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Anticoagulation activity of *Nerium indicum* specific to scorpion venom

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Abstract
Plant extract of Nerium contains some secondary metabolites. Plant has the capacity of anticoagulant specific to scorpion venom at certain moderate concentration. Traditionally some plans are used on the treatment on snake bites and scorpion bites. The anticoagulation activity can be measured by incubation time with egg yolk at certain temperature. Plant leaf extract shows best anticoagulation activity. Scropion venom is belong to chlorotoxin. Nerium plant extract is used on the treatment on scorpion bite.

Keywords: toxin, antidote, karabin, chlorotoxin

Introduction
Plant is source of different primary and secondary metabolites. The secondary metabolites play a vital role in organism and cell. Secondary metabolites are steroids, tannins, alkaloids, flavonoids, etc. Plant belongs to family Apocyneceace. Plant is rich source of neriodorin, karabin, nerioderin, odorin. These substance play a role in defiance mechanism. Nerium is evergreen shrub grows up to of 4 m height and bearing leaves all the year around. The leaves are long, linear-lanceolate, 10-15 cm in length. Flowers are hermaphrodite, white, pink or red in color, sweet smelled and 4-5 cm in diameter. Fruit is long about 15-20 cm, cylindrical and paired growing with the stem. In fruit there are numerous seeds, compressed and white in color having smooth hairs. The present study was to understand the effect of anticoagultent property of different plants parts extracts with the scorpion venom.

Preparation of suspension
Take 9 ml of egg yolk mix with 2.5 ml of $NaCl_2$, such mixture are mixed with 1.49 ml of 0.5 EDTA and 4.44 ml of $CaCl_2$, this mixture finally mixed with 2ml of tris-HCl buffer which P^H 7.5 and 0.56 ml of isosaline. The above suspension is used with scorpion venom and plant extract for to calculate the anticoagulant activity with respect to time in second.

Material and methods
Nerium indicum was collected from Hingoli district (m.s.) India. Identify plant on the basis of morphological characters up to the species level [2]. The plant material separately dried in oven at 50^0c. Each plant material separately crushed to make powder. The 50 gms. Powder of sample extracted with 500ml.of methanol. Soak the each plant material in methanol for 15 days. After soaking, filter the each plant material twice by ordinary filter paper and filter again by whatman filter paper.Discared the residue and evaporated methanol form filtrate at room temperature. These extract is used for to calculate anticoagulant activity. Collected particular species of scorpion form Hingoli district and collect venom of scorpion in glass tube containing parchment nylon paper. Venom becomes freeze dried and stored it at 2 to 8^0 c. Antidote are used as a standard. (Antidote supply by Medicare pharma, Hyderabad.) Take 19. 9ml of suspension, mixed with 20mg/ml of each plant extract separately and added 20mg/ml of scorpion venom in each extract, such mixture were incubated at 37^0c in the incubator for 60 minutes. Record the of coagulation in second. Compare the time of coagulation of each plant extract with the mixture containing 19.9ml of suspension, 25mg/ml scorpion venom and another mixture contain 19.9ml suspension, 25mg/ml scorpion venom and 20mg/ml of standard antidote.

Anticoagulation activity

Table 1

Sr.no.	Suspension (ml)	Venom(mg/ml)	Plant Extract(20mg/ml)	Incubation time.	Time in second.
01	19.9ml	25mg/ml	root	$37^0$for 60min.	112 sec.
02	19.9ml	25mg/ml	stem	$37^0$for 60min.	110 sec.
03	19.9ml	25mg/ml	Bark	$37^0$for 60min.	115 sec.
04	19.9ml	25mg/ml	seed	$37^0$for 60min.	103 sec.
05	19.9ml	25mg/ml	flower	$37^0$for 60min.	100 sec.
06	19.9ml	25mg/ml	Leaves	$37^0$for 60min.	139 sec.
07	19.9ml	25mg/ml	control	$37^0$for 60min.	92 sec.
08	19.9ml	25mg/ml	Std. antidote	$37^0$for 60min.	174 sec.

Result and Discussion

Scorpion venom is a chlorotoxin which has effect myotoxicity, necrosis, neurotoxicity, remolding of cell membrane. The venom affected too many proteins with the blood and cell. When venom interacts with the protein, it becomes coagulate and it destroys the function of various proteins. It also blocks channels of cell membrane as well as signal transduction. The standard antidote reduced the coagulation property of venom at greater extent. Traditionally different plants are used on snake bite and scorpion bite.*Nerium indicum* belong to Apocyneceae family which is used in the treatment of intestinal disorder, blood disorder, and in cancer treatment. Plant has some secondary metabolites, so traditionally it also used on scorpion bite particular in Maharashtra state. The leaves extract shows best anticoagulation activity as compare to other plant parts at 20mg/ml is 139 seconds. When it compare with standard antidote, the activity of standard antidote is 174 seconds. This experiment proves that *Nerium indicum* leaves have property of anticoagulation respected to scorpion venom.

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