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SIDDHA FORMULATION-POTENTIAL SOURCE FOR ANTI - VENOM AGAINST SNAKE BITE

Medicine

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KEYWORDS

INTRODUCTION

Traditional Medicinal plants are one of the major resources of Ethno medicines. There are about 54 million indigenous people living in India which still uses medicinal plants for various types of ailments, including snake bites (1). Globally, it is estimated that 1.2 to 5.5 million snakebites occur every year (2). There are about 35,000 to 50,000 reported deaths in India alone every year (3, 4). However, an effective treatment regimen to treat lethal snakebite envenomation remains elusive due to insufficient attention. The snake and scorpion bite is a neglected health hazard worldwide (Alirol et al., 2010). Most snakebite of the more than 3000 species of snakes are considered dangerous to humans (Brian, 2015) [8]. Agricultural and tropical regions report more snake bites than anywhere else. In India alone more than 2, 00,000 cases are reported and it is estimated 35,000 to 50,000 people die each year. In India, particularly in the rural areas snake bite victims turn to traditional medicine men and healers, due to lack of availability of antiserum Majority of the victims are agriculturists and their families living in rural areas of the country. Snake venoms consist of a mixture of enzymatic and non-enzymatic proteins and peptides and small organic compounds such as citrates, nucleosides & acetylcholine (5). Snakebite envenomation often causes severe local damage, including edema, hemorrhage, myonecrosis, and systemic toxic response, including organ failure and coagulopathy. (6, 7).

The only treatment available is animal derived polyclonal anti-snake venom (ASV) therapy. However, ASV development is a costly, time-consuming process requiring ideal storage conditions and several cases showed that this treatment is imprecise and less effective in treatment of snakebite victims. Therefore, there has been increased interest to examine the presence of snake venom antidote from plant sources using traditional knowledge such as Siddha System of Medicine. Siddha Systems of Medicines have been practicing more than 2000 years. It was originated from Dravidian (Tamil) culture. SSM has wide range of medicnes for several diseases deadly insects and snake bite since Siddhar period. Hence in the present study was carried out to explore and investigate a Siddha formulation Paambu Maruthu (Parpam) made by a Siddha physician against Indian Cobra (*Naja naja*).

MATERIALS AND METHODS:

Snake venom:

The Indian cobra venom is obtained from Irula Snake Catcher's Industrial Co-operative Society Ltd., c/o Chennai Crocodile Bank, Vadanemmeli Village, Perur P.O, East Coast Road, Kancheepuram Dist. – 603104, Tamilnadu and was preserved at 4^{o}C. The venom was then dissolved in normal physiological saline and centrifuged at 200 xg for 10 minutes and the supernatant was used for antivenom studies.

Extraction:

The ingredients of Siddha Formulation Bharath Pambu Maruthu (Parpam) for Snake bite is given below, Each 10 gm contains,

Valam	3.0 gm
Durusu	3.0 gm
Vengaram	1.0gm
Veeram	1.0gm
Puram	1.0 gm
Sulphur	0.5 gm
Erasam	0.5gm

500 grams of dried powdered given Siddha Formulation was extracted with 1000 ml of methanol for 24 h with continuous stirring. The extraction was repeated for 3 days by changing the solvent every 24 h. The extract was concentrated using rotary vacuum evaporator under reduced pressure at 40 ◦C. Known quantity of the dried extract was subsequently dissolved in 10 mM phosphate buffered saline (PBS pH 7.4). Centrifugation at 10,800 × g (for 10 min) was performed for any insoluble. The clear solution was used for the enzyme inhibition studies. The venom activity in absence of plant extract served as the negative control.

Phospholipase (PLA_2) Inhibition:

PLA2 inhibition was evaluated using egg yolk as substrate in 1% w/v agarose plates according to the method of Gutierrez (Gutierrez *et al*., 1988) (8). 100 µg of *N. naja* venom was pre-incubated with various concentrations of plant extracts for 1 h at 37ºC. The pre-incubated samples were then loaded in 3 mm diameter wells of 1% agarose plates containing 0.6% v/v egg yolk and 5 mM CaCl2 followed by overnight incubation at 37ºC. The PLA2 inhibition was calculated by measuring the zone of clearance in the presence/absence of plant extract. PLA2 activity of venom in the absence of plant extract served as control.

Hemolytic Inhibition:

The hemolytic inhibition assay was carried out as per the method described previously (9), with slight modifications. 100 µg of *N. naja* venom was pre-incubated with various concentrations of plant extract in a volume of 200 µl PBS for 1 h at 37 ◦C. Briefly, human erythrocytes and 10 mM PBS (pH 7.4) were mixed (1:8 v/v) and 100 µl of this suspension was incubated with pre-incubated venom samples for 2 h at 37 ◦C. The reaction was stopped by adding 1 ml of ice cold PBS and centrifuged at 2500 rpm for 10 min at 4 ◦C. The amount of hemoglobin released in the supernatant was measured at 540 nm.

RESULT:

Phospholipase (PLA2) Inhibition:

The results of the Phospholipase (PLA2) Inhibition assay of methanol extract are shown in Table

1. Four plates were used for the study. Plate A, B, C and D is done for concentrations of 0.5 mg, 1.0 mg, 1.5 mg and 2.0 mg of the drug respectively.

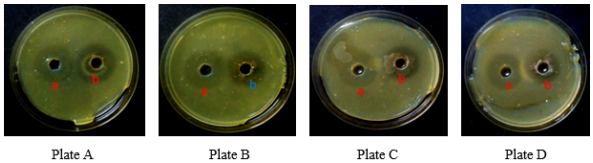

Plate A Plate B Plate C Plate D

Table 1: In vitro anti-snake venom activity of the Drug

Plate A		Plate B		Plate C		Plate D	
a (0.1mg venom)	b (0.5mg extract)+(0.1mg venom)	a (0.1mg venom)	b(1.0mg extract)+(0.1mg venom)	a(0.1mg venom)	b(1.5mg extract)+(0.1mg venom)	a(0.1mg venom)	b(2.0mg extract)+(0.1mg venom)
26	26	26	25	26	23	25	21

Hemolytic Inhibition:

The Methanolic extracts of the drug inhibited the hemolytic activity of 50% of the venom at a ratio of 100:5000 w/w (Fig. 3). Hemolytic activity is another distinct feature of cobra venoms greatly induced by multi components including metalloproteases, PLA2, and more specifically, cardio toxins and cytotoxins of venom (10, 11). This drug protect the RBC from direct hemolytic activity of the *N. naja* venom.

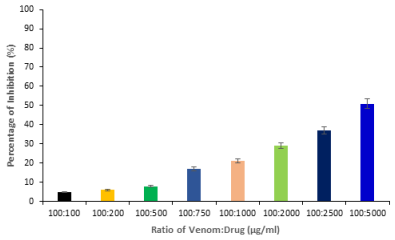

Fig. 3: Inhibition of *N. naja* venom hemolytic activity by methanolic extract of the Drug.

DISCUSSION:

Due to unavailability of proper drugs, snakebites were thought to be an ignorant global public health conundrum that leads to high mortality and permanent disability (12). Recently the PLA_2 family has been regarded as a promising target for the development of a wide range of antivenom (13). PLA2s has also been found to act with other toxic components in a synergistic manner, mostly zinc-dependent metalloproteinases, which are responsible for hemorrhage, blistering, and necrosis (14). Therefore, PLA2 became a valuable target in solving snakebite envenomation. Hence from the present study we have found that the methanolic extracts of the drug inhibits the PLA_2 activity. By calculating the diameter of the transparent circles, we found that the higher the concentration of the drug, the smaller the diameter of the circles, which shows that the drug inhibit the hydrolysis of lecithin (egg yolk) by PLA2 enzyme present in venom and vice versa.

For hemolytic inhibition the methanolic extracts of the drug has the potential to prevent the lysis of RBC from *N. naja* venom. However, we found that the extract require high ratio (100:5000) for inhibiting even 50% of the venom (fig. 3). From the present result we can confirm the drug has the potential to cure snake bites and more studies on the drug has to be done in order to understand it full potential against the venom.

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