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EFFECT OF TURMERIC (*CURCUMA LONGA*) PROTEINS AGAINST RUSSEL VIPER PHOSPHOLIPASE A2

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ABSTRACT

Specific plants have demonstrated efficacy in experimental studies. *Andrographis paniculata* inhibits phospholipase A2 activity, reducing inflammation. *Azadirachta indica* (Neem) neutralizes proteolytic and hemolytic effects, while *Curcuma longa* (Turmeric) and its active compound, curcumin, exhibit antioxidant, anti-inflammatory, and enzyme-inhibiting properties. Additionally, curcumin has been reported to promote cellular repair and reduce venom-induced cytotoxicity. This study investigates the ability of crude turmeric protein (5 to 35ug) to inhibit or neutralize the venom of *Daboia russelii* (Russell's viper), a highly venomous snake in Asia, which contains destructive enzymes such as phospholipases and hyaluronidases. Exploring plant-based solutions like turmeric offers a potential adjunct or alternative to conventional antivenoms, addressing the need for effective and accessible treatments for snakebite envenomation.

KEYWORDS: Phospholipase A2; Turmeric; Proteins; Russell viper.

INTRODUCTION

The potential of plant extracts to nullify snake venom is an interesting crossroad between traditional medicine and modern science (Vallejo et al., 2024). For centuries, many indigenous systems of medicine have used plants for snakebite remedies. With the recent scientific studies, its efficacy in countering snake venoms or at least easing the effects of snakebites has been studied (Gopal et al., 2023).

The mechanism of neutralization by plant extracts may be achieved through enzyme inhibition of snake venom, which contains enzymes like phospholipases, proteases, and hyaluronidases (Aldriao et al., 2022). Flavonoids, tannins, and alkaloids in plants inhibit these enzymes, thus decreasing the toxicity of venom. Free radicals and toxic components of venom are neutralized by polyphenols and other antioxidant compounds in plants. Some plant extracts inhibit inflammation, hemorrhage, and necrosis due to venom. Some plant substances act as venom toxins receptor antagonists, thereby inhibiting their activity (Costa et al., 2021).

Some experimental studies have shown that a number of plants possess marked antivenom properties. The plant *Andrographis paniculate* possesses phytochemicals which inhibit phospholipase A2 activity and reduce inflammation (Nayak et al., 2020). The plant *Azadirachta indica* (Neem) possesses antivenom activity as it neutralizes proteolytic and hemolytic effects (Ajjsebiola et al., 2021). *Curcuma longa* (Turmeric) contains Curcumin, the active compound in turmeric, exhibits antioxidant and anti-inflammatory properties, counteracting venom effects (Rathore et al., 2020). The plant *Mimosa pudica* that was traditionally used to treat snake bites reduces both local tissue damage as well as systemic toxicity (MS et al, 2021).

Turmeric is a medicinal spice, and its antivenom properties have been explored mostly because of curcumin's anti-inflammatory, antioxidant, and anti-toxin activity (Majeed et al., 2021). It is also an anti-inflammatory. Snake venom causes severe inflammation in many cases. Curcumin inhibits pro-inflammatory cytokines and enzymes such as COX-2, thereby minimizing the damage caused by the venom (Adinortey, 2021). Snake venom creates oxidative stress, which causes tissue damage. Curcumin is free radical scavenger and, therefore, protects the cells. Studies indicate curcumin is able to suppress venom enzymes, such as phospholipase A2, metalloproteinases, and hyaluronidases that cause localized tissue destruction and systemic toxicity (Cui et al., 2024). The curcuma extracts can thus improve cellular repair and reduce cytotoxic venom effects. A particular 14kDa peptide in turmeric

was found to inhibit the activity of the phospholipase A2 enzyme in the venom of cobra, *Naja naja* (Chethankumar et al., 2010).

This study focuses on the neutralization of *Daboia russelii*, one of the venoms of Asia's most fatal snake, by crude protein in turmeric. Russell's viper venom is quite a complex mixture of enzymes and toxins. Main phospholipases are destructive enough to muscle and nerve cells. Hyaluronidases allow spreading of the toxin in the body by degrading other materials between cells. Other compounds such as polypeptide toxins, glycoproteins, and low-molecular-weight compounds contribute to the overall effects of venom, some of which are unknown (Tan et al., 2015). The understanding of these mechanisms and exploring plant-based inhibitors like turmeric hold promising avenues for the development of effective antivenom therapies.

MATERIALS AND METHODS

Source of venom

Venom was procured from Hindustan Park, Calcutta. The venom samples were dissolved in 0.9% saline, and all the chemicals and reagents employed during the study were of analytical grade. Furthermore, all chemicals were redistilled before use.

Caseolytic activity

Protease activity was determined according to the method of Murata et al. (Murata et al, 1963; Shivalingu et al., 2015) by using casein as a substrate. A reaction mixture with a final volume of 1 mL contained 0.4 mL of casein (2% in 0.2 M Tris-HCl buffer, pH 8.5) and venom, incubated at 37°C for 2 hours and 30 minutes. The reaction was stopped by adding 1.5 mL of 0.44 M TCA and left to incubate for 30 minutes to precipitate undigested casein. The mixture was centrifuged at 1500×g for 15 minutes and 1 mL of the supernatant was taken. The supernatant was mixed with 2.5 mL of 0.4 M sodium carbonate and 0.5 mL of Folin reagent (1:2 v/v). The intensity of the colour was read at 660 nm. One unit of enzyme activity was defined as the amount of enzyme required to increase the absorbance by 0.01 at 660 nm at 37°C. Inhibition studies were conducted using 0.02 M EDTA, EGTA, and PMSF. A constant amount of venom was pre-incubated with each inhibitor for 10 minutes before assessing protease activity.

Clotting assay

Recalcification time

Recalcification time was a study in which fresh human blood was mixed with 0.11 M trisodium citrate in a 9:1 (v/v) ratio, and the sample was centrifuged at 1500 rpm for 15 minutes at 4°C. The supernatant obtained was platelet-poor plasma (PPP) that was used for the clotting assay. A 200 µl aliquot of the sample was mixed with Tris buffer (pH 7.4), and the reaction mixture was incubated for 15 minutes. After that, 20 µl of 0.25 M $CaCl_2$ was added to the test sample, and the clotting time in seconds was noted. The control used was a solution of 0.25 M $CaCl_2$. (Rosenberg et al., 1983; Rajesh et al., 2005) Various concentrations of venom samples from 0.5 µg to 25 µg were tested. Time for clotting of plasma in seconds.

Fibrinogenolytic assay

In the study of fibrinogenolytic activity the reaction mixture of 40 µl was composed of 50 µg of bovine fibrinogen and 10 mM Tris-HCl buffer at a pH of 7.4, coagulation buffer. This reaction mixture was then incubated with 20 µg of venom samples in 0.9% saline solution for one hour at 37°C. Dose-dependent, time-dependent, and inhibition studies were performed. The reaction was terminated by the addition of 40 µl denaturing buffer containing 1 M urea and 4% β-mercaptoethanol. Inhibition experiments were carried out with PMSF, EDTA, and EGTA. The hydrolysed products were analyzed on 10% SDS-PAGE as reported by Laemmli, 1990. The gels were stained with Coomassie Brilliant Blue R-250 (Ouyang and Teng, 1976; Hamed et al., 2020).

Isolation crude protein from Turmeric

10gm of turmeric powder is taken in 100ml of boiling distilled water and kept in shaker about 2hr after which it is centrifuged at 10000 rpm for 20min at 4^0C. The supernatant stored at refrigerator and transferred into cold acetone. The protein content of turmeric extract precipitated. The precipitated protein pellets separated and stored for further studies.

Inhibition of protease activity

The protease activity was determined using casein as the substrate. The assay was performed by incubating 0.4 ml of 2% casein prepared in 0.2 M Tris-HCl buffer (pH 8.5) with venom in a total reaction volume of 1 ml containing 0.2 M Tris-HCl buffer (pH 8.5) at 37°C for 2 hours and 30 minutes. After incubation, the reaction was stopped by the addition of 1.5 ml of 0.44 M TCA and letting the mixture stand for 30 minutes to precipitate undigested casein. The

mixture was then centrifuged at 1500 × g for 15 minutes. An aliquot of 1 ml of the supernatant was mixed with 2.5 ml of 0.4 M sodium carbonate and 0.5 ml of Folin reagent (1:2 v/v). The absorbance was measured at 660 nm. One unit of enzyme activity was defined as the amount of enzyme required to increase the absorbance by 0.01 at 660 nm at 37°C (Ebata and Murata, 1961; Iriki et al., 2023). For inhibition studies, 100 µg of venom was preincubated with or without varying concentrations of extract (5–40 µg) at 37°C for 15 minutes, followed by the assay as described above. The percentage inhibition was calculated by considering the activity of venom alone as 100%.

RESULTS AND DISCUSSION

The main pharmacological activities of Russell viper venom involve hemorrhage, necrosis of cells, and disorder of hemostasis, that is essentially due to the activities of the proteases in the venom. Caseinolytic activity was used for proof that the venom exhibits casein hydrolyzing activity, and inhibition study was further used to identify different protease types in the venom. PMSF proved to be more inhibitory than EDTA, but EGTA was found to be ineffective, thus implying that metalloproteases as well as serine proteases existed.

The plasma clotting time is around 5 minutes, but the rate varies from one person to another. The coagulation of blood takes place both through intrinsic and extrinsic pathways, which eventually come into a common cascade by the activation of Factor X, finally forming the fibrin clot. The intrinsic pathway's role can be explored using the recalcification time. Proteases that affect coagulation act on either pathway (White, 2005). Recalcification time was determined to evaluate the effect of eastern Russell viper proteases (Thakur et al., 2022). The venom significantly shortened clot formation time from 253 seconds to 42 seconds. This shows that the venom protease has procoagulant activity since it hastens clot formation.

The venom proteases hydrolyze fibrinogen to produce fibrin, depleting clotting factors, resulting in hemorrhage. In this study, the researchers have been able to establish that the hydrolysis of fibrinogen by Russell viper venom is dose-dependent. When 20 µg of venom protease is incubated with fibrinogen, the Aα chain of the latter, which is a nonglycoprotein, gets selectively degraded; it further demonstrates time-dependent hydrolysis at various time points, indicating the sequence of hydrolysis of Aα chain first and Bβ subunit subsequently.

Turmeric is a spice widely used in India for culinary and medicinal purposes, which has been known to promote wound healing both internally and externally among other uses. The utility

of this has led to the discovery of a water-soluble peptide in turmeric. Proteases are major agents that cause hemorrhage, bleeding, and procoagulation. The crude proteins isolated from turmeric inhibited venom protease activity in a dose-dependent manner (5–35 µg). Inferences from the study conclude that crude proteins from turmeric effectively neutralize the protease activity of eastern Russell viper venom.

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