International Journal of Phytomedicine 2 (2010) 217-220

http://www.arjournals.org/ijop.html

Research Article

ISSN: 0975-0185

In vitro and *in vivo* anti-snake venom (*Daboia russelli*) studies on various leaf extracts of *Acalypha indica* Linn.

K. Rajendran[1]*, Annie Shirwaikar[2], Maneesh Mehta[2], R. Vijaya Bharathi[3]

***Corresponding author:**

K. Rajendran

1. Department of Pharmacognosy, College of Pharmcay, 7th Al- Jabal Al-Gharbi University, Al-Zawia, Libya
email address: rajejesus@gmail.com

2. Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, India.

3. Department of Pharmacognosy, Madras Medical College, Chennai, Tamil Nadu, India.

Abstract

The study aims to examine the *Daboia russelli* venom neutralization potential of the various leaf extracts of *Acalypha indica* (Euphorbiaceae) by *in vitro* and *in vivo* antisnake venom studies. *In vitro* HRBC membrane stabilization properties of these extracts at concentrations ranging from 2-400 µg/ml revealed inhibition of haemolysis induced by Russell's viper venom, in a concentration dependent manner. The neutralizing potency of different leaf extracts *viz*., petroleum ether, benzene, chloroform and acetone extracts of *Acalypha indica* prepared by successive solvent extraction at intraperitoneal dose levels of 250, 500 and 750 mg/kg revealed that the acetone extract possessed the most significant activity on venom-induced lethality.

Keywords: *Acalypha indica; Daboia russelli;* anti-snake venom; mice.

Introduction

Acalypha indica Linn. (Family: Euphorbiaceae), known in Telugu as Kuppichettu or Harita Manjiri, in Tamil as Kuppimeni and in Kannada as Kuppigida, is a small annual shrub which generally occurs as a troublesome weed in gardens, roadsides and throughout the plains of India [1]. The plant is used as an antidote [2] for snakebite in the Hardoi District of Northern India [3], by the Gonda tribes of Tamil Nadu and by the Nakkala tribe of Chittor and in the East Godavari Districts of Andhra Pradesh [4]. We had previously reported on the neutralization potential of *Daboia russelli* (Russell's viper) venom by the ethanol leaf extract of *Acalypha indica* [5]. As an extension of our previous report an attempt was made to localize and further define the earlier reported activity. In the present work, we report neutralization potential of different leaf extracts i.e. petroleum ether, benzene, chloroform and acetone extracts of *A. indica* by *in vitro* and *in vivo* antisnake venom studies.

Materials and Methods

Venom

The lyophilised snake venom of *Daboia russelli* was obtained from the Irula Snake Catcher's I.C.S. Ltd., Vadanemmeli Village, Kancheepuram Dist., Tamil Nadu, India and was preserved at 4ºC. Before use the venom was dissolved in saline and centrifuged at 2000 rpm for 10 min. The supernatant was used for anti-venom studies. Venom concentration was expressed in terms of dry weight (µg/ml).

doi:10.5138/ijpm.2010.0975.0185.02032

Plant material

The leaves of *A. indica* were collected from Erode District, Tamil Nadu, India, in the month of September 2006 and authenticated by botanist Dr. Gopalakrishna Bhat, Professor of Botany, Poorna Prajna College, Udupi, India. A herbarium specimen bearing voucher No. PP. 523 has been deposited in the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, India.

Animals

Healthy Swiss albino mice weighing about 20-25 g between 2-3 months of age were used for the study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light and 12 h dark cycle; 25± 30 ^{o}C; 35 - 60% humidity), the animals were fed with standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The study was approved by the Institutional Animal Ethical Committee of KMC, Manipal, India (IAEC/KMC/03/2005 - 2006).

Preparation of different extracts

The shade dried coarsely powdered leaves (1kg) of *A. indica* were extracted successively and exhaustively with solvents in increasing order of polarity i.e. petroleum ether (60-80^{0} C), benzene, chloroform and acetone. After the extraction, each extract was freed of solvent by distillation under reduced pressure. The yield of each extract was recorded *viz.,* pet ether (48 g), benzene (12 g), chloroform (18 g) and acetone (13 g). The extracts were used for phytochemical, *in vitro* and *in vivo* antisnake venom studies.

Phytochemical screening

Preliminary phytochemical screening of the various extracts of *A. indica* was carried out according to the procedure of Harborne [6].

Snake venom antiserum

Lyophilised polyvalent snake venom anti-serum (as reference serum) was obtained from Kasturba Hospital Pharmacy, Manipal India. Before use the anti-serum was dissolved in 10 ml of water for injection. Each ml of antiserum shall neutralize 0.60 mg of dried Russell's Viper (*Daboia russelli*) venom.

In vitro antisnake venom activity

Anti snake venom activity of the various extracts of *A. indica* was assessed through inhibition of *in vitro* Human Red Blood Corpuscles (HRBC) lysis. The hyposaline induced haemolysis was evaluated *in vitro* by the method of Balu et al. [7] This method was modified in the present study by venom induced haemolysis. Blood was collected from healthy human volunteers by vein puncture and EDTA was used as an anticoagulant. The collected blood was washed three times with saline and 1% HRBC was prepared as described by Murugesh et al. [8]. Lyophilized venom of Viper Russelli was dissolved in physiological saline solution to make a stock solution of 100µg/ml. Then 1ml of venom (100 µg), 1ml of phosphate buffer (pH 7.4) and1ml of 1% HRBC was taken in various tubes.

To these tubes different concentrations of the various extracts of *A. indica* (20, 40, 60, 80, 100, 200, 400 µg/ml) were added. The drugs were prepared using physiological saline while the control samples were mixed with drug free solutions. The mixtures were incubated at 37ºC for 30 mins and centrifuged at 1000 rpm for 3 mins. The absorbance of the supernatant was measured at 540 nm using spectrophotometer. The percent inhibition of haemolysis was calculated according to the equation $\{(A_{Control} - A_{test}) / A_{Control}\} X 100$.

Neutralization of lethal venom effect of different leaf extracts of A. indica

The toxicity of *Daboia russelli* venom was assessed by i.p. administration of different concentrations of venom dissolved in 0.2 ml of physiological saline to groups (n =10) of Swiss albino mice (18 - 20 g). The median lethal dose

(LD_{100}) of venom was determined 24 h later by the method of Theakston and Reid [9]. The neutralizing potency of the different leaf extracts (petroleum ether, benzene, chloroform and acetone) was assessed by i.p. administration of LD_{100} dose of venom into groups of mice (n=10), followed by i.p. administration of different doses (125, 250 and 500 mg/kg body weight) of the plant extracts. The standard reference group i.e. snakevenom anti-serum was administered after administration of LD_{100} dose of venom. All the experiments were performed in duplicate.

Results and discussion

In earlier studies we reported the potent viper venom neutralizing properties of the ethanolic extract of *A. indica* [5]. As a sequel to this, different extracts of *A. indica viz.,* petroleum ether, chloroform, benzene and acetone extracts were prepared by successive solvent extraction in increasing order of polarity as to determine the most active extract.

The various extracts of *A. indica* at concentrations ranging from 20-400 µg/ml were evaluated for their *in vitro* anti snake venom activity through inhibition of Human Red Blood Corpuscles (HRBC) lysis. Though all the extracts inhibited the venom induced heamolysis to varying extent, the acetone extract showed significant inhibition of heamolysis in a dose dependent manner with maximum inhibition of 77.9 % at a concentration of 400µg/ml (Table 1). Most of the snake venoms contain phospholipase and haemolysin which will act on membrane associated phospholipids liberating lysolecithin. Lysolecithin act on the membrane of HRBC causing haemolysis [10]. Protection of acetone extract against venom induced haemolysis may be caused by stabilizing of proteins in the membrane of HRBC [11].

The LD_{100} of *Daboia russelli* venom were established at 71µg/ mouse (20 g body weight). In acute toxicity studies the benzene, chloroform and acetone extracts were found to be safe when tested up to a dose level of 3000 mg/kg body weight. However the petroleum ether extract was found to be safe only up to a dose of 580 mg/kg body weight. Though all the extracts tested reduced the venom-induced mortality in mice, the most significant anti-venom activity was exhibited by the acetone extract at a dose level of 500 mg/kg body weight. The viper venom-induced lethality was significantly antagonized by only the acetone leaf extract in a dose-dependent manner; with a dose of 500mg/kg acetone leaf extract showing the most significant activity i.e. equal protection as compared to snake venom anti-serum (Table 2). Preliminary phytochemical screening revealed the presence of cyanogenetic and anthraquinone glycosides, amides and tannins in acetone extract (Table 3). Hence, the significant antisnake venom activity exerted by the acetone extract of *Acalypha indica* in our study may be attributed by the presence any of these compounds. Further work relating to the isolation of the active constituent(s) responsible for the above said activity and the essential mechanisms are underway in our laboratory.

Table 1. In vitro antisnake venom activity of various extracts of A. indica

Concent-ration (µG/ML)	% Inhibition of Haemolysis			
	PE extract	Benzene extract	Chloroform extract	Acetone extract
20	2.0	4.6	3.4	5.8
40	4.2	5.1	4.3	9.0
60	5.5	6.9	7.6	13.7
80	7.3	10.2	9.1	19.5
100	11.0	12.7	14.3	25.0
200	13.4	18.5	20.0	38.5
400	17.0	21.2	31.5	77.9

Table 2. Effect of the various leaf extracts of *A. indica* on the lethality of *Daboia russelli* venom.

Groups (n = 10)	Dose	% Survival	% Increase in survival rate
Control	-	0	0
Snakevenom antiserum (standard)	-	87.5	87
Pet. Ether extract	125mg/kg	0.0	0
	250mg/kg	12.5	12
	500mg/kg	25.0	25
Benzene Extract	125mg/kg	12.5	12
	250mg/kg	25.0	25
	500mg/kg	37.5	37
Chloroform Extract	125mg/kg	0	0
	250mg/kg	37.5	37
	500mg/kg	37.5	37
Acetone Extract	125mg/kg	25.0	25
	250mg/kg	37.5	37
	500mg/kg	87.5	87

Table 3. Preliminary phytochemical screening of the various leaf extracts of *A. indica*

Test	PE extract	Benzene extract	Chloroform extract	Acetone extract
Phytosterols	+	+	-	-
Alkaloids	+	+	+	
Terpenes	-	-	-	-
Volatile oil	-	-	-	-
Lipids and fats	-	-	-	-
Saponins	-	-	-	-
Alkaloids	-	-	-	-
Phenolic compounds and tannins	-	-	-	+
Flavanoids	-	-	-	-
Amides	-	-	+	+
Cyanogenetic glycosides	-	-	-	+
Anthraquinone glycosides	-	-	-	+

+ denotes the presence of the respective class of compounds.
- denotes the absence of the respective class of compounds

References

1. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants, CSIR Publication, New Delhi; 1956. p. 40.
2. Peter J Houghton, Ibironke M, Osibogun. Flowering plants used against snakebite. J Ethnopharmacol 1993; 39: 1-29.
3. Siddiqui MB, Husain W, Traditional antidotes of snake poison in Northern India. Fitoterapia 1990; 61: 41 - 44.
4. Suryanarayana Raju M. Native plats used in snakebite and other poisonous animals among the tribals of East Godavari District of Andhra Pradesh. Aryavaidyan 1996; 9: 251-255.
5. Annie Shirwaikar, Rajendran K, Ramgopal Bodla, Dinesh Kumar C. Neutralization potential of *Viper russelli russelli* (Russell's viper) venom by ethanol leaf extract of *Acalypha indica.* J Ethnopharmacol 2004; 93(2-3): 267-273.
6. Harborne JB, Phytochemical Methods, 2nd Edition Chapman and Hill, London; 1984. p. 123- 175.
7. Balu S, Alagesaboopathy. Antisnake venom activities of some species of *Andrographis. Ancient Sciences of Life* 1995; 14 (3): 187-190.
8. Murugesh N, Vembar S, Damodaran C, Studies on erythrocyte membrane IV: *in vitro* haemolytic activity of oleander extract. *Toxicology Letter* 1981; 8: 33-36.
9. Theakston, RDG, Reid HA. Development of simple standard assay procedures for the characterization of snake venoms. Bull World Health Organ 1983; 61 (6): 949-956.
10. Maeno H, Mitishashi S, Konosi I, Hoshi S, Honuna M. Phosphoprotein phosphatases from rat cerebral cortex. Japanese J Exp Med 1962; 32: 55-64.
11. Abi D. Effects of calcium antagonists on the erythrocyte membrane. J Pharm Pharmacol1991; 43: 22-25. Ethanopharm 1994, 43:167–71.