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Lethality assay of *Daboia russelli* and *Naja naja* venom for determination of anti-snake venom potential of *Helicteres isora* and *Bixa orellana*

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Abstract
The purpose of this study is to investigate the anti-snake venom properties of indigenous herbs traditionally used in the western ghats of India. It was discovered that traditional healers in Maharashtra, India utilised these plants to treat patients bitten by snakes. The *Daboia* russelli and *Naja naja* freeze-dried venom powder was collected from the Hindustan Snake Park in Kolkata. A botanist recognised and confirmed the species *Helicteres isora* and *Bixa orellana*. Extraction of leaves from both plants using a hot extraction process, followed by evaluation for antivenom activity in vivo utilising a lethality assay as a research model.

Keywords: *In-vivo* antivenom activity, *Daboia russelli* venom, *Naja naja* venom

Introduction

Snake bites continue to pose a threat to public health in tropical countries, particularly India. Insufficient records exist to ascertain the exact epidemiology or even mortality of snake envenomation cases. Due to the reliance on traditional healers and practitioners, hospital records fall well short of the real amount. An average of 2,500,000 snakebites are reported annually in India. In India, there are 52 venomous snake species, with the bulk of bites and deaths attributable to Ophiophagus hannah (king cobra), *Naja naja* (spectacled cobra), *Daboia* russelli (Russell's viper), Bungarus caeruleus (common krait), and Echis carinatus (saw-scaled viper). Despite the difficulty in determining the precise number of occurrences, snake bites continue to be a public health concern in many nations. The true incidence of snake envenomation is expected to reach 5 million per year. Approximately one million of these acquire severe squeal. The global gap in epidemiological statistics reflects differences in the accuracy of health reporting and the diversity of economic and ecological factors. Rarely are accurate records available to ascertain the exact epidemiology or even fatality of snake bite cases. Due to the reliance on traditional healers and practitioners of witchcraft, hospital data regarding the real number are insufficient, especially in developing countries. In the majority of underdeveloped nations, up to 80 percent of those bitten by snakes consult traditional practitioners before consulting a medical centre. Due to the delay, a number of people die en route to the hospital. The goal of this study is to investigate the anti-venom properties of indigenous plants from India's Western Ghats. These plant species are native to India. It was discovered that these herbs were utilised by traditional healers in the Indian state of Maharashtra [1-5].

Materials and Method

Venom procurement [6]

A Lyophilized powder of snake venom was purchased from Hindustan Snake Park in Kolkata and kept at 4 degrees Celsius.

Medicinal plants and preparation of extracts [7-8]

Based on a more in-depth literature review, the anti-venom activities *Helicteres isora* and *Bixa orellana* were chosen for the current experiment. The selected plants' capacity to act as anti-venom agents was documented in the written survey. The *Helicteres isora* Grah. and *Bixa orellana* specimens were identified and authenticated by Dr. T. Chakraborty, Scientist D and Joint Director of the Botanical Survey of India in Koregaon Park in Pune, India. Voucher specimens from both collections were shipped to their respective Institutes for safekeeping. *Helicteres isora* Grah. voucher specimen sample number is TARHELI-4, and *Bixa orellana* voucher specimen sample number is TARBIXO-3. Certificates required for authentication could be obtained. *Helicteres isora* and *Bixa orellana* plant powder (250 g) was extracted using a hot extraction procedure using petrol ether, chloroform, 90% ethanol, and water. After filtering out the biomass reserves, the waste was collected into a central pool and centred on a spinning vacuum evaporator.

In vivo anti-venom Activity [9]

Lethal Toxicity determination

- Median Lethal Dose (LD_{50}) and Median Effective Dose (ED_{50}) was determined as follows,

1. Various doses of venom were prepared by mixing with physiological saline.
2. All the different doses were injected intravenously to the mice through the tail vein.
3. The Lethal dose (LD50) was calculated after 24 hours.

4. 2 LD_{50} of the venom sample was incubated with plant extracts at 37^0C for 30 mins.
5. Again this venom-antivenom mixture was administered to the animals and the median ED_{50} was calculated after 24 hours.

Result and Discussion

Lethal Toxicity Determination

Lethality Assay of *Vipera russelli* Venom in Mice

The findings of lethality studies on *Vipera russelli* venom to determine the median lethal dosage (LD50) and minimum lethal dose (MLD) following intravenous injection in mice are presented in Table 1. The median lethal dosage (LD50) of the venom was assessed based on the data, and it was determined to be 50% effective (mortality). The computed LD50 and MLD were 2 mg/kg and 1.5 mg/kg, respectively.

Table 1: Results of Lethality tests on *Vipera russelli* venom

Group	Venom Dose mg/kg	No. of Deaths/ No. of Mice Used	% Death
1	0	0/6	0
2	0.5	0/6	0
3	1	0/6	0
4	1.5	1/6	16.67
5	2	3/6	50
6	2.5	6/6	100

Lethality Assay of *Naja naja* Venom in Mice

The findings of lethality studies on *Naja naja* venom to determine the median lethal dosage (LD50) and minimum lethal dose (MLD) following intravenous injection in mice are presented in Table 2. The median lethal dosage (LD50) of the venom was assessed based on the data, and it was determined to be 50% effective (mortality). The computed LD50 and MLD were 0.85 mg/kg and 0.75 mg/kg, respectively.

Table 2: Results of Lethality tests on *Naja naja* venom

Group	Venom Dose mg/kg	No. of Deaths/ No. of Mice Used	% Death
1	0	0/6	0
2	0.5	0/6	0
3	0.7	0/6	0
4	0.75	1/6	16.67
5	0.80	2/6	33.33
6	0.85	3/6	50
7	1	6/6	100

Effective Dose Determination

Table 3: Inhibitory effect of the Pet ether extracts of *Helicteres isora* on *Vipera russelli* venom ($2LD_{50}$) in mice

Group	Venom: Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1: 0	6/6	0
2	1: 1	5/6	16.67
3	1: 2	4/6	33.33
4	1:4	3/6	50
5	1:6	0/6	100

Table 4: Inhibitory effect of the Chloroform extracts of *Helicteres isora* on *Vipera russelli* venom ($2LD_{50}$) in mice

Group	Venom: Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1 : 0	6/6	0
2	1 : 1	5/6	16.67
3	**1 : 2**	**3/6**	**50**
4	1 : 4	1/6	83.33
5	1 : 6	0/6	100

Table 5: Inhibitory effect of the Methanol extracts of *Helicteres isora* on *Vipera russelli* venom ($2LD_{50}$) in mice

Group	Venom: Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1: 0	6/6	0
2	1: 1	4/6	33.33
3	1: 2	4/6	33.33
4	1: 4	3/6	50
5	1: 6	0/6	100

Table 6: Inhibitory effect of the Aqueous extracts of *Helicteres isora* on *Vipera russelli* venom ($2LD_{50}$) in mice

Group	Venom: Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1: 0	6/6	0
2	1: 1	5/6	16.67
3	1: 2	4/6	33.33
4	1: 4	4/6	33.33
5	1: 6	3/6	50

Table 7: Inhibitory effect of the Pet ether extracts of *Helicteres isora* on *Naja naja* venom ($2LD_{50}$) in mice

Group	Venom: Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1: 0	6/6	0
2	1: 1	5/6	16.67
3	1: 2	5/6	16.67
4	1: 4	3/6	50
5	1: 6	1/6	83.33

Table 8: Inhibitory effect of the Chloroform extracts of *Helicteres isora* on *Naja naja* venom ($2LD_{50}$) in mice

Group	Venom: Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1: 0	6/6	0
2	1: 1	5/6	16.67
3	1: 2	4/6	33.33
4	1: 4	4/6	33.33
5	**1: 6**	**3/6**	**50**

Table 9: Inhibitory effect of the Methanol extracts of *Helicteres isora* on *Naja naja* venom ($2LD_{50}$) in mice

Group	Venom : Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1 : 0	6/6	0
2	1 : 1	5/6	16.67
3	1 : 2	4/6	33.33
4	1 : 4	3/6	50
5	1 : 6	2/6	66.67

Table 10: Inhibitory effect of the Aqueous extracts of *Helicteres isora* on *Naja naja* venom ($2LD_{50}$) in mice

Group	Venom: Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1: 0	6/6	0
2	1: 1	5/6	16.67
3	1: 2	4/6	33.33
4	1: 4	3/6	50
5	1: 6	0/6	100

Table 11: Inhibitory effect of the Pet ether extracts of *Bixa orellana* on *Vipera russelli* venom ($2LD_{50}$) in mice

Group	Venom: Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1 : 0	6/6	0
2	1 : 6	6/6	0

Table 12: Inhibitory effect of the Chloroform extracts of *Bixa orellana* on *Vipera russelli* venom ($2LD_{50}$) in mice

Group	Venom: Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1: 0	6/6	0
2	1: 1	5/6	16.67
3	1: 2	4/6	33.33
4	1: 4	3/6	50
5	1: 6	2/6	66.67

Table 13: Inhibitory effect of the Methanol extracts of *Bixa orellana* on *Vipera russelli* venom ($2LD_{50}$) in mice

Group	Venom: Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1: 0	6/6	0
2	1: 1	5/6	16.67
3	1: 2	4/6	33.33
4	1: 4	4/6	33.33
5	1: 6	3/6	50

Table 14: Inhibitory effect of the Aqueous extracts of *Bixa orellana* on *Vipera russelli* venom ($2LD_{50}$) in mice

Group	Venom: Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1: 0	6/6	0
2	1: 1	5/6	16.67
3	1: 2	5/6	16.67
4	1: 4	4/6	33.33
5	1: 6	3/6	50

Table 15: Inhibitory effect of the Pet ether extracts of *Bixa orellana* on *Naja naja* venom ($2LD_{50}$) in mice

Group	Venom: Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1 : 0	6/6	0
2	1 : 6	6/6	100

Table 16: Inhibitory effect of the Chloroform extracts of *Bixa orellana* on *Naja naja* venom ($2LD_{50}$) in mice

Group	Venom: Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1 : 0	6/6	0
2	1 : 1	5/6	16.67
3	1 : 2	4/6	33.33
4	1 : 4	4/6	33.33
5	1 : 6	3/6	50

Table 17: Inhibitory effect of the Methanol extracts of *Bixa orellana* on *Naja naja* venom ($2LD_{50}$) in mice

Group	Venom: Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1: 0	6/6	0
2	1: 1	5/6	16.67
3	1: 2	4/6	33.33
4	1: 4	3/6	50
5	1: 6	1/6	83.33

Table 18: Inhibitory effect of the Aqueous extracts of *Bixa orellana* on *Naja naja* venom ($2LD_{50}$) in mice

Group	Venom: Plant Extract	Mortality(24hr) No. of death/No. of mice used	% Survival
1	1: 0	6/6	0
2	1: 1	4/6	33.33
3	**1: 2**	**3/6**	**50**
4	1: 4	1/6	83.33
5	1: 6	0/6	100

Summary and conclusion

Snakebites are a major health danger that contribute to India's high mortality rate. *Vipera russelli* and *Naja naja* are common snakes in India, and their venom is responsible for a significant number of fatalities. Antisnake venom continues to serve as the specialised antidote for snake venom intoxication. Typically, this antivenom is generated from horse serum. They contain horse immunoglobulins, which usually induce complement-mediated adverse effects, as well as other proteins that occasionally induce serum sickness and anaphylactic shock. Although the use of plants to counteract the consequences of a snake bite has been known for decades, the past two decades have seen an increase in scientific interest[10]. Numerous Indian medicinal plants are suggested for treating snakebites. In this work, the antivenom capabilities of *Helicteres isora* fruits and *Bixa orellana* seeds were evaluated against *Vipera russelli* and *Naja naja* venom.

Efforts have been undertaken in this study to determine the pharmacological activity and fatal toxicity of a substance utilising mice as test subjects[11]. The pharmacological properties of the extracts suggest that their inhibitory effect on the snake venom-induced pathological alterations may be attributable, in part, to the phytochemical compounds' active components. In addition to isolating and characterising the active chemical components from these plants, it is necessary to investigate their mode of action.

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