Cobra Venom Neutralization Effect by *Hemidesmus indicus* Root Extract

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Abstract: *Hemidesmus indicus* (Apocynaceae) has been utilized traditionally in the Indian subcontinent for the treatment of snakebites and scorpion stings. It has also several beneficial bioactivities in human beings such as antidiabetic, anti-inflammatory and skin diseases. The purpose of this study was to assess the anti-venom capability of *Hemidesmus indicus* root extract against cobra venom (*Naja nigricollis*). Albino mice were utilized to test the plant's efficacy using the venom of *N. nigricollis. H. indicus* root extract (600 mg/kg) neutralized cobra venom and generated 100 % survival, suggesting its antivenom potential. *H. indicus* showed a substantial antivenom effect (p0.05) against the venom of *N. nigricollis,* indicating that it might be a useful drug for treating snake venom. The present study provides scientific basis for use of root extract of *H. indicus* against *N. nigricollis* venom.

Keywords: Antivenom, *Hemidesmus indicus*, *Naja nigricollis*, Venom Neutralization, Albino Mice

1. Introduction

In India, snake envenomation is one of the most serious hazards. Venomous attacks are more likely to leave double tooth marks than non-venomous bites. One of the most remarkable and unusual adoptions of snakes in the animal kingdom is reptile venom. Venoms are mostly complicated combinations of compounds known as enzymes found in snake poison. Toxins are divided into two categories: neurotoxins, which affect the nervous system, and haemotoxins, which affect the circulatory system. Cobras, mambas, sea snakes, kraits, and coral snakes all have neurotoxic venom. Rattlesnakes, copperheads, and cottonmouths are among the snakes having hemotoxic venom (Blanchard, 2001). Envenomation by snakes can be fatal, although only approximately 300 of the 2,700 species of snakes are poisonous (Wang et al., 1997; Sharma et al., 2004). Fang marks in the skin and inflammation at the bite site, pain, diarrhea, burning, withdrawal leading to convulsions, faintness, blurry vision, profuse sweating, fever, extreme thirst, nausea or vomiting, numbness, irritation, and quick pulse are the most common symptoms of hazardous snake bites (Mishal, 2002). It is estimated that 30,000 to 40,000 people die each year as a result of snake bites around the world. Roughly 25,000 people die in India, largely in rural areas, about 10,000 in the United States, and the rest in other countries. All snakes are protected under the 1972 Wild Life Protection Act (the venomous snake being at the top of the list of protected species), and the sale of snake skins has been prohibited since 1976.

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Antivenins extracted from horses are used to treat persons who have been bitten by snakes. When antibodies are injected into the bloodstream, they attack the venom and counteract its effects. However, due to its high cost and scarcity, the usage of snake venom antiserum has its own set of limitations. Antiserum is therefore difficult to obtain for non-urban victims (Mishal, 2002). Recent medical research has verified the effectiveness of many plants with phytoconstituents in combating snake toxins. The use of plants to deal with snake bites are more becoming practice. There are also several historical data that show that plant products employed by native practitioners are effective in treating snake bites, with a higher success rate among snake bite victims in advanced stages of venom poisoning. These help medicinal studies leading to the synthesis of novel compounds or isolation of bioactive substances from therapeutically useful plants with reduced toxicity (Dravidamani et al., 2008). This study is done to determine the antisnake venom activities of *Hemidesmus indicus* on the venom of *Naja nigricollis* (Ṭhakara, 1989).

Hemidesmus indicus (L.) R. Br. (Apocynaceae) is commonly known as Indian Sarsaparilla. Due to the many biological activities attributed to its various sections, particularly the roots, it is frequently utilized in traditional medicine in several parts of the Indian subcontinent. Snakebites, scorpion stings, diabetes, urinary diseases, dyspnea, menorrhagia, oligospermia, anorexia, fever, abdominal colic and pain, dysentery, diarrhea, cough, rheumatism, headache, inflammation, pyrosis, skin diseases, leprosy, sexually transmitted diseases, and cancer have mostly been treated with it in ancient times.

In Ayurveda, the plant is used in the treatment of bone loss, low body weight, fever, stress, topical wound and psoriasis. Besides, Ayurvedic literature also depicts its use as anti-atherogenic, anti-spasmodic, memory enhancing, immunopotentiating and anti-inflammatory agents (Kumar et al., 2007b; Kundu, and Mitra, 2013; Kundu et al., 2012). Additionally, *H. indicus* root extract provides several health advantages for conditions including diarrhea, dysentery, skin conditions, diabetes, indigestion, inflammation, edema, toothaches, syphilis, impotence, snake bites, and scorpion stings. (Madhu et al., 2017; Uthirapathy, 2021). It's also utilized as a blood purifier, a body cooler, an appetizer, and a health and energy booster" (Lakshman et al., 2006; Mahalingam and Kannabiran 2009c). The aim of the present study was to assess the anti-venom capability of *H. indicus* root extract against cobra venom (*N. nigricollis*).

2. Material and methods

2.1 Venom and Plant Materials

A voucher specimen of the *Hemidesmus indicus* plant was kept in our lab after being identified and authenticated by a department of botany on September 30, 2021, in Rajagoplalapeta village, Siddipet district, Telangana state, India. Irula Snake Catchers ICS in Kovalam, Chennai collects *Naja nigricollis* venom. *H. indicus* powdered roots were extracted with ethanol using a Soxhlet apparatus, and the extract was concentrated under reduced pressure. The extract was thoroughly dried until it achieved a stable weight. The resulting ethanol-free extract was employed in the experiment.

2.2 Acute Toxicity Study

The acute oral toxicity of an ethanolic extract of *H. indicus* was determined using the Up & Down method (OECD recommendation No. 425) and the fixed-dose method, according to the guidelines of the Organization for Economic Co-operation and Development (OECD) (OECD guideline No. 420). A test was carried out to categorize the compound's toxicity class using these approaches. The mice

(nulliparous and non-pregnant female albino mice) were fasted overnight with free access to water, weighed, and given a single oral dosage of the test drug. Individual animals were examined for the first 30 minutes, then every 48 hours during the next 14 days, with specific attention paid to the first 4 hours (short-term toxicity). There were no symptoms of toxicity in any of the animals, and the LD50 was found to be larger than 2000 mg/kg. (Zarei and Javarappa, 2012; Fatani et al., 2006; Goje et al., 2013; Anas et al., 2010).

2.3 Antivenom Activity- Oral Administration of the Extract Prior to the Injection of *Naja nigricollis* Venom

A total of twenty-four adult Wister albino mice of both sexes were divided into four groups, each with six animals. The control group received only venom (lethal dose 900g/kg) intramuscularly (I.M), whereas the other three groups received *Naja nigricollis* venom (900g/kg) following 30 minutes of oral administration of *H. indicus* root extract (200, 400, and 600 mg/kg). The mice were monitored for symptoms of toxicity, as well as the time they died. ANOVA and the student's t-test were used to examine the mean death time (Ode and Asuzu, 2006).

2.4 Evaluation of *H. indicus* Extract for Protective Activity Against *N. nigricollis* Venom

In this study, albino rats (150-180 gm) of both sexes were given a daily dose of H. indicus extract 200 mg/kg orally, followed by 45 minutes of intramuscular injection of 1/3 LD_{50}, 2/3 LD50, and 1/2 LD_{50} for 45 days.

2.5 Administration of Both *N. nigricollis* Venom (900 µg/kg) and H. indicus Extract i.p After pre-incubation of the Mixture for 30 Minutes

The experiment utilized six mice per group, divided into four groups. Only *Naja nigricollis* venom (lethal dose, 900µg/kg i.p) was given to the control group. The other groups were given a mixture of *N. nigricollis* venom and the plant extract *H. indicus*, which had been pre-incubated for 30 minutes at 37o C before being given. The average death time and the time it took the mice to die were recorded and evaluated using ANOVA and student t-test.

2.6 Intra Peritoneal Administration of the Extract 30 Minutes Before Injection of *N. nigricollis* Venom

The experiment involved four groups of mice, each with six mice. Group A received only a lethal dose (900µg/kg i.m.) of *N. nigricollis* venom, which served as the control. *H. indicus* root extract, 200 mg/kg i.p., was given to Group B 30 minutes before *N. nigricollis* venom was administered. Group C received 400mg/kg i.p. of *H. indicus* root extract 30 minutes before receiving *N. nigricollis* venom (lethal dose, 900 µg/kg i.m.). Group D received the extract at 600 mg/kg i.p., followed by *N. nigricollis* venom (lethal dose, 900 mg/kg i.p.) after 30 minutes. The number of mice that died during the course of the experiment were counted, as well as the average time of death was recorded.

3. Result and Discussion

3.1 *H. indicus* Extract Was Tested for Protective Effect Against *N. nigricollis* Venom

The extract was given orally 30 minutes before the venom was injected. In the control group, which was treated with only *N. nigricollis* venom (900 µg /kg), all of the mice died showing severe signs of toxicity. Mice treated with *H. indicus* extract at 200 mg/kg and 400 mg/kg recorded 33.3% and 50%

survival. The death time of mice treated with 600 mg/kg of the extract was significantly ($p<0.01$) prolonged compared to the control (Table 1). In *H. indicus* extract (600 mg/kg) treated four animals survived with minimal signs of toxicity and offered 66.67 % protection indicating its antivenom potential against *N. nigricollis.*

3.2 Evaluation of *H. indicus* Extract for Protective Activity Against *N. nigricollis* Venom

After 45 days of therapy, mice that were daily orally treated with *H. indicus* extract (200 mg/kg b.wt) following injection with 1/3, 2/3, or 1/2 LD50 of the venom revealed normal ideal histological structures, but mild erosion of a few villi. However, the findings of the study indicated that *N. nigricollis* can disrupt rat metabolism. In albino rats, the extract of *H. indicus* was found to be efficient in mitigating the deadly effect of *N. nigricollis* venom.

3.3 After A 30-Minute Pre-Incubation Period, A Mixture of *N. nigricollis* Venom and *H. indicus* extract was administered

Table 2 shows the findings of this research. Mice survived 33.33 %, 50 %, and 66.67 % when pre-incubated cocktails of venom (900µg/kg) and various doses (200, 400, and 600 mg/kg) of *H. indicus* extract were used. When compared to the control, the mean death time of *H. indicus* extract 600 mg/kg treated animals reduced significantly ($P<0.01$).

3.4 30 Minutes Before Injecting *N. nigricollis* venom (900 g/kg, i.m.) into the Peritoneal Cavity, the extract was administered Intraperitoneally

The control mice, which were simply given 900 g/kg of *N. nigricollis* venom, killed all six of their animals. In the envenomed mice 600 mg/kg, *H. indicus* extract increased the time to death significantly ($P<0.01$). All animals treated with *H. indicus* extract survived with 83.33 % survival, while animals treated with 200 mg/kg and 400 mg/kg survived with 33.34 % and 50.00 % survival, respectively shown in the Table 3.

Snake venom's principal function is to help the snake immobilize and digest its meal. Snake venom is a complex molecule with several components, the majority of which are proteins. Enzymes and polypeptide toxins are the most prominent venom components that cause major clinical consequences after a bite. Snake venom poisoning still has a unique antidote in the form of anti-snake venom. Horse sera are commonly used to make anti-snake venom. They contain horse immune globulins, which can produce complement-mediated adverse effects, as well as other proteins that might cause serum sickness and anaphylactic shock. Plants have been used to counteract the effects of snake bites for the past 22 years. Snake bites can be treated with a variety of Indian medicinal plants.

The venom of the Indian cobra contains neurotoxins, cardiotoxins, enzymes, and proteins. Respiratory paralysis is the most common cause of death from cobra envenomation. Anti-snake venom or aided ventilation can save lives in most cases (Banejee, 1978). Anti-snake venom is notorious for causing severe side effects and making dosage management difficult. Plants have been used to cure snake venom poisoning for over 20 years, but only recently have they been acknowledged (Meier, 1991; Warrell, 1991). It has only been given more scientific attention in the last 20 years.

Although there are several claims from various countries claiming to be able to nullify the effects of snake venom, only a handful relate the activity to chemical components found in plant materials. Many plants in India are known to be effective against snake envenomation. Many traditional healers,

particularly in India's southern area, used *A. paniculata.* There is also various Ayurvedic snake bite treatments that contain *A. paniculata* as one of the ingredients. Snake venom's pharmacological activities are mostly linked to proteins, particularly enzymes (Uthirapathy et al., 2021; Okanogi et al., 1970; Mors et al., 2000).

The venom of the *N. nigricollis* is a combination of proteins that comprises highly harmful post synaptic neurotoxins and diffuses fast in circulation due to its low molecular weight. Toxic principle phospholipases in cobra venom have pre-synaptic neuromuscular inhibiting action. The major component responsible for harmful effects such as neurotoxicity, cardiotoxicity, heamotoxicity, and disruption to cellular membranes is phospholipase A2 (Fattepur and Gawade, 2007; Nazimuddin et al., 1978). *A. paniculata* has been shown to offer some protection against the venom's deadly dose. Protein-binding and enzyme-inhibitory properties have been found in naturally occurring chemicals such as sitosterol, pentacyclicterpines, nitro compounds (aristolochic acid), cinnamic acid derivatives, curcuminoids, polyphenolic compounds, and flavonoids. Nahed et al., (2011) have proved that the various secondary metabolites, such as phenols, flavonoids, terpenoids, xanthenes, and quinonoids, have previously been found to mask various enzymatic effects of cobra venom (Otten, 1998; Yingprasertchai et al., 2003). These secondary metabolites prevent different snake venom enzymes from attaching to their potential targets, resulting in an antivenom action (Chatterjee et al., 2006). These active ingredients, such as *A. paniculata*, are responsible for the antivenom characteristic of snake venom by altering the function of proteins and enzymes. Lupeol, a pentacyclic triterpenoid inoculated from the roots of *H. indicus*, has been shown to exhibit antivenom activity in terms of haemorrhagic, coagulant, and anticoagulant properties. Asad et al., (2013) have explained that the plant's molecules distribute positively at the site of a snake bite before hospitalization and block the rapid spread of toxins (Meier, 1991), this method is a great for initial treatment. Alkaloid, flavonoid, saponin, carbohydrate, protein, phenolic compounds, steroids, terpenoids, glycoside, resins, tannin, and thiols are all found in the roots of *H. indicus*. It is urgently needed to isolate harmful components of cobra venom that are responsible for its primary toxicities. Furthermore, identification of antivenom compounds derived from medicinal plant extracts would be beneficial in the future for complete and effective snake bite therapy. In this study, it was also discovered that the mice's survival rose (66.7-100 %) in a dose-dependent manner as the extract dose was raised. As a result, *H. indicus* root extract is believed to have anti-snake venom activity, neutralizing the harmful effects of *N. nigricollis* venom.

Table 1: Oral administration of *H. indicus* extract, 30 minutes prior to injection of *N. nigricollis* venom (900 µg/kg, i.m.)

Groups	Dose of extract administered	No. of dead mice out of the total in the groups	Percentage (%) of mice alive	Mean death time (sec)
1	Control	6/6	--	333.75±34.37
2	200	4/6	33.33	424.67±51.19
3	400	3/6	50	487.00±51.48
4	600	2/6	66.67	678.00±33.19*

*Significant at P<0.01 when compared with the control group.

Table 2: Intraperitoneal (i.p.) administration of a mixture of *N. Nigricollis* venom and *H. indicus* extract after pre-incubation for 30 minutes.

Groups	Dose of extract administered	No. of dead mice out of the total in the groups	Percentage (%) of mice alive	Mean death time (sec)
1	Control	6/6	--	287.00±51.48
2	200	4/6	33.33	533.75±34.37
3	400	5/6	16.67	724.67±51.19*
4	600	0/6	100	--

*Significant at $p<0.01$ when compared with the control group.

Table 3: Intraperitoneal Administration of the extract, 30 minutes before the administration of *N. nigricollis* venom (900 µg/kg, i.m)

Groups	Dose of extract administered	No. of dead mice out of the total in the groups	Percentage (%) of mice alive	Mean death time (sec)
1	Control	6/6	66.67	203±14.1
2	200	4/6	33.34	605±19.2
3	400	3/6	50.67	733.00±34.60*
4	600	1/6	83.33	812.71±18.18*

*Significant at $p<0.01$ when compared with control.

4. Conclusion

In Swiss albino mice, neutralization of the venom of the *N. nigricollis* was investigated. The antivenom ability of plant extracts was tested in vitro and in vivo at three dose levels of 200, 400, and 600 mg/kg, indicating considerable neutralization of *N. nigricollis* venom. In terms of venom neutralization, survival rate, and increase in the mean death time, *Hemidesmus indicus* extract at 600 mg/kg has shown promising antivenom activity. To elucidate the structure of the bioactive chemicals and additional investigations to determine the mechanism of action of *H. indicus* extract on venom neutralization, the phytoconstituents of *H. indicus* extract must be isolated, identified, and characterized. The current research suggests that *H. indicus* could be developed as a possible cobra envenomation medication.

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