Research Article

Neutralization Property of Datura Metel Root Extract on Indian Russell's Viper Venom in Experimental Animals

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Received: 27-03-2019; **Revised:** 30-04-2019; **Accepted:** 06-05-2019.

ABSTRACT

The main aim is to determine the Neutralization property of Datura Metel root extract against Russell's viper venom using swiss albino mice and Wistar rats. Male swiss albino mice and Wistar rats were used for the study. Albino mice are divided into 5 groups (A, B, C, D, E) each group contains six animals. Group A and B is pretreated with Datura metel root extract 20 and 50 mg/kg body weight of the animal orally for 21days, on 21st day injected with 0.075mg/kg Russell's viper venom subcutaneously. Group B is treated with Antisera i.v 5min after injection of 0.075mg/kg of venom. Group D and E is used as a control and negative control group. Blood clotting, bleeding time, WBC, RBC, HGB is determined by in vitro method. Kidney, brain, heart and liver, is dissected out and histopathological studies were carried out. The Wistar rats are induced with MHD and MND of venom sac and also hemorrhagic and necrotizing activity is determined.

Keywords: Datura metal, Russells, antisera, hemorrhagic, venom.

INTRODUCTION

Snake bite is a public health hazard in India. In India at an average 250,000 snakebites are recorded in a single year. The snakes found in India show great biodiversity and their length varies from 6 mm to 10 mm, while weight ranges between few grams to several kilograms [1]. To treat envenoming, the production and clinical use of antivenom must be improved. Since some research articles prove that snake venom contains several neurotoxic, cardiotoxic, cytotoxic, nerve growth factor, lectins, disintrigins, haemorhagins and many other different enzymes. These proteins not only inflict death to animals and humans, but can also be used for the treatment of thrombosis, arthritis, cancer and many other diseases[2, 3]. The treatment for snake bite varies from snake to snake. The main accessible treatment is the use of anti-venom against snake bite. The first anti-venom (called an anti-ophidic serum) was discovered by Albert Calmette, a French researcher of the Pasteur Institute in 1895, against the Indian Cobra (Nagoya) [4]

The medicinal plant *Datura metel (DM) (Fam. Solanaceae)* has been used in ethano-therapeutic management of asthma, insomnia and rheumatic pain. The smoke from the burning leaf is inhaled for the relief of asthma and bronchitis. Seeds and leaves of *D. metel* were reportedly used to sedate hysterical and psychotic patients[5]. Decoction of *D. Metal* leaf has been reported to be effective in management of madness, epilepsy and depression. The bitter narcotic plant relieves pain and encourages the healing process[6]. Phytochemical studies of the plant revealed the presence of Scopolamine in the plant, which makes it a potent cholinergic-blocking hallucinogen that has been used to calm schizoid patients[7]. *D. metel* contains flavonoids, phenols, tannins, saponins and sterols as major phytoconstituents[8].

The Russell's viper is considered a highly venomous snake throughout its range in Asia In Russell's viper bite, the reported incidence of dry bite has been low, with most bites resulting in significant envenoming. At present there is no early sign or symptom that has been identified in Russell's viper bite that could be considered a clinical predictor of significant systemic envenoming [9].

Medicinal plant extracts are widely used as a remedy for treating snakebite. In medicinal plant extracts, rich source of natural inhibitors and pharmacologically active compounds, have been shown to antagonize the activity of some venoms and toxins. Anecdotal evidence abounds to indicate that plant remedies used are effective, and there appears to be a high rate of survival among snakebite patients with advanced clinical stages of venom toxicity[10].

The present study is to analyze the anti-snake venom activities of *Datura metal* which is native to India and to assess how significant the folklore, traditional medicine is (*Datura metal Linn*) to neutralize the snake venom activity of a typical and highly poisonous snake such as Russell's viper.

MATERIALS AND METHODS

Venom

The lyophilized venom of Russell's viper was purchased from the Government licensed Irula Snake Catcher's Cooperative Society, Crocodile park, Chennai. The venom was suspended in physiological saline and centrifuged at

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2000 g for 10 min. The supernatant was used for further analysis and they are stored at 4°C.

Anti-Snake venom

Anti-snake venom was purchased from A.G. Pharmacy, West Tambaram Chennai-45, Tamilnadu.

Plant Material

The fresh roots of *Datura metel* were obtained in the month of July2018 from Thiruvananthapuram District, Kerala. The plant material has been identified and authenticated by National Dietetics and Ayurveda Research Centre, Thiruvananthapuram, Kerala, India

Ethanol Extraction

The *Datura metel* root was cleaned from earthly matters and extracted using alcoholic solvent ethanol at (60-80^{0}c) in a soxhlet extractor, and the residue was derived by evaporating the solvent using rotovap evaporator.

Experimental Animals

Swiss albino mice (male) with an average weight of 25-30 g and Wistar rats weighed between 200-250kg were used for experiments. The animals were obtained from Biogen Enterprises, Bangalore, were housed in cages at room temperature of 28-32°C and under 12-hour light/dark cycle daily *ad libitum* feeding was given. The experiments were performed at C.L Baid Metha College of Pharmacy with the approval from Institutional animal ethical committee clearance.

Phytochemical Screening

The ethanolic extract of D. Metal Roots was screened for the presence of phytochemical constituents[11]. The stock solution was prepared from the crude extract of ethanol and dissolved in 10 ml of ethanol solvent. The stock solution was used for preliminary phytochemical screening. The color intensity or the precipitate formation was used as analytical responses to these tests.

Test for alkaloids

The stock solution was taken in a test tube and ammonia solution (3 ml) was added to it. They were allowed to stand for a few minutes. Then chloroform (10 ml) was added to the test tube samples which was shaken and then filtered to remove the powder samples. The chloroform was evaporated using a water bath and Mayer's reagent (2 ml) was added. A cream colored precipitate was immediately produced which indicates the presence of alkaloids.

Test for flavonoids

A few drops of diluted sodium hydroxide solution were added to the stock solution of D. Metal (0.5 ml). An intense yellow color appeared in the plant crude extract, which became colorless upon the addition of a few drops of diluted H_2SO_4 acid. This shows the presence of flavonoids.

Test for saponins

The stock solution from crude extract of D. Metal (0.5 ml) was diluted with distilled water (20 ml) and then the test tube was shaken by hand for 15 min. The formation of a foam layer on the top of the test tube showed the presence of saponins.

Test for steroids

The powder samples of D. Metal (1 GM) were dissolved in chloroform (10 ml) and added concentrated sulfuric acid (1 ml) into the test tube by sides of the wall. The color of the upper layer turned red and the sulfuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for tannins

The crude extract solution (0.5 ml) was dissolved in chloroform (5 ml) and added acetic-anhydride (1 ml). Finally sulfuric acid (1 ml) was added carefully to the solution along the wall sides of the vessel. A green color was formed, showing the presence of tannins.

Test for triterpenoids

The plant extract (5 mg) was dissolved in chloroform (2 ml) and then aceticanhydride (1 ml) was added to it. One milliliter of concentrated sulfuric acid was added to the solution. The formation of reddish violet color shows the presence of triterpenoids.

Neutralization

Male swiss albino mice of body weight ranging between 15-30gm were used for this research study. The mice were randomly grouped into 5 groups (A, B, C, D and E) of six mice each. Group D, E were all treated with ethanolic extracts of the *Datura metel* root of 20 mg/kg, and 50 mg/kg body weight orally to animals respectively for 21 days, on the 21th day the pretreated animals were challenged with Russell's viper venom 0.075 mg/kg body weight. The factors like Hematological, Death Rate and Biochemical parameters are determined.

Grouping

Group Name Treatment

Group A: Normal control: No Induction, No treatment.

Group B: Test control: Induced with 0.075mg/kg of RSV (Russell'sViper venom)

Group C: Standard control: Induced with 0.075mg/kg body weight of venom and Treated with Antisera (standard drug).

Group D: Venom+20mg/kg: Induced with 0.075mg/kg body weight of RSV and treated with 20mg/kg EEDMR (Ethanolic extract of Datura metel root).

Group E: venom+50mg/kg: Induced with 0.075mg/kg body weight of RSV and

Treated with 50mg/kg EEDMR.

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Neutralization of hemorrhagic activity

The minimum haemorrhagic dose (MHD), i.e. The least amount of venom (g/dry weight) which, when injected intradermally into rats results in a hemorrhagic lesion of 10-mm diameter 24 h later was determined by the method of Kondo (1960). The MHD dose, intradermally injected into the shaved dorsal skin of the rats was followed after 5 min by IP administration of the different doses of EEDMR extract [12, 13].

Neutralization of necrotizing activity

The minimum necrotizing dose (MND), i.e., the least amount of venom (gm/dry weight) which, when injected intradermally into rats results in a necrotic lesion of 5-mm diameter 3 days later, was determined by the method of Theakston and Reid (1983). The MND dose of venom, intradermally injected into the shaved dorsal skin of the rats was followed after 5 min by i.p. administration of different doses of the EEDMR [14].

Histopathological study

Kidney, Liver, Heart and Brain tissues were collected from the respective group after acute exposure of Russell's viper venom (0.075mg/kg body weight of mice). 10% buffered formalin was used for fixation. Graded Ethanol (50-100%) was used for dehydration followed by clearing in Xylene. Tissues were embedded in paraffin (56-58°c) at 51+-1°c for 4h. Paraffin sections were deparaffinized with xylene, stained with hemoxylene-eosin, followed by mounting in DPX with a cover slip. Histological changes were observed with a bright field microscope (Miotic Germany) and photographs was captured for documentation (Magnification 150x).

Determination of Bleeding and Clotting Time

Bleeding Time

Blood is taken from the Retro orbital vein of Wistar rats. The blood is allowed to flow, stop watch is immediately started. The blood is soaked in a filter paper (While soaking the filter should not touch the skin). This is repeated every 10 seconds till no blood appears on the paper. The time from which the first appearance of the blood to the bleeding occurs is called as bleeding time.

Clotting Time: Wright's Method

The blood is taken by puncturing the tail vein of rat as soon as the blood comes out the stop watch is started and the capillary tubes is filled up to three fourth of the total length. After every 30sec a portion of the capillary tube is broken until a thin line of unbroken is seen stretched between the two broken ends. The time is noted and the difference between the time when the blood comes out from the finger and the unbroken coagulum seen is the coagulation time.

Total RBC and WBC Count

The Neubauer's counting chamber is adjusted and observed in RBC and WBC squares until the low power of the microscope, keeping the Thomas cover slip resulting on the platform of the slide. The blood is collected by puncturing the retro-orbital of the Wistar rats. Make a 1:200 dilution of blood. Place 20µl of whole blood in the tube. Mix with 3.98 ml of the Gower's solution to get 1:200 dilutions. Mix well by using a glass rod. Clean the counting chamber & cover glass. Fill 10µl to each side of the counting chamber. Once the counting chamber is filled, allow approximately 3minutes for the red blood cell to settle prior to counting. Carefully place the filled counting chamber on the microscope stage. Lower the condenser on the microscope and scan by using the low power (10x) objective lens. The cells should be evenly in all of the squares. Count all the cells in the squares (5R) using the 45X objective lens. Repeat the count on the other side of counting chamber. The difference the total cells counted on each side should be less than 10%.

Haemoglobin (Hgb) Estimation

Place N/10 Hcl into Hb pipette up to the mark 20. Blood is taken from the tail vein of Wistar rat. A blood sample is pipetted by using Shali's Hb pipette up to 20cubic mm. Add blood sample to Hcl acid solution. Mix with a stirrer allow standing for 10 min. Add distilled water drop by drop till the color of the solution matches the brown glass standard. Take the reading of the lower meniscus from the graduated tube in grams[15, 16,17].

Statistical Analysis

The values are expressed as mean±S. E. M followed by student t test, P values less than 0.05 was considered significant, statistical analysis was made by Graph 7.2

RESULTS

The preliminary phytochemicals test reveals that the major phytochemical constituents in Datura metal are alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, cardiac glycosides and anthraquinones (Table 2). flavonoids, phenols, tannins, saponins and sterols.

The results of this study shown in Table 2 shows group II treated with antisera in comparison with the group I control produced 100% survival from lethality (death), Group IV animal produced 80% survival, group III animals when compared with group II showed a significantly lower survival rate.

Table 4 and 5 reveal group II treated with antisera in comparison with the group I produced significant reduction in the lesion diameter. Group IV animals and group III animals when compared with group II showed less significance in the reduction of lesion diameter.

Table 6, 7 and 8 reveals Group II, Group III, Group IV treated with EEDMR in comparison with group I, produced significantly low RBC, WBC and HGB level.

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Table 1

Phytochemicals	Presence in Datura metal leaves
Alkaloids	++
Flavonoids	++
Steroids	++
Saponins	++
Tannins	+
Terpenoids	++

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

Graph 1:

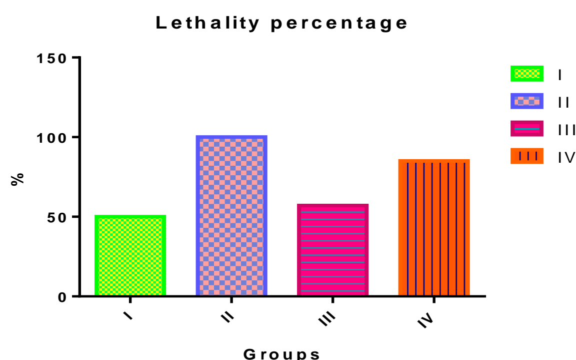


Table 2: Effect of EEDMR on Vipera Russeli induced Necrotizing activity

S. No	Grouping of Animals	Diameter Of Necrotic Lesion (mm) m
1.	Group I Test control (venom)	5.11± 0.0071
2.	Group II Standard (antisera)	1.03±0.0105*
3.	Group III 20mg/kg DMRE.	3.23±0.0106*
4.	Group IV 50mg/kg DMRE	1.81±0.0244

Effect of Eedmr on RBC Count Before and After Russell's Viper Venom Induction:

Graph: 2

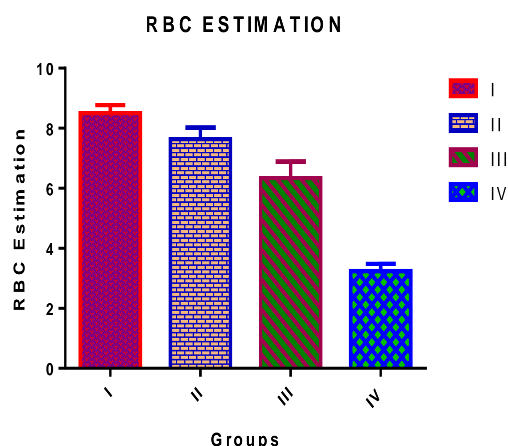
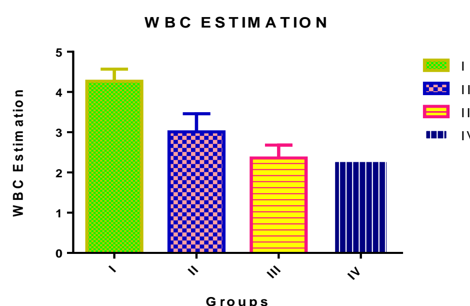


Table 3: Effect of EEDMR on Vipera russelli's induced Hemorrhagic activity

S. NO	Grouping of Animals	Diameter Of Hemorrhagic Lesion (mm) m
1.	Group I Test control (venom)	9.60±0.2145
2.	Group II Standard (antisera)	2.153± 0.0242
3.	Group III 20mg/kg DMRE.	6.05±0.0349
4.	Group IV 50mg/kg DMRE	2.66±0.0349

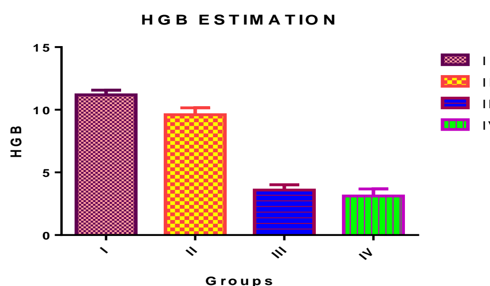
Effect of Eedmr on WBC Count before and after Russell's Viper Venom Induction

Graph-3



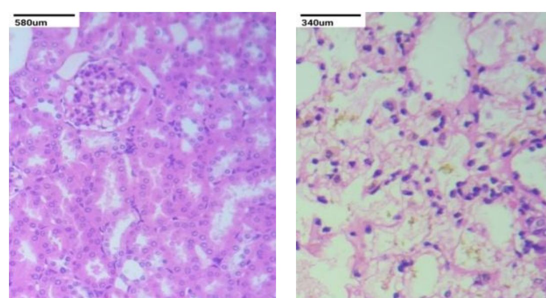
Graph-4

Effect of Eedmr on Hgb Level Before and after Russell's viper Venom Induction:



RESULTS OF HISTOLOGICAL PARAMETERS:

KIDNEY



Group I -Kidney Group II-Kidney

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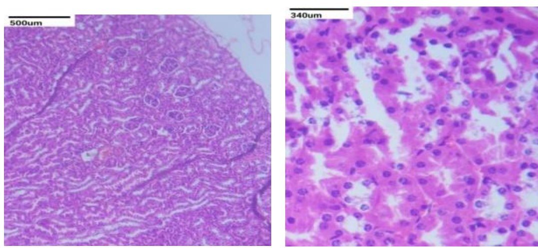
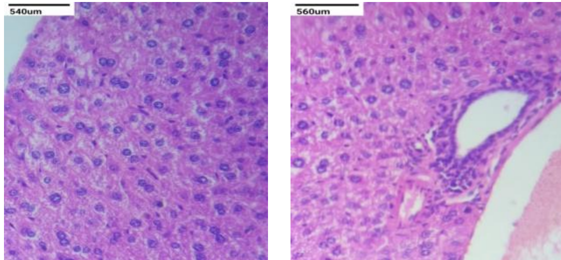
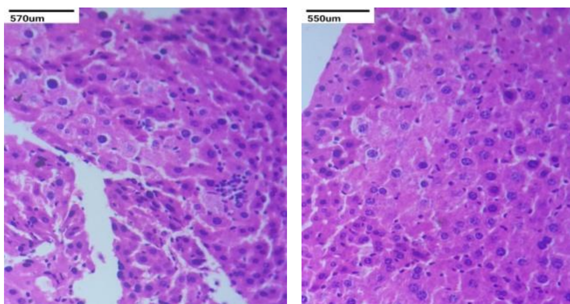


Group III- kidney Group IV- Kidney

LIVER:

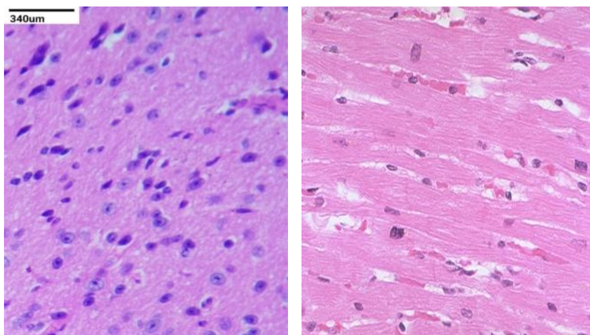


Group I Group II

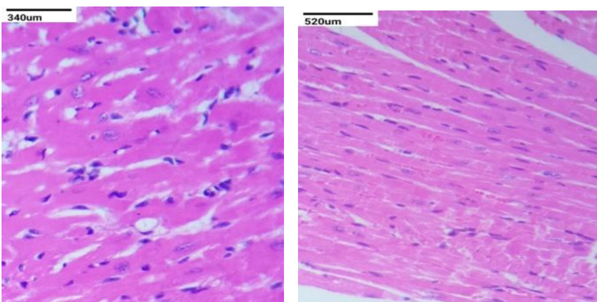


Group III Group IV

HEART

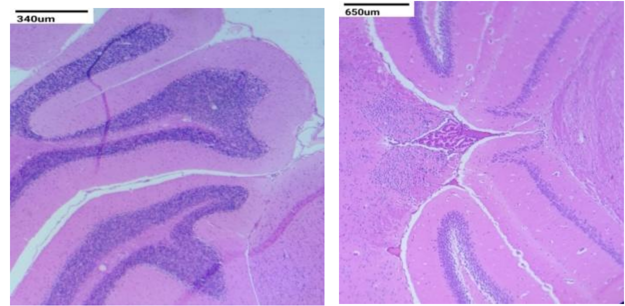


Group I Group II

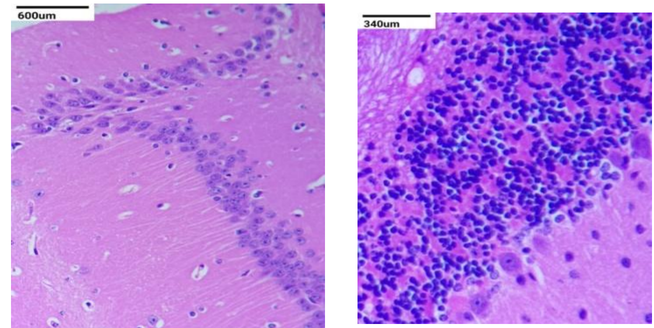


Group III Group IV

BRAIN



Group I Group II



Group III Group IV

Histopathology Report

After fixation for 24 hours, tissues are dehydrated, embedded in paraffin, sectioned at 7 µm thickness, and stained with haematoxylin and eosin (HE) for histological examination

Kidney

Apoptosis is a pathway of cell death, and excessive apoptosis leads to various kinds of organ injury. No histological structure changes in glomeruli, in group I and Group II, but atrophy in group III and Group IV tubules, atrophic glomeruli, glomerular capsule and tubules dilatation; moreover, severe tubular leakage appeared in group III and Group IV

Liver

Group I- Liver section of male control rat. Cords of hepatocytes well preserved and essentially normal, cytoplasm, not vacuolated, sinusoids well demarcated, no area of necrosis, no fatty degeneration and change.

Group II- In the Liver section of mice, treated with antisera cords of hepatocytes are distinctly and essentially normal, no fatty change, cytoplasm not vacuolated

Group III- mice treated with 20mg of EEDMR shows abnormal hepatocytes and mild cellular edema congestion in the portal veins, fibrosis of liver cells seen

Group IV- mice treated with 50mg of EEDMR shows normal hepatocytes and mild cellular edema portal congestion absent, feathery degeneration absent

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Heart: all groups show regular cell distribution and normal myocardium architecture no myofibrillar loss, cytoplasmic vacuolization, inflammatory cell infiltration, edema, congestion and nuclear pyknosis

Brain: In all treated groups no visible lesions seen, mild diffused spongiosis of the parenchyma and neuronal cells, normal cerebral cortical neuronal arrangement is seen the ventricular zones, though the indistinguishable less cellular population, protects against neuronal damage to the frontal cortex

DISCUSSION

Datura has alkaloids which have anti-cholinergic action which acts against Russell's viper venom which has cholinergic action. Datura metel has a strong affinity towards muscarinic receptor, so the effect of the receptor will be reduced. Datura metal will reduce the level of RBC, WBC and HGB. HGB is a carrier for oxygen it also exchanges CO_2. In this study HGB level has been reduced by the use of Datura metal so absorption of venom in the Datura metel is decreased and antivenom effect is achieved. Most venom has the ability to cause local necrosis and hemorrhage when given intradermally, so the minimum necrotizing dose and minimum hemorrhagic dose estimation will prove to be a test for accessing the antivenom activity. Intravascular haemolysis may contribute to the development of acute tubular necrosis and bilateral cortical necrosis in victims of Russell's viper bites. Hemorrhagins cause death because of bleeding by vital organs by damaging vascular endothelial[22] EEDMR was found that it significantly reduces the viper venom induced Necrotic and Hemorrhagic lesions. According to the anti-snake venom primarily possessing compound should be tested for its capacity to neutralize venom effects using procedures such as lethality, hemorrhagic and necrotizing effects[23]. The ethanolic extract of Datura metel 50mg/kg was found to prevent the lethality up to 85% 20mg/kg survival rate was 57%.

CONCLUSION

In conclusion, the present experimental results indicate that Datura metel root extract was effective in neutralizing the toxic effects of Russell's viper venom and or has an alternative or complementary treatment strategy of envenomation by Russell's viper. Further experiments could address the fractioning of the Datura metel root extract in order to identify the bioactive compounds responsible for these observations, their efficacy, safety and the mechanism of action which could possibly lead to the development of pharmaceutical formulations for treating snake bite accident victims. Our study on the ethanolic root extract of Datura metel has demonstrated some useful activities that support its traditional use against snake bite.

Acknowledgement: The author wishes to thank C. L. Baid Metha college of pharmacy, Chennai for providing necessary facilities to carry out this study and Mr. PrassanaKumar B. Pharm for helping to procure Vipera russelli venom

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Source of Support: Nil, **Conflict of Interest:** None.

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