Vol 4|Issue 3| 2014 | 145-147.

e-ISSN 2248 - 9169
Print ISSN 2248 - 9150

International Journal of
Experimental Pharmacology

www.ijepjournal.com

ANTI-SNAKE VENOM ACTIVITY OF ROOT AND RHIZOME OF *CORALLOCARPUS EPIGAEUS*

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ABSTRACT

Invivo and *invitro* antisnake venom activity of the aqueous and alcoholic extract of root and rhizome of *Corallocarpus epigaeus* was studied through inhibition of *invitro* HRBC lysis and using albino mice in modifying the lethal effect of the test dose of Russell's viper venom. The effectiveness of these two extracts was evaluated by i.p administration at a dose level five minutes after administration of the snake venom. The LD_{50} of Russell's viper venom was found to be 8µg/18g bodyweight. Both the methanolic and aqueous extract of root and rhizome of *Corallocarpus epigaeus* markedly decreased the percentage mortality in venom induced toxicity in mice at dose level 500mg/kg bodyweight. The methanolic and aqueous extract of root and rhizome of *Corallocarpus epigaeus* is endowed with significant antisnake venom activity there by justifying its use in the indigenous system of the medicine.

Keywords: Anti-snake venom activity, *Corallocarpus epigaeus*, Aqueous and alcoholic extract, Root and rhizome.

INTRODUCTION

The *Corallocarpus epigaeus* belonging to family *Cucurbitacea* is claimed to possess Antisnake venom, Analgesic, Antihelminthic properties and in treatment of dysentery [1,2]. Most of the species belonging to the genus were having therapeutic value.

Of about 216 species of snakes in India, the Russell's viper is one of the most common poisonous snakes. Though administration of an antisnake venom serum is the only remedy available today, this itself possesses a risk, as it is associated with manifestation of severe allergic reactions. Literature review reveals that the antisnake venom activity of root and rhizome of *Corallocarpus epigaeus* has not been reported and hence in the present study was conducted to investigate *invitro* and *invivo* effectiveness of root and rhizome of *Corallocarpus epigaeus* as an antidote for snake poison.

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MATERIALS AND METHODS

Plant Material

The root and rhizome of *Corallocarpus epigaeus* were collected from Vellore district during month of December 2005 and identified, authenticated by the botanist Prof.P.Jayaraman, Ph.D., Director Medicinal Plant Research Unit, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai.

Preparation of Extracts

The root and rhizome were dried in shade, powdered and extracted with methanol and water by soxhlet extraction [3] to yield the respective extracts. The extracts were vacuum dried and the extractive values are calculated as 2.704% and 20.776% respectively.

Snake venom

The lyophilized venom of the snake Russell's viper was collected from Irula snake catcher's Industrial co-operative society Ltd, Vadanemeli village, Perur post, East Coast Road, Kancheepuram Dist and preserves at 2°C, until further use. The snake venom was dissolved in 0.9 %(w/v)

saline, centrifuged and the supernatant was used whenever required. The venom concentration was expressed on terms of dry weight (mg/ml).

***Invitro* Anti-snake Venom Activity**

Antivenom activity of *Corallocarpus epigaeus* was assessed through inhibition of *invitro* HRBC lysis. In this study in vitro HRBC membrane stabilization activity of methanolic and aqueous extract of *Corallocarpus epigaeus* was studied. The hyposaline –induced haemolysis was evaluated *invitro* by the method of Roelofsen *et al* (1971) [4] and Balu *et al* (1995) [5], this method was modified in present study by venom induced heamolysis. Blood was collected from healthy human volunteers by vein puncture and heparin was used as anticoagulant. The collected blood was washed three times with physiological saline. 1% HRBC was used for the study. Lyophilized venom of Russell's viper was dissolved in physiological saline solution to make a stock solution of 100µg/ml.

Then 1ml of diluted venom, phosphate buffer (PH 7.4) and 1% HRBC were taken in each of labelled various tubes. To the above labelled tubes, different concentrations of methanolic and aqueous extracts of Corallocarpus epigaeus were added. Drug solutions were prepared by using physiological saline. The control samples were the drug free saline solution.

The mixtures were then incubated at 37^{o}C for 30 mins and centrifuged at 1000rpm for 3 min. The supernatant solution was collected and its absorbance was measured at 540 nm using spectrophotometer.

The percentage inhibition of heamolysis was calculated according to the equation

$$\text{Percentage inhibition} = \frac{100 - (A_1 - A_2)}{A_3 - A_4} \times 100$$

Where

A_1= Absorbance of test drug in venom solution
A_2= Absorbance of test drug in physiological saline
A_3= Absorbance of control in venom solution
A_4= Absorbance of control in physiological saline

Pharmacological Study

Acute Toxicity Studies

Animals were fed with increasing doses of methanolic and aqueous extracts of roots and rhizome of *Corallacarpus epigaeus* suspended in 1% Acacia. The animals were observed continuously for 2 hours for the gross behavioral changes and then intermittently once every 2 hours and finally at the end of 24 and 72 hours to note any other toxic signs including death.

***Invivo* Anti-snake Venom Activity**

For the determination of the LD50 of venom four groups of swiss albino mice comprising of six in each were used. In acute toxicity studies, the aqueous and methanolic extract of *Corallocarpus epigaeus* was found to be safe up to a dose level of 3000mg/kg body weight.

The venom was dissolved in 0.9% saline before use and administered by i.p route. The animals were monitored closely for 2 hrs and thereafter for a period of 24 hrs. The effectiveness of the methanolic and aqueous extract in modifying the lethal effect of the test dose of venom was investigated by i.p administration of the extracts at a dose level of 500mg/kg bodyweight five minutes after the administration of snake venom. Experiment was performed in duplicate and values are the mean of two such determinations.

RESULTS AND DISCUSSION

***Invitro* Anti-snake Venom Activity**

Results on effects of methanolic and aqueous extracts of root and rhizome of *Corallocarpus epigaeus* at concentration ranging from 25–500mg/ml in *Invitro* HRBC membrane stabilization properties inhibits haemolysis induced by Russell's viper venom (Table1). It accelerated the process of haemolysis at the given concentration to a great extent. Most of the snake venom contains phospholipase and haemolysin which will act on membrane associated phospholipids liberating lysolecithin. Lysolecithin acts on the membrane of HRBC causing haemolysis [6]. Protection against venom induced haemolysis is thought to be caused by stabilization of proteins in the membrane of HRBC. (Table-1).

Table 1. *In vitro* anti-snake venom activity of methanolic and aqueous extract of *Corallocarpus epigaeus*

Concentration of methanolic extract (µg/ml)	Percentage inhibition of heamolysis (%)	Concentration of aqueous extract (µg/ml)	Percentage inhibition of heamolysis (%)
25	10.32	25	6.5
50	15.78	50	9.83
100	21.80	100	14.75
200	25.56	200	18.85
400	33.83	400	23.77
500	39.09	500	27.04

Table 2. *In vivo* anti-snake venom activity of methanolic and aqueous extracts of *Corallocarpus epigaeus*

Treatment	Dose mg/kg	Envenomation	
		No.of Survival out of 6	% of Survival
Vehicle + venom	-	0	0
Methanolic extract	500 mg/Kg	4	66.66%
Aqueous extract	500mg/Kg	3	50%

***Invivo* anti-snake venom activity**

Antisnake venom activity of the methanolic and aqueous extract of *Corallocarpus epigaeus* was studied at a dose level of 500mg/kg. Mortality of the mice reduced progressively. The administration of methanolic and aqueous extract at dose level of 500mg/kg protects the mice against the lethal effect of Russell's viper venom [6]. Significant protection ($P<0.05$) was observed at 500mg/kg dose. The percentage survival was observed as 66.66% and 50.0% at dose level of 500mg/kg for methanolic and aqueous extracts respectively (Table-2).

Invivo antisnake venom studies of the methanolic extract of *Corallocarpus epigaeus* reveals significant antisnake venom activity and could have a promising role in the treatment of Russell's viper snake bite. The exact mechanism of antisnake venom and isolation of active constituents has to be evaluated.

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