Med and Hyg. 2000; 31:408-410.

399 Sofowora. A Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd,
400 Ibadan, 1993.

401 Sonpetkar JM, Nipate SS, Shenoy PA, Chaudhari PD. In vitro antioxidant activity of
402 ethanolic extract of *Mucuna pruriens* (L.) DC. Seeds. J Pharm Res. 2012;5 (5):2769.

403 Sathyanarayanan G, Garg PK, Prasad H, Tandon RK. Elevated level of interleukin-6
404 predicts organ failure and severe disease in patients with acute pancreatitis. J
405 Gastroenterol Hepatol. 2007;22:550-4.

406 Tan NH, Fung SY, Sim SM, Marinello E, Guerranti R, Aguiyi JC. The protective effect of
407 *Mucuna pruriens* seeds against snake venom poisoning. J Ethnopharmacol. 2009; 123:
408 356–358.

409 Tietz NW. Clinical Guide to Laboratory Tests, Second Edition W. B. Saunders
410 Company, Philadephia, USA,1990.

411 Treatise GE, Evans WC. Pharmacognosy.11th edn. Brailliar Tiridel Can. Macmillan
412 publishers. Ltd, Ibadan, 1989.

413 Tripathi YB, Updhyay AK. Antioxidant property of *Mucuna pruriens* Linn. Curr. Sci.
414 2001; 80, 1377-1378.

415 Trinder P. Determination of glucose in blood using glucose oxidase with an alternative
416 oxygen acceptor. Anal Clin Biochem. 1969;6: 24-27.

417 Warrell DA. Snake venoms in science and clinical medicine. Russell's viper: biology,
418 venom and treatment of bites. Trans Roy Soc Trop Med Hyg. 1989;83, 732–740.