B-5013
[1-5]

RESEARCH ARTICLE Indian Journal of Animal Research

Antivenom Activity of *Solanum xanthocarpum* Hydroethanolic Root Extract Targeting the Enzymes Present in Naja Snake Venom

Pradeep Kumar Choudhary[1], Vishal Khandelwal[1]

10.18805/IJAR.B-5013

ABSTRACT

Background: The work reported in this paper targets the ethnobotanical role of one of the traditional medicinal plant *Solanum xanthocarpum*, whose medicinal value was reported in Ayurveda. Traditionally *Solanum xanthocarpum* was used as the antidote for treatment of snake bites by different tribal community of India, to explore and scientifically validate these concept this work was planned.

Methods: We have prepared the hydro ethanolic root extract (HERE) of *Solanum xanthocarpum* and investigated its pharmacological activity both *in vitro* and *in vivo*, targeting the enzymes present in Naja snake venom. We have used the wistar albino rat as the animal model in our study.

Result: It was inferred by our study that *Solanum xanthocarpum* is safe for wistar albino rats upto 2000 mg/kg body weight. Phospholipase A2 (PLA2) and anti-fibrinogenolytic activity are significantly neutralized by *Solanum xanthocarpum*. Dose dependent inhibition of human red blood cell (HRBC) lysis was also observed. In the future, bioactive compounds from HERE could be isolated and standardized to act as an antidote to Naja venom.

Key words: Antivenom, Hydroethanolic root extract (HERE), *Solanum xanthocarpum*.

INTRODUCTION

Snakebite is one of the most important public health problem in tropical countries, including India. Every year on an average 200000 persons fall prey to snake bite in India and approximately 35 000 to 50000 lives are lost per year. In India, snakes are commonly associated with religious sentiments (White, 2002). Snakes are considered very dangerous and produce systemic poisoning because the rapid action of their neurotoxin causes respiratory paralysis and death. Anti-snake venom (ASV) is a specific antidote to snake venom's actions and the mainstay of treatment. Monovalent ASV is preferable to the polyvalent type since it is less hazardous to the patient and likely to be more effective in the treatment of the particular bite; however, a species diagnosis must be made before the right treatment can be chosen. Polyvalent ASV is commonly used against snakebite, but it is expensive and contains the animal antibodies of immunized animals; hence, there are possibilities of cross-reactions inside the host immune system that affect about 20% of patients (Alirol *et al*. 2010, Gowtham *et al*. 2014).

The WHO mentioned on record that 80% of the global population depends upon traditional medicine for their primary healthcare needs. It was also stated that seventy percent population depend upon the plants as source of their medicines. At present, a large number of traditional medicinal plants have been tested for their pharmacological activity against different diseases (Choudhary *et al.* 2019; Srivastava *et al*. 2022).

Solanum xanthocarpum has different enthono medicinal properties it has been used for centuries as antiasthmatic, hepatoprotective, anti-inflammatory, anticancer, hypochole sterolaemic, antianaphylactic, antiandrogenic, antispermato genic, insecticidal, antiaccelerator, antioxidant and many other diseases. (Vadnere 2008, Sridevi *et al*., 2007).

In contrast to the difficulty of availability of modern treatment in large areas of developing world there are many plant species are used as traditional medicine against the snake bite (Janardhan *et al.* 2014; Khandelwal *et al.* 2022). In India, several medicinal plants are reported to treat snake bites (Kumarapppan *et al.* 2011). *Solanum xanthocarpum*, is one of the plants that has long been used in traditional herbal medicine against snakebite (Asad *et al.* 2014). However, only few attempts have been made to scientifically correlate and compare its anti-cobra venom activity with respect to ASV. The current study looks into the neutralizing effects of Naja venom enzymes in hydroethanolic extracts of *Solanum xanthocarpum* roots.

MATERIALS AND METHODS

Present study was conducted in department of Biotechnology, GLA University Mathura, Uttar Pradesh

[1]Department of Biotechnology, GLA University, Mathura-281 406, Uttar Pradesh, India.

Corresponding Author: Pradeep Kumar Choudhary, Department of Biotechnology, GLA University, Mathura-281 406, Uttar Pradesh, India. Email: pradeep.choudhary@gla.ac.in

How to cite this article: Choudhary, P.K. and Khandelwal, V. (2023). Antivenom Activity of *Solanum xanthocarpum* Hydroethanolic Root Extract Targeting the Enzymes Present in Naja Snake Venom. Indian Journal of Animal Research. doi: 10.18805/IJAR.B-5013.

Submitted: 05-09-2022 **Accepted:** 04-02-2023 **Online:** 24-02-2023

(India) during january to may 2022. Material used in this study were following.

Venom

Lyophilized Naja venom is purchased from Sigma and stored at 4°C inside a dessicator for further use. It was mixed in a 0.9% saline solution and centrifuged at 2000 rpm for 15 minutes. Supernatant of this was stored at 4°C for further use as venom on experimental wistar albino rat. The venom concentration was denoted in terms of dry weight.

Plant extracts

Solanum xanthocarpum plants was collected from Mathura (27°33′05.0″N and 77°38′11.3″E) and its adjoining areas was identified and authenticated by Dr Anuradha Upadhye of Agharkar research institute, Pune (Boucher deposition no. 26). The roots of this plant were separated and dried. A coarsely powdered root was used for hydroethanolic extract preparation.

Snake venom antiserum

Polyvalent antiserum of venom purchased from Mediclone Biotech pvt. ltd, chennai. Before use, the antiserum solution was prepared in 0.9% saline.

Animals

Wistar albino rats, male and female, were collected from the GLA University animal house in Mathura, weighing 60-100 gm with reference GLAIPR/CPCSEA/IAEC/2014/ Biotech 02. Five groups of rats were made, with six rats in each group. Safe doses were determined according to OECD guidelines No. 423. The control group was fed rat pellets and water only.

Acute toxicity study

To determine the acute toxicity standard, the “up and down” method was used, in which 2000 mg/kg body weight dose was administered to a female wistar albino rat. All the albino rats were observed after 3 hours for behavioral change, walking pattern and change of fur texture up to 24 hours. For determination of aniti venom activity three dose 125 mg/kg, 250 mg/kg and 500 mg/kg of HERE were selected. These different doses were prepared by dissolving HERE in 0.05% carboxymethyl cellulose.

Determination of LD_{50} and lethality neutralization activity

The medium lethal dose (LD_{50}) of Naja venom was calculated as per standard protocol (Parveen *et al.* 2017). The mortality concentration of Naja venom was calculated by using the different concentrations of venom dissolved in a 0.9% sterile normal salt solution. Venom solution of different concentrations prepared in 0.2 ml of sterile normal saline and its i.p. administration to different groups (n = 5) of wistar albino rats. Probit analysis was performed to calculate the LD_{50} with limit of 50% of mortality wistar rat taking place within 24 h after the oral dose of venom given to animal (Akçay 2013). The venom neutralization potential of HERE was assessed by standard protocol (Theakston *et al*. 2003). Double doses of venom to LD_{50} ($2\times LD_{50}$) were injected ip into different groups of wistar albino rats (n = 5) just after the oral doses of 125, 250 and 500 mg/kg. Same dose of venom given to control group without HERE poly valent anti serum given to other group followed by oral administration of $2\times LD_{50}$ of Naja venom dose. Lethality was analyzed by probit analysis after 24 hours.

Neutralization of HRBC lysis induced by venom

The anti-human red blood cell lysis activity of the HERE of *Solanum xanthocarpum* was investigated by suppression of human erythrocyte lysis (HRBC). Hemolysis was performed by using the Naja venom. A mixture of 50 µg venom with different concentrations of HERE 125, 250 and 500 µg was prepared and placed in an incubator for 60 min at 37°C. 1 ml of HRBC (1% of the total) was mixed with venom along with HERE suspension in centrifuge tubes, while the control tubes contained only saline free from HERE. All the tubes were placed in the incubator at standard conditions (37°C for 30 min). Further centrifugation was done at 5000 rpm for 5 min, resultant supernatant absorbance was observed at 540 nm by using a spectrophotometer. The hemolytic inhibition in percentage was determined by the following equation:

$$\frac{\text{Control-Test}}{\text{Control}} \times 100$$

(Rajesh *et al.* 2017).

Anti phospholipase A_2 activity by the here

Following the method of Rajesh *et al.* (2017), Phospholipase A_2 assay was performed. 1% lecithin, 0.1 mM $CaCl_2$ and 1.2% erythrocytes were suspended in 0.8% agarose to form a plate and were used for the study. 50 µg venom was mixed with 125, 250 and 500 µg of HERE and placed in an incubator for 30 min at 37°C These mixtures were then placed in the agarose gel wells. The agarose gel were placed inside incubator for 24 h at 37°C, after which haemolytic halos were investigated, venom mixed with HERE free solution used as control.

Assay of fibrinogenolytic activity

Fibrinogenolytic activity was assayed by the standard protocol (Slagboom *et al*. 2017, Bittenbinder *et al.* 2019). The test sample was prepared by mixing the 60 µg fibrinogen obtained from bovine plasma with 25 µg Naja venom and a Tris-HCl buffer (pH 7.4). This mixture was incubated for 2 hours. By adding the 20 µl stoping buffer (4% SDS, 4% β-mercaptoethanol and 1 M Urea) the reaction was terminated after that samples were loaded on 12% SDS-PAGE and analyzed. To determine the anti-venom studies, venom and HERE are mixed in ratio 1:1 and 1:2 and placed in an incubator at standard conditions (37°C for 30 min) and the above process was repeated.

RESULTS AND DISCUSSION

Determination of the toxicity of here

The net percentage yield of the hydro-ethanolic root extract was collected at 12% and the safe dose of here was

estimated at 2000 mg/kg body weight in wistar albino rat. All the wistar albino rats expressed the normal texture of fur and skin color and no toxicity symptoms were observed.

Determination of LD_{50} and ati mortality of venom

The LD_{50} of Naja venom was calculated was 10 µg/20 gm wistar albino rat by i.p. rout. Dose concentrations of here 125, 250 and 500 mg/kg were observed to effectively neutralize the venom induced by $2\times LD_{50}$ Naja venom (Table 1). Absolute neutralization of venom-induced mortality was expressed at 250 and 450 mg/kg of here (Table 1).

Anti HRBC lysis induced by venom

50 µg naja venom suspension mixed with 1 ml HRBC (1%) produced erythrocyte lysis compared with haemolysis occured in hypotonic saline. Dose-dependent protection by HERE was observed for the venom-catalyzed erythrocyte lysis up to a dose of 500 µg (Fig 1).

Anti phospholipase A_2 (PLA-2) activity of here

Here was used to neutralize the venom-induced hemolysis by using 10 µg of venom that form the 10 mm diameter haemolytic halo. A dose concentration of 500 µg of here shows the significant anti-haemolytic activity of Naja venom (Fig 2).

Assay of fibrinogenolytic activity

60 µg fibrinogen was mixed with 25 µg Naja venom in a buffer containing 5 mM Tris HCl (pH 7.4) and 10 mM NaCl and incubated for 2 h. Resultant mixture after adding the 20 µl denaturing buffer were analyzed by loading it on 12% SDS-PAGE. Fig 3 clearly depict that well A contain the intact fibrinogen, BandC well show the fibrinolytic activity of naja venom on fibrinogen as its Aá,Bâ and ã subunits were separated. In the well D antifibrinogenolytic activity of 250 µg HERE was shown that neutralize the naja venom fibrinogenolytic activity.

There are several plants species reported that have been used in traditional medicine all over the world against the snakebites. At present, limited plants and plant products have been assayed in a controlled manner and approximately three dozen plant species have been reported to neutralize the enzymes present in Naja venom. The plants contain more than one secondary metabolite responsible for venom neutralization, among them vanillic acid, solesonin and solasodine, which is reported for the first time in the fruits of *Solanum xanthocarpum* and is widely used in fragrances and licensed as a food additive. It is also bestowed with a variety of pharmacological activities such as inhibiting snake venom activity, carcinogenesis, apoptosis and inflammation, while chlorogenic acid is an ester of caffeic acid and quinic acid and is long known as an antioxidant (Sood *et al.* 2020, Kumar and Pandey 2014).

In previous studies, different researchers reported that methanolic extracts from the roots of Vitex and Emblica significantly inhibit the mortality activities of several snake venoms as they exhibit anti hemorrhagic, anti-coagulant, defibrinogenating and anti-inflammatory activity (Alam 2014). It was also reported that the seed extract of Tamarindus inhibits the PLA2, protease, hyaluronidase, L-amino acid oxidase and 5′-nucleotidase enzyme activities in a

Table 1: *In vivo* effect of here of *Solanum xanthocarpum* on mortality induced by $2xLD_{50}$ of Naja venom in wistar albino rat.

Groups	Dose	After 24 h mortality (no. of death wistar rat/no. of wistar rat used)	% survival after 24 h	*Modified %	Probit
1 (control)	20 µg venom	5/5	0	5	3.75
2	20 µg venom + 5 µg polyvalent antivenom	0/5	100	95.0	6.85
3	125 mg/kg HERE+20 µg venom	1/5	20	65.33	5.55
4	250 mg/kg HERE+20 µg venom	0/5	100	95.0	6.85
5	250 mg/kg HERE+20 µg venom	0/5	100	95.0	6.85

*Modified formula: for no dead: [100*0.25/n]; for the 100% mortality: 100 [n-0.25)/n]. When n is the no of animals in the group.

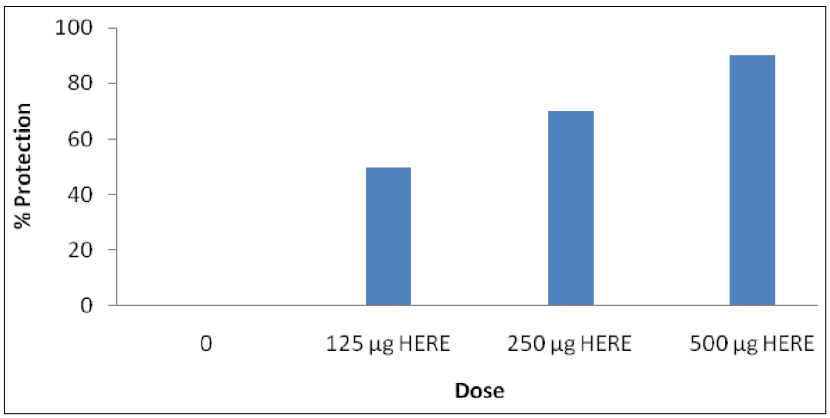

Fig 1: Effect of Hydroethanolic Root Extract of *Solanum xanthocarpum* on Naja Venom-Induced degradation of HRBC.

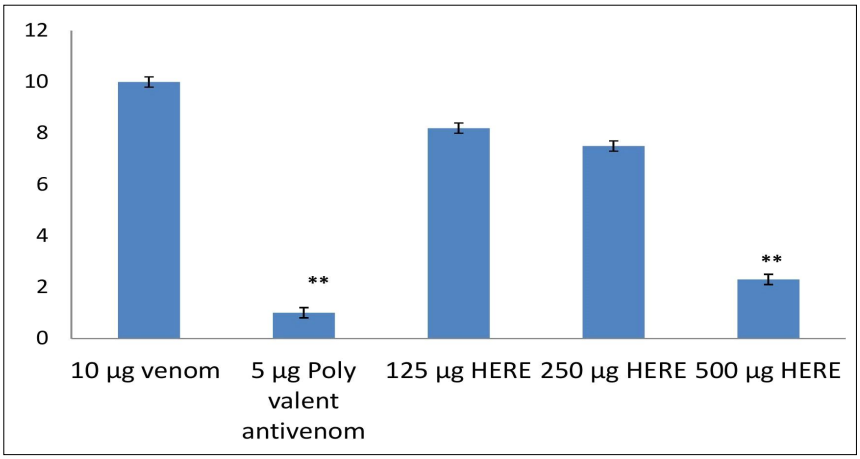

Fig 2: Anti phospholipase activity of HERE of *Solanum xanthocarpum* against the Naja venom. 125, 250 and 500 µg of HERE were used to investigate anti-PLA-2 activity. The values are shown as Mean±SEM, 5 rats in one group (n = 5).*P value ≤0.05 show significant, **P value ≤0.01 show highly significant, as compare to control group.

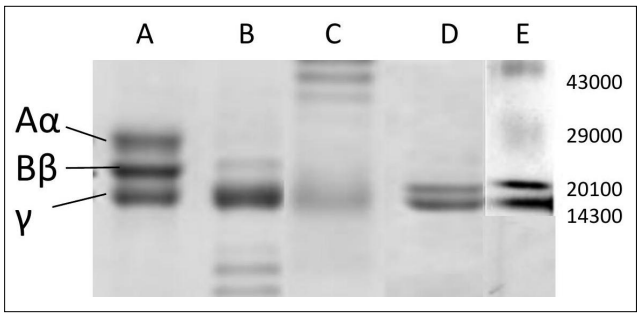

Fig 3: Anti-fibrinogenolytic activity of *Naja* venom demonstrated by SDS-page.

dose-dependent manner against russel venom (Asuzu and Hervey 2003). In the present work, we have selected the plant *Solanum xanthocarpum* because in our previous study, its hot aqueous extract showed significant anti-inflammatory activity with reference to cytokines IL-2, IL-10 and IL-4. (Choudhary *et al.* 2019). *Solanum xanthocarpum* fruits and roots are used by several tribal population of north east against the snake bite (Ozukum *et al*. 2019). In the present investigation the hydroethanolic extract of roots (HERE) was prepared for further study to investigate the anti-venom activity targeting the enzymes present in Naja Venom (both *in vitro* and *in vivo*).

In our studies, it was also inferred that hydroethanolic root extract (HERE) of *Solanum xanthocarpum* significantly neutralized the venom enzymes of Naja. In the future, we will have to isolate the bioactive compound from *Solanum xanthocarpum* so that it can be formulated as an antidote for the treatment of Naja snake bite.

CONCLUSION

It was well known fact that lethality of Naja venom depends upon its hemolytic, fibrinolytic, phosphor lipase (PLA) activity. It was long time challenge for all the scientists who are working to find anti venom activity of certain snake venoms because every venom has its unique composition. Some of work become successful to find out certain peptide from plants that show the antivenom activity by targeting the enzymes present in snake venoms. The tribal populations worldwide using the plants to treat the snake bite but this knowledge confined to that population only so it must be scientifically approved by different pharmacological experiments. Present study prove that *Solanum xanthocarpum* has the anti PLA, antifibrinolytic and anti hemolytic activity that can be further investigated to explore the photoactive compounds to formulate as the antidot against the Naja venom.

Conflict of interest: None.

REFERENCES

Akçay, A. (2013). The calculation of LD50 using probit analysis.

Alam, M.I. (2014). Inhibition of toxic effects of viper and cobra venom by Indian medicinal plants. Pharmacology and Pharmacy. 2014.

Alirol, E., Sharma, S.K., Bawaskar, H.S., Kuch, U., Chappuis, F. (2010). Snake bite in South Asia: A review. PLoS Negl Trop Dis 4: e603.

Asad, M.H.H.B., Sabih, D.E., Chaudhory, B.A., Ahmad, I., Hussain, M.S., Izhar, N. and Hussain, I. (2014). Anti-hemolytic property of local medicinal plant (s) upon Pakistani cobra venom induced hemolysis. J. Anim Plant Sci. 24: 1701-8.

Asuzu, I.U. and Harvey, A.L. (2003). The antisnake venom activities of Parkia biglobosa (Mimosaceae) stem bark extract. Toxicon. 42(7): 763-768.

Bittenbinder, M.A., Dobson, J.S., Zdenek, C.N., Brouw, B.O.D., Naude, A., Vonk, F.J. and Fry, B.G. (2019). Differential destructive (non-clotting) fibrinogenolytic activity in Afro-Asian elapid snake venoms and the links to defensive hooding behavior. Toxicology *in vitro*. 60: 330-335.

Choudhary, P.K., Khandelwal, V., Gupta, S., Chaubey, K.K., Singh, S.V., Goel, A. and Bhatia, A.K. (2019). Immunosuppressive and anti-cancer potential of aqueous extract of *Solanum xanthocarpum*. Indian Journal of Traditional Knowledge. 18(3): 451-457.

Gowtham, Y.J., Mahadeswaraswamy, Y.H., Girish, K.S.K.K. (2014). Cross-reactivity and neutralization of Indian King cobra (*Ophiophagus hannah*) venom by polyvalent and monovalent antivenoms. International Immunopharmacology. 21(1): 148-155.

Janardhan, B.H.A.V.Y.A., Shrikanth, V.M., Dhananjaya, B.L. and More, S.S. (2014). Antisnake venom properties of medicinal plants. Int J. Pharm Pharm Sci. 7(S1): 21-6.

Khandelwal, V., Sharma, T., Gupta, S. and Singh, S.V. (2022). Molecular diagnostic strategies for the detection of toxicological changes in animals: A review. Indian Journal of Animal Research. 56(9): 1055-1062.

Kumar, S. and Pandey, A.K. (2014). Medicinal attributes of *Solanum xanthocarpum* fruit consumed by several tribal communities as food: An *in vitro* antioxidant, anticancer and anti HIV perspective. BMC Complementary and Alternative Medicine. 14, 112. https://doi.org/10.1186/1472-6882-14-112.

Kumarapppan, C., Jaswanth, A. and Kumarasunderi, K. (2011). Antihaemolytic and snake venom neutralizing effect of some Indian medicinal plants. Asian Pacific Journal of Tropical Medicine. 4(9): 743-747.

Ozukum, A., Changkija, S. and Tripathi, S.K. (2019). Ethnobotanical studies on the Khiamniungan tribe in Tuensang district of Nagaland, Northeast India: Ethnomedicinal plants. Pleione. 13(1): 70. DOI: 10.26679/Pleione.13.1.2019.070-081.

Parveen, G., Khan, M.F., Ali, H., Ibrahim, T. and Shah, R. (2017). Determination of lethal dose (LD50) of venom of four different poisonous snakes found in Pakistan. Biochem Mol Biol J. 3(3): 18. doi: 10.21767/2471-8084.100046.

Rajesh, K.S., Bharath, B.R., Rao, C.V., Bhat, K.I., Bhat, K.C. and Bhat, P. (2017). Neutralization of Naja naja venom induced lethality, edema and myonecrosis by ethanolic root extract of Coix lacryma-jobi. Toxicology Reports. 4: 637-645.

Slagboom, J., Kool, J., Harrison, R.A. and Casewell, N.R. (2017). Haemotoxic snake venoms: Their functional activity, impact on snakebite victims and pharmaceutical promise. British Journal of Haematology. 177(6): 947-959.

Sood, A., Kumar, B., Singh, S.K., Prashar, P., Gautam, A., Gulati, M. and Porwal, O. (2020). Flavonoids as potential therapeutic agents for the management of diabetic neuropathy. Current Pharmaceutical Design. 26(42): 5468-5487.

Sridevi, M., Chandramohan, G. and Pugalendi, K.V. (2007). Protective effect of Solanum surattense leaf-extract on blood glucose, oxidative stress and hepatic marker enzymes in STZ-diabetic rats. Asian J. Biochem. 1: 117-121.

Srivastava, V., Navabharath, M., Gupta, S., Singh, S.V., Ahmad, S. (2022). Exploration of *Solanum xanthocarpum* schrad. and wendl. against *Mycobacterium avium* subspecies *paratuberculosis* and assessment of its immunomodulatory and anti-inflammatory potential. Pharmaceuticals. 15(11): 1367.

Theakston, R.D.G. and Reid, H.A. (1983) Development of simple standard assay procedures for the characterization of snake venom. Bulletin of the World Health Organization. 61: 949-956.

Theakston, R.D.G., Warrell, D.A. and Griffiths, E. (2003). Report of a WHO workshop on the standardization and control of antivenoms. Toxicon. 41(5): 541-557.

Vadnere, G.P., Gaud, R.S. and Singhai A.K. (2008) Evaluation of Anti-asthmatic property of Solanum xanthocarpum flower extracts. Pharmacologyonline. 2008;1: 513-522.

White, J. (2002). Bites and Stings from Venomous Animals: A Global Overview. Therapeutic Drug Monitoring. 22: 65-68.