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EVALUATION OF ANTIVENOM ACTIVITY OF *CALOTROPIS GIGANTEA* PLANT EXTRACT AGAINST *VIPERA RUSSELLI* SNAKE VENOM

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ABSTRACT

Ethnopharmacological relevance: *Calotropis gigantea* is used traditionally to treat common diseases such as fever, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhoea, either alone or with other medicines

Aim of the study: To evaluate the antivenom activity of *Calotropis gigantea* plant extract against *Vipera russelli* snake venom

Materials and methods: The lyophilized snake venom of *Vipera Russelli* was dissolved in saline and required concentrations were prepared. Lyophilized polyvalent snake venom antiserum was used as reference serum. The methanolic extract of *Calotropis gigantea* was evaluated for its efficacy to neutralize various actions of the venom like lethality, necrotizing activity, edema forming activity and haemorrahgic activity.

Results: Oral administration of *C. gigantea* plant extract at dose levels 200 and 400 mg/kg body weight effectively neutralized the lethal effect of $2LD_{50}$ and $3LD_{50}$ of *V. russelli* venom in mice (*in-vivo* neutralization). In *in-vitro* studies, the plant extract at all dose levels, i.e. 100, 200 and 400mg/kg body weight effectively neutralized $2LD_{50}$ and 3 LD_{50} of *Vipera russelli* venom. Oral administration of the plant extract at various dose levels was found to effectively inhibit the induction of haemorrhage and necrosis by the venom. At doses 200 and 400 mg/kg, the antinecrotic effect of plant extract was significant. The effect of methanolic extract of *C. gigantea* against edema induced by viperid venom was studied at 60, 120, 180 and 240 minutes. Plant extract at dose levels 200mg/kg and 400mg/kg showed significant anti-inflammatory activity at 240 min, and effect was comparable with that produced by the antivenom.

Conclusion: Present study confirms the anti snake venom activity of alcoholic extract of *C. gigantea.*

INTRODUCTION: There are estimated to be around 25000 effective plant based formulations which are available in the indigenous medical texts. In India, medicinal plants have made good contribution to the development of ancient Indian *Materia Medica*. One of the earliest treatises on Indian medicine, the Charak Samhitha records the use over 340 drugs of vegetable origin. Herbal Medicine sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. Herbal medicine is a major component in all indigenous people's traditional medicine and a common element

in Ayurvedic, Homeopathic, Naturopathic, Traditional oriental and Native American Indian medicine. The World Health Organization (WHO) estimates that around 4 billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care. In some countries like India and china, herbal drugs are used traditionally and a major portion of their population still prefers to use plant medicines over synthetic pharmaceuticals [1].

Snakebite is a global medical problem especially in the rural areas of the tropics with about 40,000 deaths each year [2]. In India, more than 200000 cases are reported and an estimated 35000 to 50000 people die of snakebite every year [2]. Approximately 330 species of snakes exist in India, of which about 70 species are venomous (40 land snakes and 30 sea snakes).The commonest Indian venomous snakes are common krait (*Bungarus caeruleus*), common cobra (*Naja naja*), saw-scaled viper (*Echis carinatus*), and Russell's viper (*vipera russelli*) [3].

Russell's viper is a nocturnal snake, but unfortunately for humans, during the day time it often rests up under bushes, at the base of trees, and in leaf litter. It is therefore frequently encountered by rural workers as they carry out general agricultural activities[4]. Snake venom is a complex mixture of enzymes, peptides and proteins of low molecular mass with specific chemical and biological activities. Snake venom contains several neurotoxins, cardiotoxins, cytotoxins, nerve growth factor, lectines, disintrigrins, haemorrhagins and many other different enzymes [5].

Russell's viper venom is predominantly vasculo-and haemotoxic, but is able to produce neurotoxic effects also. Acute renal failure and adrenal insufficiency have also been associated with these snake envenomations. Pit less as well as pit vipers cause marked local manifestation which develops rapidly, usually within half an hour. Swelling appears around the bitesite and spreads quickly the whole limb and adjacent trunk. There is associated pain, tenderness and regional lymphadenopathy. Persistent bleeding from the bitesite is a constant feature. Blisters begin to appear in about 12 hours in and around the bitesite. In about 10-15% of the cases, extensive necrosis of the skin, subcutaneous tissue and muscles may occur [4].

Systemic effects like haematurea, gingival bleeding, epistaxis, ecchymosis, intracranial and subconjuctival haemorrhages, bleeding from the tympanic membrane, gastrointestinal tract and genito-urinary tract may be seen in viper envenomations. Intravascular haemolysis causing haemoglobinurea and renal failure is a frequent occurrence, especially in *Russell's viper* bites. Hypotension is an important manifestation in all viper bites and is usually accompanied by tachycardia, in which case pulse may be slow or irregular. Hypotension has been responsible for 38% of deaths related to Russell's viper envenomation [4].

Antiserum is the only therapeutic agent available throughout the world. Antiserum sometimes does not provide enough protection against venom-induced haemorrhage, necrosis and often produces hypersensitive reactions. Antiserum development in animal is time consuming, expensive and requires ideal storage condition [6].

Traditional remedies from plants used in the treatment of snakebite patients have a number of potential advantages. They are not expensive, are readily available, can be grown locally and hypersensitivity reactions to plant extracts are rare. Herbal antivenom may alleviate the local effects of the venom, which is difficult to achieve with conventional antivenoms.

Over the years, many attempts have been made for the development of snake venom antagonists especially from plant sources. India has a rich tradition of the usage of medicinal plants. Many Indian medicinal plants are mentioned in literature, which are used to treat snakebite victims especially in rural areas [7].

Calotropis gigantea, commonly known as milkweed or swallow-wort, is a common wasteland weed and belongs to family *Asclepiadaceae*. Being native to India, it grows wild, up to 900 m, throughout the country. Traditionally it is used to treat common diseases such as fever, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhoea, either alone or with other medicines. The whole plant, root bark, roots, leaves and flowers are used to treat many diseases and abnormalities in humans [7].

MATERIALS AND METHODS:

Snake Venom: The lyophilized snake venom of *Vipera Russelli* was obtained from Calcutta Snake Park, Calcutta, India and was preserved at 4^{o}C. Before use, the venom was dissolved in saline and required concentrations were prepared.

Snake Venom Antiserum: Lyophilized polyvalent snake venom antiserum (as reference serum) was obtained from Justice KS Hegde Charitable Hospital, Deralekatte, Mangalore.

Plant Material: The plant material was collected from Mangalore, Karnataka, India during May 2010 and was authenticated by Dr. Krishna Kumar, Associate Professor, Department of Applied Botany, Mangalore University.

Animals: Healthy adult Wistar albino rats, weighing about 180-220g and Swiss albino mice, weighing about 18-22g between 2 and 3 months of age obtained from KSHEMA, Deralakatte, Mangalore, were used for the study. The study was approved by the Institutional Ethics Committee for animal experimentation KSHEMA, Deralakatte, Mangalore. Rats and mice were housed individually in polypropylene cages, maintained under standard conditions (12 hrs light and 12 hrs dark cycle; 25^{o}C and 45-55% relative humidity).They had been given Standard pellet diet supplied by Hindustan Lever Co. Mumbai and water *ad libitum* throughout the course of the study.

Preparation of Extract: The fresh plant material of *Calotropis gigantea* (*Asclepiadaceae*) was washed and shade dried at room temperature. The air dried plant material was ground (1kg) and subjected for maceration. For extraction, the powdered plant material was soaked in methanol and kept aside for 7 days with occasional stirring. After 7 days, the methanolic layer was filtered. The solvent from the total extract was distilled off and the concentrate was evaporated on a water bath to a syrupy consistency and then evaporated to dryness [8]. The yield obtained was approximately 8.5% (85g).

Phytochemical Screening: The preliminary phyto-chemical studies were performed according to procedure given by Khandelwals, revealed the presence of alkaloids, tannins, flavonoids, triterpinoids, saponin and steroids.

Acute Toxicity Studies: Acute toxicity study was conducted to determine the median lethal dose (LD_{50}) of the methanolic extract of plant *Calotropis gigantea*. The toxicity studies were carried out according to OECD guidelines- 425. Rats of either sex (three females and three males, weight: 200-220 g, age: 1.5-2 months) received methanolic extract of the plant *Calotropis gigantea,* suspended in 0.6% Na CMC starting dose at 2000mg/kg of body weight orally. The animals were observed for toxic symptoms continuously for the first 4 hrs after dosing. Finally, the number of survivors was noted after 24 hrs.

Evaluation of LD_{50} of venom: The median lethal dose (LD_{50}) of Vipera Russelli venom was determined according to the method developed by Theakston and Reid 1983. The toxicity of *Vipera Russelli* venom was assessed by i.p administration of different concentrations of the venom dissolved in 0.2 ml of physiological saline to groups (n = 6) of Swiss albino mice (18-22g). The LD_{50} was calculated with the confidence limit at 50% probability by the analysis of deaths occurring within 24 hrs of the venom administration [9].

Neutralization of Lethality: The *in-vivo* neutralization potency of *Calotropis gigantea* plant extracts were assessed by i.p. administration of $2LD_{50}$ and $3LD_{50}$ dose of venom into different groups of mice immediately after the administration of various doses of the plant extract per oral (po) [9]. To assess *in-vitro* neutralization, various amounts of the plant extracts were mixed with $2LD_{50}$ and $3LD_{50}$ of the venom sample and incubated at 37^{o}C for 30 minutes and then injected i.p. in to the mice. 6 mice were used in each group. Control mice received same amount of venom without plant extracts. The standard reference group i.e. snake venom anti serum was administered after the administration of $2LD_{50}$ and $3LD_{50}$ dose of venom and the results were calculated by probit analysis.

Neutralization of Haemorrhagic Activity: The minimum haemorrhagic dose (MHD) of *Vipera russelli* venom was determined by the method described by Theakston and Reid, 1983. The minimum haemorrhagic dose is defined as the least amount of

venom which when injected intradermally (i.d.) in to rats results in a haemorrhagic lesion of 10mm diameter in 24 hrs. The MHD of the venom was intradermally injected in to the shaved dorsal skin of the rats followed after 5 min by oral administration of different doses of the plant extract [9, 10].

Neutralization of Necrotizing Activity: The minimum necrotizing dose (MND) of *Vipera Russelli* venom was determined by the method described by Theakston and Reid, 1983. The minimum necrotizing dose (MND) is defined as the least amount of venom (µg dry weight) which, when injected intradermally into rats, results in a necrotic lesion of 5 mm diameter 3 days later. The MND of venom was intradermally injected into the shaved dorsal skin of the rats followed after 5 min by oral administration of different dose of the plant extract.

Neutralization of Edema forming activity: The minimum edematic dose (MED) of venom/ carrageenan is defined as the least amount of venom/carrageenan which, when injected in to male albino rats**,** produced inflammation (edema) in the paw. To assess MED, Non fasted albino rats (180-220g) were treated with different dose of venom (in 0.1 ml) and were injected into sub-plantar area of the paw. Test group received MED of venom (sub-plantar) followed by different dose of plant extract per oral. As a control, only the venom was injected (sub-plantar). The edematogenic response was evaluated by the use of plethysmograph. Results were expressed as the percentage decrease in edema volume of the treated group compared to control [11, 12].

Statistical Analysis: The lethal dose (LD_{50}) of the venom was expressed as µg/mice and was calculated by probit analysis. The other datas were expressed as mean±SEM, analyzed by one way ANOVA followed by Dunnett's multiple comparison test.

RESULTS:

Acute Toxicity Studies: The alcoholic extract of the plant *Calotropis gigantea* was found to be safe upto 2000mg/kg body weight by oral route. After 24 hours animals were found well tolerated. There was no mortality and no signs of toxicity and extract were found to be safe.

Evaluation of LD_{50} of the Venom: The median lethal dose (LD_{50}) of *Vipera Russelli* venom was determined according to the method developed by Theakston and Reid 1983. The LD_{50} was calculated with the confidence limit at 50% probability by the analysis of deaths occurring within 24 hrs of the venom administration. The LD_{50} of *Vipera russelli* venom was found to be **12 µg/20gm** mice (i.p.).

***In-vivo* neutralization of Lethality: (Table 1 & 2)**

TABLE 1: EFFECT OF *CALOTROPIS GIGANTEA* EXTRACT IN MICE ADMINISTERED WITH $2LD_{50}$ (24µg) OF *VIPERA RUSSELLI* VENOM

Group	Dose of the drug	Mortality (after 24hrs) [no. of death/no. of mice used]	% Survival after 24hrs	% Corrected	Probit
1	Control (only venom)	6/6	-	4.16	3.25
2	Venom + Std (polyvalent antivenom)	0/6	100	95.83	6.75
3	Venom + *C. gigantea* extract 100 mg/kg	4/6	33.33	33.33	4.56
4	Venom + *C. gigantea* extract 200 mg/kg	3/6	50	50	5.00
5	Venom + *C. gigantea* extract 400 mg/kg	1/6	83.33	83.33	5.95

TABLE 2: EFFECT OF *CALOTROPIS GIGANTEA* EXTRACT IN MICE ADMINISTERED WITH $3LD_{50}$ (36µg) OF *VIPERA RUSSELLI* VENOM

Group	Dose of the drug	Mortality (after 24hrs) [no. of death/no. of mice used]	% Survival after 24hrs	% Corrected	Probit
1	Control (only venom)	6/6	-	4.16	3.25
2	Venom+ Std (polyvalent antivenom)	0/6	100	95.83	6.75
3	Venom + *C. gigantea* extract 100 mg/kg	4/6	33.33	33.33	4.56
4	Venom + *C. gigantea* extract 200 mg/kg	4/6	33.33	33.33	4.56
5	Venom + *C. gigantea* extract 400 mg/kg	3/6	50	50	5.00

The neutralization potency of *Calotropis gigantea* plant extracts by *in-vivo* method were assessed by i.p. administration of $2LD_{50}$ and $3LD_{50}$ dose of venom into different groups of mice (n= 6) immediately after the administration of various doses of the plant extract per oral (po). The plant extract at doses 200 and 400mg/kg body weight were found to effectively neutralize the lethal activity of $2LD_{50}$ and $3LD_{50}$ of *Vipera russelli* venom.

***In-vitro* neutralization of Lethality:** To assess *in-vitro* neutralization, various amounts of the plant extracts were mixed with $2LD_{50}$ and $3LD_{50}$ of the venom sample and incubated at 37°C for 30 minutes and then injected i.p in to the mice. The plant extract at doses 200 and 400mg/kg body weight were found to effectively neutralize the lethal activity of $2LD_{50}$ and $3LD_{50}$ of *Vipera russelli* venom (**table 3 & 4**).

TABLE 3: EFFECT OF *CALOTROPIS GIGANTEA* EXTRACT IN MICE ADMINISTERED WITH $2LD_{50}$ (24µg) OF *VIPERA RUSSELLI* VENOM

Group	Dose of the drug	Mortality (after 24hrs) [no. of death/no. of mice used]	% Survival after 24hrs	% Corrected	Probit
1	Control (only venom)	6/6	-	4.16	3.25
2	Venom+ Std (polyvalent antivenom)	0/6	100	95.83	6.75
3	Venom + *C. gigantea* extract 100 mg/kg	0/6	100	95.83	6.75
4	Venom + *C. gigantea* extract 200 mg/kg	0/6	100	95.83	6.75
5	Venom + *C. gigantea* extract 400 mg/kg	0/6	100	95.83	6.75

TABLE 4: EFFECT OF *CALOTROPIS GIGANTEA* EXTRACT IN MICE ADMINISTERED WITH $3LD_{50}$ (36µg) OF *VIPERA RUSSELLI* VENOM

Group	Dose of the drug	Mortality (after 24hrs) [no. of death/no. of mice used]	% Survival after 24hrs	% Corrected	Probit
1	Control (only venom)	6/6	-	4.16	3.25
2	Venom+ Std (polyvalent antivenom)	0/6	100	95.83	6.75
3	Venom + *C. gigantea* extract 100 mg/kg	1/6	83.33	83.33	5.95
4	Venom + *C. gigantea* extract 200 mg/kg	0/6	100	95.83	6.75
5	Venom + *C. gigantea* extract 400 mg/kg	0/6	100	95.83	6.75

Neutralization of Haemorragic Activity: The minimum haemorrhagic dose (MHD) of *Vipera Russelli* venom was determined by the method described by Theakston and Reid, 1983. The MHD of the venom was found to be 24µg/200g rat. The plant extract showed significant neutralization of haemorrhage at all the dose levels, when compared with the standard polyvalent antivenom (**table 5, figure 1**).

TABLE 5: EFFECT OF METHANOLIC EXTRACT OF *CALOTROPIS GIGANTEA* ON *VIPERA RUSSELLI* VENOM INDUCED HAEMORRHAGIC ACTIVITY IN RATS

Group (n = 6)	Mean Area of the lesion ± S.E
Control (Venom only)	9.833±0.3073
Std (Polyvalent antivenom)	9.667±0.3333
Venom + *C. gigantea* extract 100mg/kg	7.167±0.3073*
Venom + *C. gigantea* extract 200mg/kg	5.167±0.3073**
Venom + *C. gigantea* extract 400mg/kg	2.667±0.8819**

The Values are expressed as Mean ± SEM, n=6 rats in one group. *P<0.05 significant, **P< 0.01 highly significant, when compared with control group.

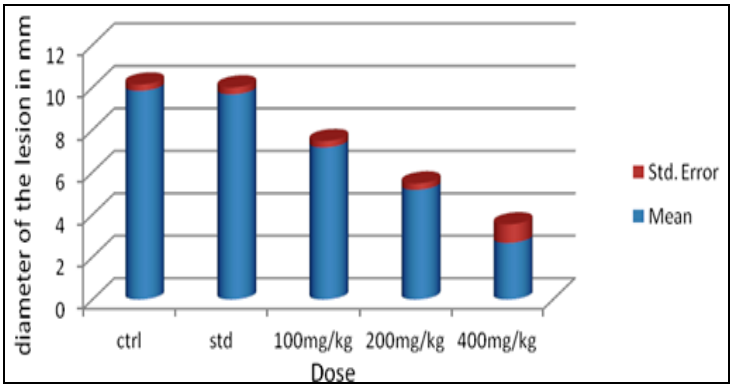

FIGURE 1: EFFECT OF METHANOLIC EXTRACT OF *CALOTROPIS GIGANTEA* ON THE *VIPERA RUSSELLI* VENOM INDUCED HAEMORRHAGIC ACTIVITY IN RATS

Neutralization of Necrotizing Activity: (Table 6, fig. 2)

TABLE 6: EFFECT OF METHANOLIC EXTRACT OF *CALOTROPIS GIGANTEA* ON *VIPERA RUSSELLI* VENOM INDUCED NECROSIS IN RATS

Group	Mean area of the lesion ± S.E
Control (Venom only)	4.833± 0.3073
Std (Polyvalent antivenom)	4.833± 0.3073
Venom + *C. gigantea* extract 100mg/kg	2.833± 0.3073*
Venom + *C. gigantea* extract 200mg/kg	1.333± 0.4216**
Venom + *C. gigantea* extract 400mg/kg	0.500± 0.3416**

The Values are expressed as Mean ± SEM, n=6 rats in one group. **P< 0.01 highly significant, when compared with control.

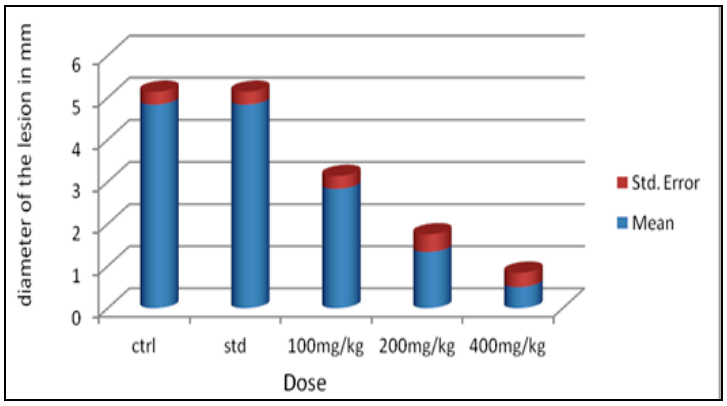

FIGURE 2: EFFECT OF METHANOLIC EXTRACT OF *CALOTROPIS GIGANTEA* ON *VIPERA RUSSELLI* VENOM INDUCED NECROSIS IN RATS

Effect of methanolic extract of *Calotropis gigantea* on the *Vipera russelli* venom induced Necrotizing Activity in rats: The minimum necrotizing dose (MND) of *Vipera Russelli* venom was determined by the method described by Theakston and Reid, 1983. The MND of the venom was found to be **30µg/200g** rat. Plant extract showed significant activity at all the dose levels when compared with standard polyvalant antivenom.

Neutralization of Edema forming activity: The minimum edematic dose (MOD) of venom/ carrageenan is defined as the least amount of venom/ carrageenan which, when injected in to male albino rats**,** produced inflammation (edema) in the paw. The minimum edematic dose of the venom was found to be **4µg** in rat. Significant inflammation was seen after 1hr of venom injection and maximum inflammation was seen at 180 min. Plant extracts at dose level 200mg/kg and 400mg/kg showed significant activity when compared with control.

TABLE 7: EFFECT OF METHANOLIC EXTRACT OF *CALOTROPIS GIGANTEA* ON THE *VIPERA RUSSELLI* VENOM INDUCED PAW EDEMA IN RATS

Group	Increase in the edema volume (ml) (%inhibition)			
	60min	120min	180min	240min
Control	0.2917± 0.0318	0.3767±0.0315	0.385±0.0209	0.3867±0.0223
Std	0.1283±0.0296** (56.01)	0.1517±0.0282** (59.72)	0.1663±0.0144** (56.80)	0.1512±0.0145** (60.89)
C. gigantea extract 100mg/kg	0.2067±0.0164 (29.13)	0.1927±0.0145** (48.84)	0.1916±0.0151** (50.23)	0.165±0.0111** (57.33)
C. gigantea extract 200mg/kg	0.1817±0.1817* (37.70)	0.1683±0.0065** (55.32)	0.1667±0.0098** (56.70)	0.1633±0.0066** (57.77)
C. gigantea extract 400mg/kg	0.1817±0.0094* (37.70)	0.165±0.0076** (56.19)	0.1668±0.0263** (56.67)	0.1617±0.0256** (58.18)

The Values are expressed as Mean ± SEM, n=6 rats in one group. *P<0.05 significant, **P< 0.01 highly significant, when compared with control group.

DISCUSSION AND CONCLUSION: The present study was undertaken to carry out the preliminary phytochemical screening and evaluation of antivenom activity of the plant *Calotropis gigantea* belonging to the family *Asclepiadaceae*. Preliminary phytochemical study revealed the presence of alkaloid, flavonoids, saponins, steroids, tannins and triterpenoids.

Snake envenomations cause different patho-physiological changes such as inflammation, haemorrhage, necrosis, edema, alterations in blood coagulation system and ultimately leading to death [4]. *Vipera russelli* venom is predominantly vasculo- and haemotoxic, but able to produce neurotoxic effects also.

Hypotension is the important manifestation in all viper bites and it has been responsible for 38% of deaths in *Russell viper* envenomations. Toxic symptoms include haemorrhage, renal failure, hypotension, local tissue necrosis, edema etc [4]. In the present study also, similar sequence of symptoms were observed after the administration of *Vipera russelli* venom [4].

Even though antiserum causes some side effects like hypersensitivity, anaphylactic reactions, it is the only therapeutic agent used for snake envenomations throughout the world. Several studies are going on to find out the suitable drug which can neutralize or antagonize the snake venom. Different plant constituents such as alkaloids, acids, flavonoids,

triterpenoids, tannins etc are responsible for the anti snake venom activity [13].

According to WHO, the anti snake venom activity possessing compound should be tested regarding its capacity to neutralize the *in-vivo* biological effects venom such as lethality, haemorrhage, necrosis, edema etc [9].

Three doses of the plant extract 100, 200 and 400 mg/kg body weight were selected based on the acute toxicity studies in rats.

Vipera russelli venom has an ability to cause local tissue damages such as necrosis and haemorrhage when injected intradermally. Hence, the minimum necrotizing, minimum haemorrhagic dose and also minimum edematic dose estimation proves a reasonable test for assessing the antivenom activity. By using the methods of Theaktson and Reid (1983), the LD_{50} of the venom was found to be 12 µg in mice.

Oral administration of *C. gigantea* plant extract at dose levels 200 and 400 mg/kg body weight effectively neutralized the lethal effect of $2LD_{50}$ and $3LD_{50}$ of *V. russelli* venom in mice (*in-vivo* neutralization). In *in-vitro* studies, the plant extract at all dose levels, i.e. 100, 200 and 400mg/kg body weight effectively neutralized $2LD_{50}$ and 3 LD_{50} of *Vipera russelli* venom.

The MHD was found to be 20 µg in rats when injected intradermally after 24hrs. Oral administration of the plant extract at various dose levels was found to effectively prevent the haemorrhage induced by the venom. The polyvalent antivenom was not able to prevent haemorrhage caused by viperid venom.

MND was found to be 30 µg in rats when injected intradermally after 3 days. Oral administration of the alcoholic extract was found to inhibit the induction of necrosis by the venom. At doses 200 and 400 mg/kg, the antinecrotic effect of plant extract was significant and was comparable with the control. The polyvalent antivenom did not show any antinecrotic activity.

MED was found to be 4µg when injected by sub-plantar route in rats. The effect of methanolic extract of *C. gigantea* against edema induced by viperid venom was studied at 60, 120, 180 and 240 minutes. Significant inflammation was seen after 1hr of venom injection and maximum inflammation was seen at 180 min in control group.

Plant extract at dose levels 200mg/kg and 400mg/kg showed significant anti-inflammatory activity at 240 min, and effect was comparable with that produced by the antivenom.

Present study confirms the potent anti snake venom activity of alcoholic extract of *C. gigantea.* The methanolic extract of *C. gigantea* was able to effectively neutralize the *in-vivo* activity of the viperid venom like lethality, edema, haemorrhage and necrosis. The antivenom activity of methanolic extract of *C. gigantea extract* suggests that chemical constituents like alkaloid, flavonoids, tannins or Triterpenoids present in the plant extract may be responsible for the activity.

Further isolation, purification and structural elucidation of active constituents from the plant extract are needed to develop the new chemical antidote for snake envenomations.

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