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Research Article

PRELIMINARY PHTOCEMICAL INVESTIGATION AND ANTI-VENOM ACTIVITY OF *COIX LACRYMAJOBI* ROOT EXTRACT AGAINST *DABOIA RUSSELLI* VENOM-INDUCED MYONECROSIS

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ABSTRACT

Objective: The aim of the study was to carry out the preliminary phytochemical investigation and to evaluate the inhibition of *Daboia russelli* venom-induced myonecrosis by root extract (RE) of *Coix lacrymajobi.*

Methods: The roots of *C. lacrymajobi* were subjected to differential extraction by soxhlet extraction using petroleum ether, chloroform, ethyl acetate, and ethanol. The resultant extracts were subjected to the preliminary phytochemical investigation to identify the different chemical groups present in the extracts. Myonecrotic activity was conducted, to assess the ability of ethanolic RE to inhibit the myonecrosis induced by *D. russelli* venom in rats.

Results: The preliminary phytochemical investigation revealed the presence of triterpenoids, resins, steroids and fixed oils in petroleum ether extract, flavonoids, triterpenoids, saponins and fixed oils in chloroform extract and alkaloids, carbohydrates, flavonoids, glycosides, resins, saponins, steroids, and tannins. Ethanolic extract was found to have maximum number of phytochemicals, and hence, it was used for further study. The ethanolic RE significantly inhibited the myonecrotic activity at dose level 200 and 400 mg/kg body weight.

Conclusion: The screening of phytochemicals presents on the different fractions of the RE was studied successfully. Supporting the use of roots by traditional healers, ethanolic extract successfully inhibited *D. russelli* venom-induced myonecrosis in rats.

Keywords: *Coix lacrymajobi, Daboia russelli,* Myonecrosis.

INTRODUCTION

Coix lacrymajobi is a tropical plant also known as Job's tear belongs to the family Poaceae is a tall, grain-bearing grass [1]. Traditional healers of coastal Karnataka have been using the root of this plant to treat snake-bite victims with great success.

Snake-bite, being a great professional hazard in the tropical countries, such as India, is cause of around 35-50 thousand mortalities annually and many more morbidity [2]. Snake-bite was included in the WHO's list of neglected tropical diseases in the year 2009 [3]. The best-known treatment for a snake-bite is the immediate administration of polyvalent anti-venom [4]. The polyvalent anti-venom faces a supply shortage and also needs specific storage conditions, which are hard to maintain in rural areas [4].

However, anti-venom therapy has several adverse effects on various organs of the human body, because of the administration of foreign proteins, sensitization to horse serum, and the presence of immune complex [5].

The risk associated with anti-venom therapy is acute anaphylaxis or anaphylactic reaction (mild to severe), which occurs within 1 hr of anti-venom administration [5], serum sickness, which occurs between 5 and 24 days after anti-venom therapy [6]. Furthermore, the conventional anti-snake venoms have not always been able to resolve the local effects of the venom [5,6] such as necrosis, hemorrhage, local swelling, and bacterial infections.

For this reason, scientists are in search for safe and effective alternative treatment for snake bite. In the present study, the root extract (RE) of *C. lacrymajobi* was subjected to different phytochemical investigation and ethanolic RE was tested for inhibition of myonecrosis induced by *D. russelli* venom.

METHODS

The roots of *C. lacrymajobi* were collected from wildly grown plants in Udupi district, Karnataka, India. The plant was authenticated, and a voucher specimen was deposited (No: NI-5396, Blatter Herbarium, St. Xavier's college, Mumbai).

The shade dried roots were then grounded into coarse powder. This powder was subjected to soxhlet extraction [7] using different solvents (petroleum ether, chloroform, ethyl acetate, and ethanol).

Preliminary phytochemical investigation of the different extract was carried out by previously explained methods [7-9]. Extracts were tested for alkaloids, carbohydrates, flavonoids, glycosides, triterpenoids, resins, saponins, steroids, tannins, and fixed oils.

Ethanolic extract yielded maximum number of phytochemicals; hence, this extract was used for pharmacological investigation by inhibition of myonecrosis induced by *D. russelli* venom.

Myonecrosis induced by *D. russelli* venom and its neutralization by the ethanolic RE was studied in rats as per previously described method [10]. The study was approved by the Institutional Ethics Committee for animal experimentation KSHEMA, Deralakatte, Mangalore (KSHEMA/AEC/38/2011). Animals were divided into five groups, each group containing six Wistar albino rats. The control group was injected with 5 µg of venom (intramuscular, i.m.) followed by per oral (p.o.) administration of vehicle (saline). The second group was administered with standard drug (polyvalent anti-venom) by

intraperitoneal (i.p.) route after venom injection. Groups 3, 4, and 5 were administered with 100, 200, and 400 mg/kg of ethanolic RE p.o., respectively, followed by i.m. injection of venom.

Statistical analysis

The results are expressed as mean ± standard error. Analyzed by one-way ANOVA followed by Dunnett's test using GraphPad Prism 5. p≤0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Soxhlet extraction of the dried, powdered root of *C. lacrymajobi* yielded 4% petroleum ether extract, 3.5% chloroform extract, 0.2% ethyl acetate extract, and 5.5% of ethanolic extract. Ehtyl acetate extract was not used for further studies, due to low yield.

The preliminary phytochemical studies were performed for testing different chemical groups present in the different RE *C. lacrymajobi*. The observations are shown in Table 1.

In this study, it was noted that the ethanolic extract of the root contains maximum number of phytochemicals when compared to petroleum ether and chloroform extracts. The results of phytochemical tests revealed the presence of triterpenoids, resins steroids, and fixed oils in the petroleum ether extract. Chloroform extract was tested positive for flavonoid, saponins, and fixed oils. The ethanolic extract of the root contains alkaloids, carbohydrate, flavonoids, glycosides, resins, saponins, steroids, and tannins, whereas ethanolic extract was tested negative for triterpenoids and fixed oils.

Myonecrosis caused by intramuscular injection of *D. russelli* venom (5 µg), assessed in terms of creatine kinase (CK) activity was significantly inhibited by the ethanolic RE at dose level 200 and 400 mg/kg, administered orally (Fig. 1).

The presence of alkaloids, steroids, tannins, glycosides, and triterpenoids and the inhibition of myonecrosis support the use of the RE by the traditional healers. Myotoxicity was evaluated by measuring the serum CK level. Intramuscular injection of *D. russelli* venom (5 µg) resulted in 3 fold increase in the serum CK level at the end of 4 hrs. Standard polyvalent anti-venom did not show any significant inhibitory action against venom-induced myonecrosis, which is an important parameter in treating snake-bite patients. Ethanolic RE of *C. lacrymajobi* at dose level 200 and 400 mg/kg significantly inhibited CK level in the serum. Previous studies have reported a flavonoid from *P. denticulate* [11], alkaloids from *A. indica* and *Dendoaspis* spp. [12], steroids for *Pluchea indica* and *E. prostrata* [13,14], tannins from *Dyospirus kaki* and *Casearia sylvestris* [15], which have shown strong inhibitory action against snake venom toxins.

CONCLUSION

The above observations suggest that the RE of *C. lacrymajobi* has potential phytochemicals, which can be used alone or in combination with polyvalent anti-venom in treating snake-bite victims. This can reduce the dose of the anti-venom, which when used alone has many unwanted effects. The phytochemicals also have the potential to

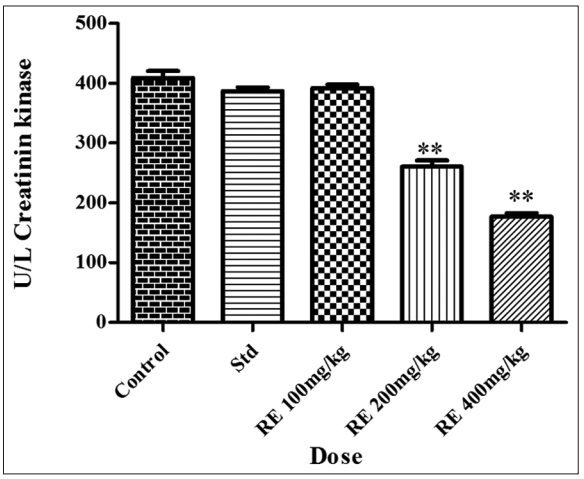

Fig. 1: Effect of *Coix lacrymajobi.* Ethanolic root extract (RE) on *Daboia russelli* venom-induced myotoxicity. The values are expressed as mean±standard error of mean, n=6 rats in one group. *p≤0.05 significant, **p£0.01 highly significant, when compared with control group. Note: Control: i.m. venom (5 µg) + vehicle(p.o), Std: i.m. venom+standard polyvalent anti-venom (i.p.), RE 100 mg/kg: i.m. venom+ethanolic root extract 100 mg/kg, RE 200 mg/kg: i.m. venom+ethanolic RE 200 mg/kg, RE 400 mg/kg: i.m. venom+ethanolic RE 400 mg/kg

Table 1: Phytochemical screening of different root extracts of *C. lacrymajobi*

S. No	Test for organic compound	Pet. Eth. extract	Chloroform extract	Ethanol extract
1	Alkaloids			
	Dragendorff's test	-	-	+
	Hager's test	-	-	+
	Wagner's test	-	-	-
	Mayer's test	-	-	+
2	Carbohydrates			
	Anthrone test	-	-	+
	Benedict's test	-	-	+
	Fehling's test	-	-	+
	Molisch's test	-	-	+
3	Flavanoids			
	Shinoda's test	-	+	+
4	Glycosides			
	Molisch's test	-	-	+
5	Triterpenoids			
	Liebermann - Burchard's test	+	-	-
6	Resins	+	-	+
7	Saponins	-	+	+
8	Steroids			
	Liebermann - Burchard's test	-	-	+
	Salkowski reaction	+	-	+
9	Tannins	-	-	+
10	Fixed oils	+	+	-

C. lacrymajobi: Coix lacrymajobi

neutralize the local effects of the venom, as observed in the above study. There is a great scope for further study on this RE. Isolation, purification, and identification of the active chemical constituent have to be done.

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REFERENCES

1. Duke JA. Coix_lacryma-jobi; 1983. Available from: https://www.hort.purdue.edu/newcrop/duke_energy/Coix_lacryma-jobi.html.
2. Whitaker R, Whitaker S. Venom, antivenom production and the medically important snakes of India. Curr Sci 2012;103(6):635-43.
3. World Health Organization. WHO Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins. Geneva: World Health Organization; 2010.
4. Warrell DA. Guidelines for the Clinical Management of Snake-bites in the South-east Asia Region. New Delhi: World Health Organization, Regional Office for South East Asia; 2005. p. 1-77.
5. Warrell DA. WHO/SEARO Guidelines for the clinical management of snake bites in the Southeast Asian region. Southeast Asian J Trop Med Public Health 2010;1:61-4.
6. Kalyan B, Nanda SS, Venkateshwarlu P, Kiran Y, Jadhav RT. Antisnake venom serum (Asvs). Int J Pharm Biomed Res 2010;1:76-89.
7. Evans WC. Trease and Evans Pharmacognosy. 15th ed. Philadelphia: Elsevier Sceince Ltd.; 2002. p. 414-5.
8. Kokate CK, Purohit PA, Gokhale SB. Pharmacognosy. Pune: Nirali Prakashan; 2010. p. 30-3.
9. Khandelwals KR. Practical Pharmacognosy-techniques and Experiments. Pune: Nirali Prakashan; 1996. p. 98-103.
10. Dhananjaya BL, Zameer F, Girish KS, D'Souza CJ. Anti-venom potential of aqueous extract of stem bark of *Mangifera indica* L. against *Daboia russellii* (Russell's viper) venom. Indian J Biochem Biophys 2011;48(3):175-83.
11. Mors WB, Nascimento MC, Pereira BM, Pereira NA. Plant natural products active against snake bite – The molecular approach. Phytochemistry 2000;55(6):627-42.
12. Mukherjee AK, Doley R, Saikia D. Isolation of a snake venom phospholipase A2 (PLA2) inhibitor (AIPLAI) from leaves of *Azadirachta indica* (Neem): Mechanism of PLA2 inhibition by AIPLAI *in vitro* condition. Toxicon 2008;51(8):1548-53.
13. Gomes A, Saha A, Chatterjee I, Chakravarty AK. Viper and cobra venom neutralization by beta-sitosterol and stigmasterol isolated from the root extract of *Pluchea indica* Less. (Asteraceae). Phytomedicine 2007;14(9):637-43.
14. Mors WB, do Nascimento MC, Parente JP, da Silva MH, Melo PA, Suarez-Kurtz G. Neutralization of lethal and myotoxic activities of South American rattlesnake venom by extracts and constituents of the plant *Eclipta prostrata* (*Asteraceae*). Toxicon 1989;27(9):1003-9.
15. Da Silva SL, Calgarotto AK, Chaar JS, Marangoni S. Isolation and characterization of ellagic acid derivatives isolated from *Casearia sylvestris* SW aqueous extract with anti-PLA(2) activity. Toxicon 2008;52(6):655-66.