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Research Article

INHIBITORY EFFECT OF ETHANOLIC ROOT EXTRACT OF *COIX LACHRYMAJOBI* ON HYALURONIDASE AND L-AMINO ACID OXIDASE OF *NAJA NAJA* AND *DABOIA RUSSELLI* VENOM

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ABSTRACT

Objective: This study was carried out to investigate the efficacy of inhibitory effect of ethanolic root extract (ERE) of plant *Coix lachrymajobi* (Poaceae) on hyaluronidase and L-amino acid oxidase (LAAO) of *Daboia russelli* and *Naja naja* venom.

Methods: The ERE of *C. lachrymajobi* is used to treat snakebite victims by traditional healers as folk medicine for centuries, was tested *in-vitro* to determine its ability to inhibit *Daboia russelli* and *N. naja* hyaluronidase and LAAO activities. *In-vitro* studies were carried out with different doses of ERE.

Results and Discussion: This effectively neutralized hyaluronidase and LAAO activities of both *D. russelli* and *N. naja* venom. ERE at doses of 2000 µg successfully inhibited *D. russelli* (50 µg) hyaluronidase activity by 67.04% and LAAO activity by 77.86%. The hyaluronidase activity of *N. naja* venom (50 µg) was inhibited by 71.83% and LAAO activity was reduced by 74.83%. From the results, it is evident that the ERE of *C. lachrymajobi* effectively neutralizes important toxic enzymes of the *D. russelli* and *N. naja* venoms.

Conclusion: The ERE of *C. lachrymajobi* has a potent inhibitory action against venom enzymes.

Keywords: *Daboia russelii*, *Naja naja*, *Coix lacryma-jobi,* Hyaluronidase, L-amino acid oxidase.

INTRODUCTION

In the year 2009, WHO included snake bite in the list of neglected tropical disease [1]. In India, every year around 50,000 people succumb to snake bite. Number of people who are permanently disabled due to snake bite are many more [2]. Important species of snakes, which are responsible for maximum snake bite cases in India, include *Naja naja, Daboia ruselli*, *Echis carinatus*, and *Bungarus caeruleus* [3].

Most of the snakebite deaths are avoidable with proper medical attention. Mostly, snake bite victims are farmers and the people working in estates who are generally far away from medical facility and the remoteness makes road transport connectivity very difficult. In the rural areas, non-availability of anti-venom, improper storage facility, and non-availability of trained doctors are the major reasons for deaths of snakebite victims [3,4].

Traditional healers of the coastal Karnataka claim the usefulness of the aqueous root extract of *Coix lachrymajobi* (poaceae) in treating snakebite victims. The study was undertaken to evaluate the effectiveness of root extract of *C. lachrymajobi* in inhibiting the two important toxins, i.e. hyaluronidase and L-amino acid oxidase in the venom of *N. naja* and *Daboia russelli*.

L-amino acid oxidase (LAAO) enzyme is highly specific for L-amino acids, and generally hydrophobic amino acids are the best substrates. Recent studies showed that LAAOs are multifunctional enzymes exhibiting edema-inducing, platelet aggregation-inducing or inhibiting, and apoptosis-inducing effects [5].

Proficient distribution of toxins into systemic circulation from the bite site in the victim is very important in envenomation. Fragmentation of hyaluronan in the extracellular matrix (ECM) by snake venom hyaluronidase is a crucial element in this diffusion progression. Studies have established that hyaluronidase not only enhances the lethal potency of the venom but also enhances the injury at the bite site, causing severe morbidity [6].

METHODS

Preparation of extract

Roots of *C. lachrymajobi* were collected from wildly growing plants in Udupi district of Karnataka, India, and were authenticated and voucher specimen was deposited (No: NI-5396 Blatter Herbarium, St. Xavier's College, Mumbai).

To prepare the ethanolic root extract (ERE), the fresh root of the plant *C. lachrymajobi* was washed and shade dried. 1 kg of dried root was ground to powder and was subjected to maceration and cold extraction in ethanol [7]. After 7 days, the ethanolic extract 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 finally evaporated to dryness and stored in a desiccator for future use.

Snake venom

The lyophilized snake venom of *N. naja* and *D. russelli* was procured from Irula Snake Catchers Industrial Co-operative Society, Kanchipuram, Tamil Nadu, India and was stored at 4°C. Before use, the venom was dissolved in saline and dilutions of the required concentrations were prepared using saline.

Hyaluronidase inhibition studies

Hyaluronidase activity of the venom and its neutralization by the root extract was estimated by method of Mahadeswaraswamy *et al.*, [8]. In brief, the venom sample (50 µg) was incubated with 100 µg of hyaluronic acid in 300 µl 0.2 M sodium acetate buffer, pH 5.5 containing 0.15 M NaCl at 37°C for 2 hrs. The reaction was terminated by adding 50 µl of 0.1 M potassium tetraborate and boiled for 3 minutes. 1.5 ml of coloring reagent (*p*-dimethyl aminobenzaldehyde [10%] in acetic acid:hydrochloric acid; 9:1; v/v) was added and incubated

for 30 minutes at 37°C. The absorbance was measured at 585 nm to measure the amount of N-acetyl glucosamine released. For inhibition study, one proportion of venom (50 µg) (*N. naja* and *D. russelli*) was incubated with ERE at 5, 10, and 20 proportions and above procedure was carried out. Drug free solutions (0.9% saline with 0.06% sodium carboxy methyl cellulose as suspending agent) served as control.

LAAO inhibition studies

LAAO activity was determined as described by Dhananjaya *et al*. [9]. In brief, peroxidase (0.05 ml of 0.007% 250; NIH units/mg) was incorporated in 1 ml of 0.2 M triethanolamine buffer, pH 7.6 having 0.1% L-leucine and 0.006% o-dianisidine, later the reaction blend was incubated for 30 minutes at room temperature.

Venom samples (50 µg) (*N. naja* and *D. russelli*) were incubated with reaction mixture for 30 minutes at 37°C and the increase in absorbance at 420 nm was measured. For inhibition studies, venom sample (50 mg) was pre-incubated with different amounts of ERE of *C. lachrymajobi* (5, 10 and 20 times the amount of venom) at 37°C for 30 minutes. Drug free solutions (vehicle only) served as control.

Statistical analysis

The data are expressed as mean±standard error, analyzed by one-way ANOVA followed by Dunnett's test. p≤0.05 was considered as statistically significant.

RESULTS

Hyaluronidase inhibition studies

In the present study, the ERE of *C. lachrymajobi* effectively inhibited hyaluronidase enzyme activity of both *N. naja* and *D. russelli* venom. The ERE at 10 and 20 times the dose of venom, significantly inhibited release of N-acetyle glucosamine by both the venoms. ERE at 5 times the dose of venom inhibited *N. naja* venom hyaluronidase activity significantly (Figs. 1 and 2).

L-amino oxidase inhibition studies

In the present study, ERE could effectively inhibit the LAAO activity of both *N. naja* and *D. russelli* venom (Figs. 3 and 4). At maximum dose of ERE used, i.e., 2000 µg (20 times the dose of venom), the LAAO action of *N. naja* was decreased to 25.16% and *D. russelli* venom LAAO activity was decreased to 22.12%.

DISCUSSION

Venomous snakes professionally capture their prey by immobilizing them through injecting the venom into the prey by striking the prey with the fangs, and quick systemic distribution of venom in the prey [10]. The distribution of toxins inside the body is aided primarily by hyaluronidases enzyme, which degrades the elements of ECM and connective tissue surrounding the blood vessels [11-13]. Hyaluronidases are endoglycosidases, which primarily act on hyaluronan of ECM [12,14].

A study carried out by Mahadeswaraswamy *et al*. [8] reported that methanolic extract of grape seeds (*Vitis vinifera*) significantly inhibited hyaluronidase activity at venom-extract ratio of 1:2. Wahby *et al*. [15] studied different plants for hyaluronidase inhibition property and reported that leaf extract of *Rosmarinus officinalis* showed 100% inhibition of hyaluronidase enzyme of Egyptian horned viper, *Cerastes cerastes* at venom to extract ratio *of* 1:10. The study also reported that *Menthapeprita, Ocimum basilicum* and *Origanum majorana* leaf extract significantly inhibited the hyaluronidase action of the same venom. In the present study, ERE of *C. lachrymajobi* showed significant inhibition of hylauronidase activity of *N. naja* and *D. russelli* venom in dose dependent manner. Even though, there was no 100% inhibition in activity of hylauronidase of both the venoms, percentage inhibition of hyaluronidase activity of *D. russelli* venom was observed to be 67.04% at 2000 µg dose and against *N. naja* venom, percentage inhibition was observed to be slightly better at 71.83% with the same dose of ERE.

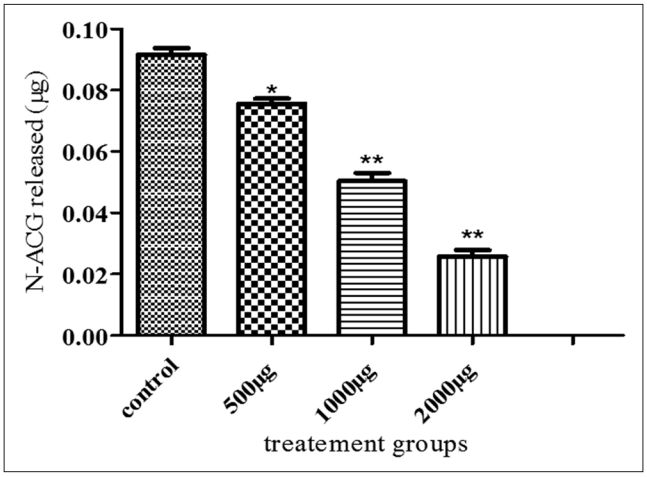

Fig. 1: Effect of ethanolic root extract of *Coix lachrymajobi* on release of N-acetyl glucosamine by the action of *Naja naja* venom hyaluronidase. The values are expressed as mean±standard error of mean, n=6 rats in one group. *p≤0.05 significant, **p≤0.01 highly significant, when compared with control group

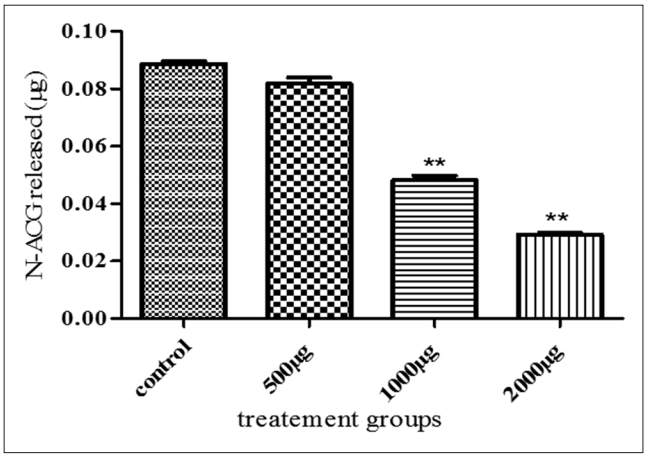

Fig. 2: Effect of ethanolic root extract of *Coix lachrymajobi* on release of N-acetyl glucosamine by the action of *Daboia russelli* venom hyaluronidase. The values are expressed as mean± standard error of mean, n=6 rats in one group. *p≤0.05 significant, **p≤0.01 highly significant, when compared with control group

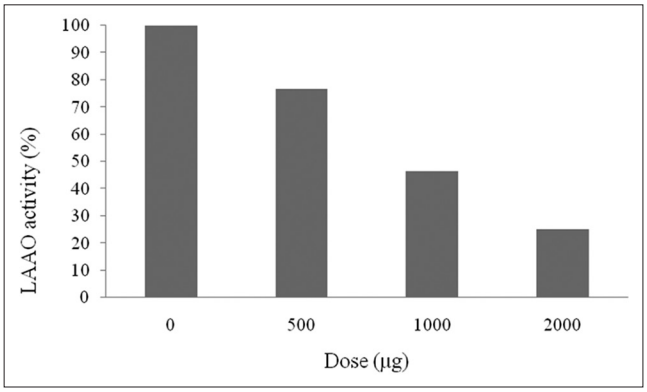

Fig. 3: Effect of ethanolic root extract of *Coix lachrymajobi* on L-amino acid oxidase activity of *Naja naja* venom. The values are expressed as % L-amino acid oxidase activity n=6

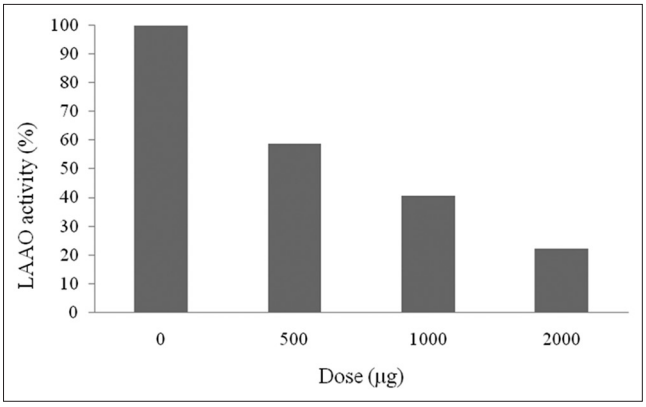

Fig. 4: Effect of ethanolic root extract of *Coix lachrymajobi* on L-amino acid oxidase activity of *Daboia russelli* venom. The values are expressed as % L-amino acid oxidase activity n=6

LAAO distributed extensively in nature, and snake venoms being the largest sources [5]. Snake venom LAAOs are very dynamic enzymes which are widely used in formulation of α-keto acids for their chemo- and stereo specificity [16,17]. α-Keto acids of essential amino acids are useful nutraceuticals as well as therapeutic agents for certain diseases. Snake venom LAAO attracts new researches in the field of biomedical studies because of its antimicrobial, anti-HIV, anticoagulant, platelet aggregation-inducing and -inhibiting, apoptosis-inducing, anticancer activities etc. [5].

In a previously published work [18], authors claimed that glycyrrhizin isolated from *Glycyrrhiza glabra* showed protection against LAAO isolated from Habu snake. 100 µg of glycyrrhizin was pre-incubated with 4 µg LAAO, which reduced the LAAO activity to 30% of the untreated group. The study also reported glycyrrhetinic acid did not have any effect on Habu snake venom LAAO activity. In the present study, ERE of *C. lachrymajobi* showed significant inhibition of LAAO activity of *N. naja* and *Daboia russelli* venom in dose dependent manner. LAAO activity of *D. russelli* was inhibited by 77.86% which was slightly better than *N. naja* LAAO inhibition, which was 74.83%.

CONCLUSION

In the background of the above discussion, it can be concluded that the ERE of *C. lachrymajobi* has the ability to inhibit the toxic enzymes present in the *D. russelli* and *N. naja* venoms. Advanced research need to be carried out to identify the chemical components responsible for these inhibitory effect by molecular docking studies.

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