INTERNATIONAL JOURNAL OF DRUG DISCOVERY AND HERBAL RESEARCH (IJDDHR)
ISSN: 2231-6078
3(1): Jan.-March.: (2013), 521-524

Research Article

Anti-Venom Activity of Ethanolic Extract of *Rauwolfia Serpentine* Against Naja Naja (Cobra) Venom

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Abstract

Ethanolic extracts of *Rauwolfia serpentina* plants were tested for antivenom activity against naja naja venom (cobra). The plant extract effectively neutralized the naja naja venom induced lethal activity. About 0.14 mg of *Rauwolfia serpentina* plant extract was able to completely neutralize the lethal activity of 2LD50 of naja naja venom. The alkaloids reserpine exerts the inhibitory action against the venom of naja naja. Naja naja venom is responsible for neuro transmitter release at cholinergic nerve terminals. *Rauwolfia serpentina* PLA2 inhibitors were purified from the ethanolic extracts of *Rauwolfia serpentina*. Pharmacological activities like pre coagulation, *In vivo* assessment of anti-venom effect of the extracts were studied and the pharmacological activities were significantly neutralized by the leaves extracts. The above observations confirmed that *Rauwolfia serpentina* plant extracts possess potent snake venom neutralizing capacity and could potentially be used for therapeutic purposes in case of snakebite envenomation.

Key Words: Anti venom, plant extracts, snake bite, rauwolfia.

Introduction

Rauwolfia serpentina plant is widely used medicinally both in Modern Western Medical system and also in Ayurveda,Unani medicine.It has been used for the relief of various central nervous system disorders. Extract of the plant are valued for the treatment of intestinal disorders like diarrhea and dysentery, anthelmintic, cholera, colic and fever. It is also used as an antidote to the bites of poisonous reptiles like snakes[1]

Snakebite is a major health hazard that leads to high mortality rate especially in India. The common poisonous snakes found in India are Cobra (Naja naja), Krait (Bangarus Caeruleus), Russell's viper (Daboia russelli) and Saw Scaled Viper (Echis Carinatus) (Bawaskar,2004). About 35,000 to 50,000 people die of snakebite every year in India. Vipera russellii and Naja kaouthia are the common snakes found throughout India and a large number of deaths occur due to envenomation by these snakes (Alam & Gomes, 2003). Antivenom immunotherapy is the only specific treatment against snake venom envenomation. Antivenoms are usually hyper immune sera collected from animals which bind and inactivate venom components. Antiserum development in animals is time consuming, expensive and requires ideal storage condition.There are various side effects of antivenom such as anaphylactic shock, pyrogen reaction and serum sickness. Most of these symptoms may be due to the action of high concentrations of non immunoglobulin proteins present in commercially available hyper immune antivenom[2].

Extracts from plants have been used among traditional healers, especially in tropical areas where there are plentiful sources, as therapy for snakebite for a long time. Several medicinal plants, which appear in old drug recipes or which have been passed on by oral tradition, are believed to be snakebite antidotes. Many Indian medicinal plants are recommended for the treatment of snakebite[3]. The present investigation explored the venom neutralizing activity of *Rauwolfia serpentina plant* extracts *by in vivo* and *in vitro* methods.

Chemical constituents[4]

The major chemical constituents present in the *Rauwolfia serpentina* are alkaloids like reserpine which varies from 1.7 to 3.0 %. Minor alkaloids are ajmalicine, ajmaline, isoajmaline, ajmalinine, chandrine, rauwolfia, renoxidine, rescin namine, reserpine, sarpagine, serpentine. Roots contain resin, starch and wax.

MATERIAL AND METHOD

Collection of Plant Material

Rauwolfia serpentina plants were collected & they were authenticated by Regional forest research centre, Rajahmundry & department of Pharmacognosy, GIET School of Pharmacy, Rajahmundry. The plants were air dried under shade & coarse powdered and used for extraction.

Chemicals

The freeze-dried snake venom powder of naja naja(cobra) was obtained from Irula's Snake Catchers Industrial Co-operative Society Limited, Chennai and was stored at 4°C. Male inbreed Swiss albino mice 18-20 g were used for efficacy studies.

Extraction of the active principle[5]

The shade dried coarsely powdered plants (1kg) of *Rauwolfia serpentina were* extracted were extracted by Soxhlet apparatus with ethanol as solvent. The extract was concentrated under reduced pressure in freeze drier & this extract was used for the Pharmacological investigation.

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Chemical Analysis of Extract[6]

Different chemical tests were carried out for the ethanolic extract of *Rauwolfia serpentine* to identify the presence of various chemical constituents like alkaloids, tannins, resins, saponins, flavonoids, steroids and terpenoids (table 1)

In vitro assessment of venom toxicity and anti-venom Effect of the extracts[7]

Enzyme activity

Phospholipase A2 activity was measured using an indirect hemolytic assay on agarose– erythrocyte–egg yolk gel plate by the methods described by Gutierrez et al., 1988. Increasing doses of Echis Carinatus venom (µg) were added to 3mm wells in agarose gels (0.8% in PBS, pH 8.1) containing 1.2% sheep erythrocytes, 1.2% egg yolk as a source of lecithin and 10mM CaCl2. Slides were incubated at 37°C overnight and the diameters of the hemolytic halos were measured. Control wells contained 15µl of saline. The minimum indirect hemolytic dose (MIHD) corresponds to a dosage of venom, which produced a hemolytic halo of 11mm diameter. The efficacy of antivenom (plant extracts) in neutralizing the phospholipase activity was determined by mixing constant amount of venom (µg) with various amount of plant extracts (µl) and incubated for 30 minutes at 37°C. Then, aliquots of 10µl of mixtures were added to wells in agarose-egg yolksheep erythrocyte gels. Control samples contain venom without plant extracts. Plates were incubated at 37°C for 20 h. Neutralization was expressed as the ratio of mg antibodies/mg venom which could reduce the diameter of the hemolytic halo by 50% when compared to the effectinduced by venom alone . The alkaloids reserpine exerts the inhibitory action against the venom of naja naja. Naja naja venom is responsible for neuro transmitter release at cholinergic nerve terminals

Procoagulant activity[8]

Procoagulant activity was assayed according to the method described by Theakston and Reid, 1983 as modified by Laing et al., 1992. Various amounts of venom dissolved in 100µl PBS (pH 7.2) was added to human citrated plasma at 37°C. Coagulation time was recorded and the minimum coagulant dose (MCD) was determined as the venom dose, which induced clotting of plasma within 60 seconds. Plasma incubated with PBS alone served as control. In neutralization assays constant amount of venom was mixed with various dilutions of leaves extracts. The mixtures were incubated for 30 minutes at 37°C. Then 0.1ml of mixture was added to 0.3ml of citrated plasma and the clotting times recorded. In control tubes plasma was incubated with either venom alone or plant extracts alone. Neutralization was expressed as effective dose (ED), defined as the ratio µl antivenom (leaves extracts)/mg venom at which the clotting time increased three times when compared with clotting time of plasma incubated with two MCD of venom alone.

***In vivo* assessment of anti-venom effect of the extracts[9]**

Lethal toxicity

The median lethal dose (LD50) of naja naja venom was determined according to the method developed by Theakston and Reid 1983. Various doses of venom in 0.2 ml of physiological saline were injected into the tail vein of mice, using groups of 3-5 mice for each venom dose. The LD50 was calculated with the confidence limit at 50% probability by the analysis of deaths occurring within 24 h of venom injection. The antilethal potentials of *Rauwolfia serpentine* plant extract were determined against 2LD50 of naja naja venom. Various amount of plant extract (µl) were mixed with 2LD50 of venom sample and incubated at 37°C for 30 minutes and then injected intravenously into mice. 3–5 mice were used at each antivenom dose. Control mice received same amount of venom without antivenom (leaves extracts). The median Effective Dose (ED50) calculated from the number of deaths within 24h of injection of the venom/antivenom mixture. ED50 was expressed as µl antivenom/mouse and calculated by probit analysis.

Result and Conclusion

The anti-venom potential of *Rauwolfia serpentina* plant extracts were tested against naja naja

Venom using *invivo* and *invitro* methods. . The alkaloid reserpine inhibits the action of phospholipase- A2 enzyme from naja naja venom. The lethal toxicity (LD50) of naja naja venom was assessed using Balb/c strain mice. About 8 µg of naja naja venom was found to be LD50 for 18g of mice. The neutralization of lethality was done by mixing constant amount of venom (2LD50) with various dilutions of *Rauwolfia serpentina* plant extracts and incubated at 37°C for 30 minutes prior to injection. We found that 0.14 mg of *Rauwolfia serpentina* plant extracts were able to completely neutralize the lethal activity of 2LD50 of naja naja venom (Table 2, Fig. 1).

Snakebite is a major health hazard that leads to high mortality rate especially in India. Saw Scaled Viper (Echis Carinatus) and Naja kaouthia are the common snakes found throughout India and a large number of deaths occur due to envenomation by these snakes. Antisnake venom remains the specific antidote for snake venom poisoning. This antisnake venom is usually derived from horse sera. They contain horse immunoglobulins, which frequently caused complement mediated side effects, and other proteins that cause serum sickness and occasionally, anaphylactic shock. Although, use of plants against the effects of snakes bite has been long recognized, more scientific attention has been given since last 20 years (Santosh et al., 2004). Many Indian medicinal plants are recommended for the treatment of snakebites (Alam et al., 2003). In this study we examined the antivenom potential of *Rauwolfia serpentine* plant

Table 1: qualitative phytochemical analysis

SN.No	Bioactive constituent	Chemical test	Colour intensity
1	alkaloids	Picric acid test	++
2	flavanoids	Ammonia test	+
3	tannins	$FECl_3$	+
4	saponins	Frothing test	++
5	Steroids and terpens	Salkowski test	+++
6	resins	Precipitation test	+

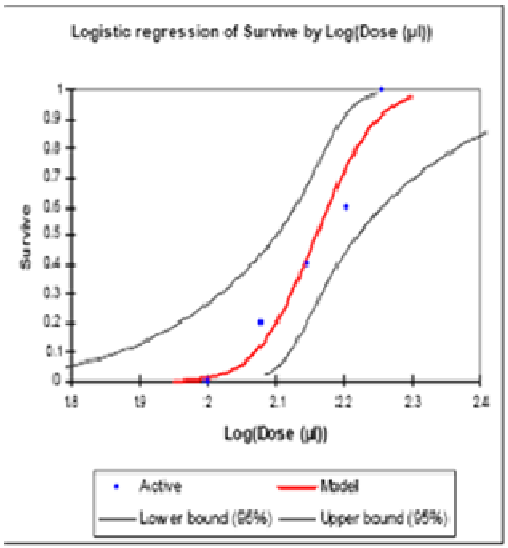
Table 2: Neutralization of naja naja venom induced lethality by plant extracts

Plant extract	Dose of venom(microgram)	Neutralization of venom by plant extract(ED 50 im mg)
Rauwolfia serpentine plant extract	16(2LD 50)	0.14mg

Fig:2, The whole plant of *Rauwolfia serpentina* plant

extracts against naja naja (cobra) venom. Neutralization of these pharmacological effects was carried out using *Rauwolfia serpentina plant* extracts. Neutralization studies can be performed by incubating of venom and plant extracts prior to testing (pre-incubation method). The results showed that the leaves extracts were capable of neutralizing the lethality induced by the venom. Procoagulant activity induced by Saw Scaled Viper (Echis Carinatus) venom was studied using human citrated plasma and *Rauwolfia serpentina* plant extracts were found to be effective in the neutralization of procoagulant activity. In conclusion the present experimental results indicate that Rauwolfia *serpentina* plant extracts were effective in neutralizing the toxic effects of the naja naja (cobra) venom.

Fig: 1,



Acknowledgement

The authors are thankful to Principal Dr.A.Rajasekaran sir, KMCH college of pharmacy for providing the necessary facilities in the Colleges, Sincerely thanks to Dr.C.Sankar, Professor Department of Pharmacuetics, KMCH college of pharmacy(Coimbatore, Tamilnadu.) for his valuable support.We would also like to thank our colleagues, lab assistants for their support.

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