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Anti-inflammatory activity study of antidote *Aristolochia indica* to the venom of *Heteropneustes fossilis* in rats

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Abstract

The dried extract of plant Aristolochia indica is used as anti-inflammatory , anti-pyretic and analgesic activity against the venom extract of H.fossilis which present in the glandular cell (Poison gland) present in base of pectoral spine. The LD_{50} of a H.fossilis extract in oral and IV doses were about 40 mg/kg/day and 29mg/kg/day respectively. The volume of inflammation caused by venom extract was seen upto 10 µl/ml safely. The viability of the 10mg/kg conc. of plant extract in 10 rats under test with conc. of venom 5 µl/ml to 100 µl/ml showed that extention of rats survival upto the concentration of 30 µl/ml and the inflammation at that point was 0.20 ml which was too less than 0.84 ml of control study with venom only. The calibration of inflammatory effect from 1 µl/ml to 10 µl/ml was extracted maximum at conc. of 4µl/ml v/v which gave an average inflammation of 0.84ml on 10 rats. The combined administration of the extract of plant and venom shows the more resistivity of venom in rats proving the extention of the conc. of venom effectiveness from 4µl/ml to 7µl/ml v/v and also the volume of inflammation gets retarded upto the range of 0.01 ml to 0.21 ml .

***Key words* :** Extracts ; Lethal dose ; inflammation ; validation ; effectiveness.

Introduction

Aristolochia Species refers to several members of genus (family- Aristolochiaceae). Aristolochia indica (Indian Birthwort) is a perennial climber with greenish white woody

stems found through out India in the plains and low hills [1]. The leaves are glabrous and very variable, usually obovate –oblong to sub-pandurate entire with somewhat undulate margins somewhat cordate acuminate. Flowers are few, in axillary racemes with a perianth up to 4 cm long having a glabrum pale green inflated.

The roots of Indian birthwort (Aristolochia indica L) have been in Indian folk medicine as an emmenagogue and an abortifacient. The aristolochic acid occurring in aristolochia species has reported to function as a phospholipase A_2 inhibitor and as antineoplastic, antiseptic, anti-inflammatory and bactericidal agent [2].

The roots of A.indica contains aristolindiquinone,[3] aristololide, 2-hydroxy-1-methoxy-4H-dibenzo quinoline-4,5-(6H)-dione, Cephradione, aristolactum IIa, β-sitosterol-β-D-glucoside aristolactam glycoside I, stigmastenones II and III, methylaristolate, ishwarol, ishwarone and aristolochene [4].

The two kinds of cell lines are known to contribute to the mucus formation by skin; the goblet cells, which contribute primarily to the production of glycoprotein rich slimy mucus and clavate cells, which contributes to the production of proteinaceous material. The latter are known to be rich in the epidermis of fishes with reduced squammation and are believed to play an important role in producing the toxic components, *ichthyocrinotoxins* (Halstead 1970) [5]. These cells get concentrated at the base of the pectoral spine (poison glands) in catfishes and aid the fish in defence mechanism (Cameron and Endcan 1973) . *Heteropneustes fossilis* is the common fresh water catfishes found in India. The crude extracts of the pectoral spine of these fishes exhibites several pharmacodynamic activities in experimental animals (Bhimacher 1994 ; and Dutta et al 1982). The biochemical composition of the mucus or that of the poisonous spin of these fishes has not been worked out . In an earlier study, it was demonstrated that the extracts of the tissue from this fish induced cardiotonic activity in isolated heart and frogs and the extracts from the *H. fossilis*. In the view of importance of extracts of epidermal secretions and poisonous spine in pharmacology, a study was made on the biochemical composition and the protein patterns of the tissues of fishes [6].The H.fossilis venom induces a severe burning pain, oedema and nacrosis observed in both clinically and experimentally. The present study was carried out in order to describe the pattern of local acute inflammation due to the presence of TNF-α, IL-1β and IL-6, response after *H. fossilis* venom injection [7]. Among the constituents of the epidermal secretion are protein and lipid components which induce alterations in cell metabolism, including an abundant lectin, a protein kinase, a large molecular weight clotting factor, a specific hemolytic factor, esterases, prostaglandins, platelet activating factors, and components which induce smooth muscle contraction. This activated enzyme causes an unregulated release of arachidonic acid, which is converted to prostaglandins at levels which result in the death of the animals [8].

Sometimes fishermans and non-veg. fish eaters has get inoculated with the hard sting accidentally. These may response badly with fever, inflammation urgently require to escape from its venom injection, needs arise to take antidote. The extract of *Aristolochia indicum* is has a promicing antidote against painful venom of *H. fossilis.*

Materials and Methods

Plants Extraction

The whole plant material was dried in shade and the air dried plant was ground and extracted first by refluxing with petroleum ether (50–60°C, for 72 hr) and then with methanol (60–80°C for 72 hr). The methanol extract was concentrated in vaccum and kept in a desiccators at room temperature for further use. Before use, it was dissolved in saline and centrifuged at 2000 rpm for 10 min at room temperature. The supernatant was used for further investigation and kept at 4°C. The plant extracts were expressed in terms of dry weight [9].

Evaluation of Toxic effects of plant extract

As earlier reported study shows the LD_{50} ranges of oral dose from 56-203 mg/kg body weight and intravenous LD_{50} range is 38-83mg/kg, of rats and mices. As in this experiment only 11 pairs of male albino rats were taken of body weight within 260-275 gram ranges. Keeping the above LD_{50} ranges, the experiment was positively response with 22 rats (1039/ac/07/CPCSEA).

The evaluation of LD_{50} ranges were carried out and observed upto 15 days . Then effective dose was checked in rats for inflammation with 0.5ml of 1mg/ml Carrageenan injection.

Venom i.e, Ichthyocrinotoxin was extracted from poison gland of H. fossilis which was located in the base of pectoral spins of that fish through venom apparatus. And it was stored in -70 °C with freezed drying technics [10].

The inflammation of hind paw was estimated by preparing the venom concentration of 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µl per ml in distilled water, administered to the 11 rats having nearly body weight.

Simultaneously, the viability of the 10mg/kg conc. of plant extract in 10 rats of near about same body weight were studied with volume of concentration between 5-100 µl of venom extract to get the survival extention limit of the poisonized rats.

Validation for the inflammatory response of venom as control (without plant extract) was evaluated in 10 rats nearly same body weight against conc. between 1-10 µl/ml of extract. The inflammation was measured with water displacement technique, help of plethysmometer.

Then conc. of 1µl, 2 µl, 3 µl, 4 µl, 5 µl, 6 µl, 7 µl, 8 µl, 9 µl, 10 µl per ml solution was made from the dried powder extract in distilled water . Then the concentration of the extracted toxin were administered to all the 10 rats out of 11 rats in the hind paw which was previously administered with plant extract orally of 10mg/kg body weight. And rest one rat was administered with control test i.e, 4µl of venom extract (after validation of its inflammatory response). The inflammation was calibrated with plethysmometer (water displaced).

Results

As the oral LD_{50} gives mean values of 51mg/kg and IV LD_{50} gives mean value of 29mg/kg body weight for 11 pairs of rats . Table 1 represents the LD_{50} for oral and IV doses with its mean ± SD

Table 1 : This table shows LD_{50} of 11 pair of rats.

No. of Rats	Body weight (gm)	LD_{50} for oral dose (mg/kg/day)	LD_{50} for IV dose (mg/kg/day)
1	267	52.1	28
2	268.21	53	30.4
3	267.43	51.7	28.2
4	268	51.2	29.8
5	266.9	51	27.9
6	268.10	52	29
7	267.56	50.4	28.4
8	268.12	49.4	30.1
9	267.61	49	29.2
10	267.27	51.8	28.1
11	268.78	50.4	30.9
Average	267.72	Mean± SD = 51 ± 1.297	Mean ± SD = 29 ± 1.067

The graphical representation of (Table1) was marked in (Figure1) which showing increasing values inflammation upto 0.87 and then gradually decreasing value of inflammation with increasing concentration.

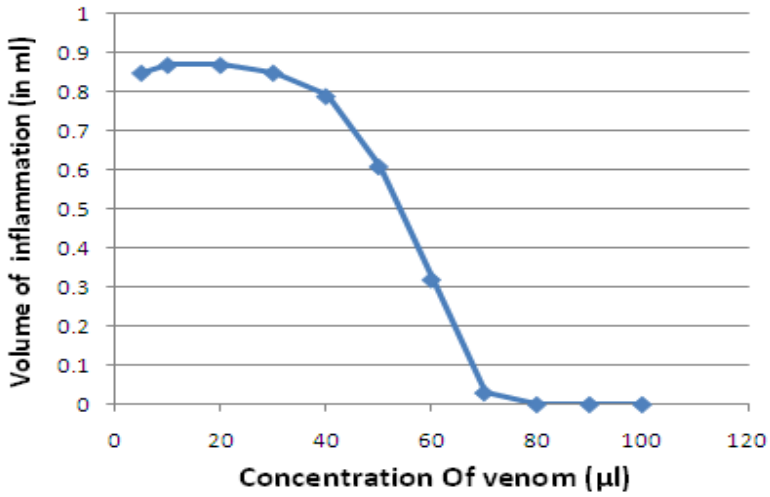

Figure 1 : Graph is plotted between the Conc. of venom (µl) and Volume of inflammation (water displaced in ml)

In order to evaluate the inflammation of hind paw with venom extract of concentration between 5µl -100 µl , it was noted that the rats were start expiring from 20 µl onwards to 100 µl . The following Table 2 shows the volume of displace water due to inflammation of paw respective with concentration of venom in selected body weight of rats.

Table 2 : Viability of the 10mg/kg concentration of plant extract in rats.

Conc. of venom (µl/100ml)	**5 µl**	**10 µl**	**20 µl**	**30 µl**	**40 µl**	**50 µl**	**60 µl**	**70 µl**	**80µl**	**90 µl**	**100 µl**
Volume of inflammation (water displaced in ml)	0.85	0.87	0.87 Dead after 5min	0.85 Dead after 1min	0.79 Dead instantly	0.61 Dead instantly	0.32 Dead instantly	0.03 Dead instantly	0.00 Dead instantly	0.00 Dead instantly	0.00 Dead instantly
Body weight (kg)	265	264.6	265.2	264.3	265.9	266	266.2	265.5	265.2	264	265.1

Viability of the 10mg/kg concentration of plant extract in 10 rats of near about same weight were studied in concentration between 5 - 100 µl of venom extract which showed the survival of rats extended as well as the resistivity of the plant extract in the following

Table 3 : Volume of inflammation resisted by pre-administered plant extract and the viability of rats against venom extract concentration (µl).

Conc. Of venom	**5µl**	**10µl**	**20 µl**	**30 µl**	**40 µl**	**50 µl**	**60 µl**	**70 µl**	**80 µl**	**90 µl**	**100 µl**
Body weight	266	265.1	266.6	265.5	264.9	266.7	265.3	266.2	265.5	265	265.2
Vol. of inflammation resisted(Extent of survival)	No infla -mation	0.20 ml Survive	0.20 ml Survive	0.20 ml Survive	0.20 ml Dead after 5 min	0.20 ml Dead after 50 min	Dead instant	Dead instant	Dead instant	Dead instant	Dead instant

This graphical representation (Figure 2) shows volume of inflammation resisted and viability of 10 mg/ kg body weight of plant extract vs concentration venom extract.

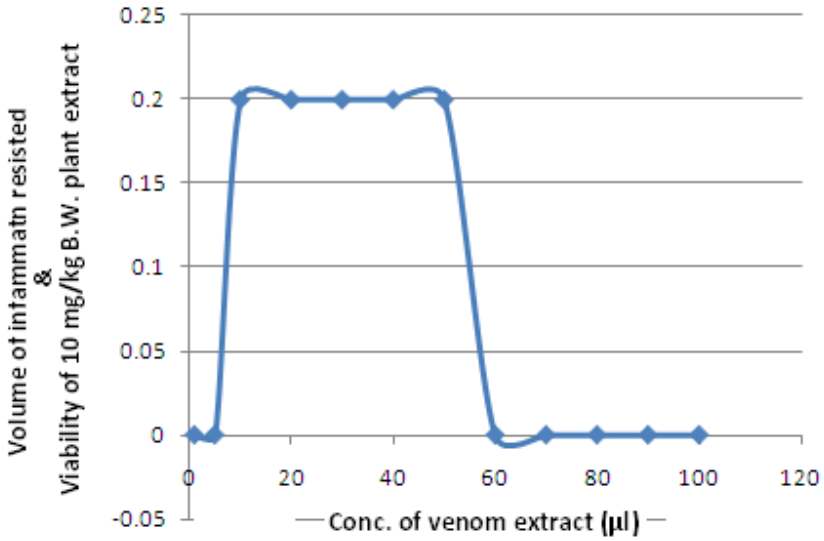

Figure 2: Graph is plotted between Conc. of venom extract (µl) & Viability and volume of imflammation resisted with 10mg/kg body weight(B.W) of plant extract

As the rats were expiring above 10 µl , it was considered to plot data between 1 µl to 10 µl which showed the maximum inflammation occured in 4 µl and average of 0.84 ± 0.043 for 10 rats. So the Table 4 was plotted while it was noted that injection to each rat was given with 48 hr interval from 1 to 10 µl. Average body weight of rats were 96 ± 0.766 gram.

Table 4 : Effectivity of the venom extract in conc. 4 µl evaluated with 1 to 100 µl.

Rats	Body Weight(gm)	1µl (ml)	2 µl (ml)	3 µl (ml)	4 µl (ml)	5 µl (ml)	6 µl (ml)	7 µl (ml)	8 µl (ml)	9 µl (ml)	10 µl (ml)
1	265	NI	NI	0.16	0.83	0.83	0.83	0.83	0.83	0.83	0.83
2	264.2	NI	NI	0.14	0.81	0.81	0.81	0.81	0.81	0.81	0.81
3	265.8	NI	NI	NI	0.89	0.90	0.90	0.90	0.90	0.90	0.90
4	264	NI	NI	0.16	0.79	0.79	0.79	0.79	0.79	0.79	0.79
5	264.4	NI	NI	0.18	0.81	0.82	0.82	0.82	0.82	0.82	0.82
6	265.1	NI	NI	0.16	0.85	0.86	0.86	0.86	0.86	0.86	0.86
7	265.7	NI	NI	0.31	0.89	0.89	0.89	0.89	0.89	0.89	0.89
8	266	NI	NI	NI	0.91	0.91	0.91	0.91	0.91	0.91	0.91
9	265.4	NI	NI	0.2	0.87	0.87	0.87	0.87	0.87	0.87	0.87
10	264	NI	NI	0.17	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Avg	**264.96± 0.766**				**0.84± 0.043**						

(Note : Avg – Average ; NI – No inflammation)

The plant extract of *Aristolochia indica* showed its effectiveness upto 7 µl with venom extract and in concentration of 8 µl , 9 µl & 10 µl it gave inflammation of 0.01ml, 0.19ml, 0.21ml respectively when it was injected previously to rats before venom extract ranges from 1 µl to 10 µl . But the control i.e inflammatory effect without plant extract shows maximum concentration upto 4 µl . So, the Table 5 shows the below readings of plants extract of A.indica against 10mg/kg concentration venom extract of H. fossilis.

Table 5 : Activity of plant extract against venom extract changes the required effective conc. from 4 µl to 8 µl

Conc. Of Venom extract	**1 µl**	**2 µl**	**3 µl**	**4 µl**	**5 µl**	**6 µl**	**7 µl**	**8 µl**	**9 µl**	**10 µl**
Conc. of plant extract (mg/kg)	10	10	10	10	10	10	10	10	10	10
Volume of inflammation(ml) (with plant extract)	NO	NO	NO	NO	NO	NO	NO	0.01	0.19	0.21
Control (without plant extract)	**0.84 ± 0.043 ml inflammation in 4 µl concentration.**									

The above table 5 gives plotted graphical representation of volume of inflammation in rats paw with and without plant extract (figure 3) below.

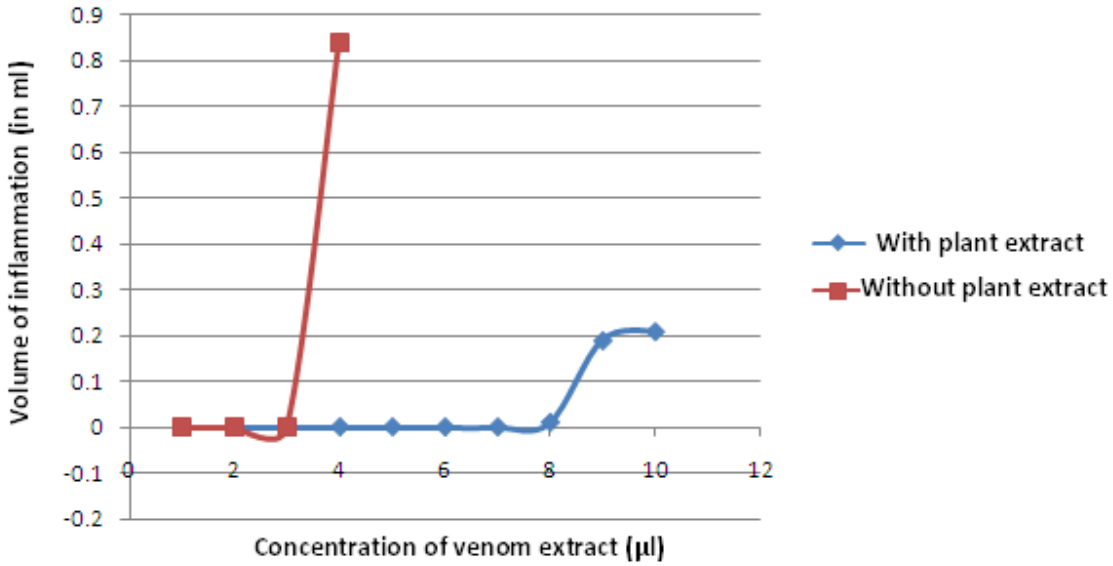

Figure 3 : Graph represents the difference between the venom inflammation with & without plant extract

Discussion

As the plant A. indica has its anti-inflammatory activity, antipyretic action and analgesic activity and simultaneously the sting of H. fossilis itself having the inflammatory, fever like action and painful stimuli, it was decided to work with the extract of plant against the venom extract .

LD_{50} of oral dose and IV dose of venom extract is Mean± SD = 51 ± 1.297 and Mean ± SD = 29 ± 1.067 mg/kg/day respectively in 11 pairs of rats which having average weight of 267.72 grams evolved. From table 2 & Figure 1, it could be observed that the gradual raising of inflammatory volume seen in 5µl i.e, 0.85 ml to 10 µl i.e, 0.87 which was

again gradually reduce to 0.85 ml in 30 µl, 0.79 in 40 µl, 0.61 ml in 50 µl, 0.32 ml in 70 µl, which was thus retarded to 0 ml in 80, 90 & 100 µl/ml v/v simultaneously. In the same time the life existence of the rats also varied from 20 µl/mg v/v concentration to 100 µl/mg v/v. In 20 µl/mg v/v the life remains about 5min after injection, and in 30 µl/mg v/v the life reduce again to 1 min but between the 40 ml/mg v/v to 100 µl/mg v/v the rats collapse within sec. And in 5 µl to 10 µl/ml v/v there is safety observed. This means the reading for viability can be taken for the experiment in between 1 µl to 10 µl only after that it is useless to take readings.

Similarly, when the viability of the plant extract conc. was judged in 10 rats in order to measure it, there was absence of inflammation on conc. 5 µl/ml v/v of venom extract pre-administered with 10mg/kg BW plant extract but in 10 µl/ ml v/v It seems to be inflammate of about 0.20ml volume constantly upto the 50 µl/ml v/v conc. which is due to the anti-inflammatory action of the plant extract . The inflammation to rats paw get steady (Table 3 & Figure 2*),* but due to higher conc. i.e, on 40 µl and 50 µl the life span retards along with the steady inflammatory response of 0.20 ml volume in plethysmometer. Due to higher conc. of the venom extract from 60 – 100 µl/ml v/v rats died with shock. These means that 0.20 ml volume is the limit of inflammation.

As the need arise to judge the inflammatory response of the venom extract in Table 4 where 10 rats of nearly same body weight were taken under the consideration for the experiment to evaluate conc. at which max. inflammation occurs with venom extract in rat's paw. It was observe that at 1 µl/ml and 2 µl/ml v/v there is no inflammation but its gets started slowly in 3 µl/ml and finally 4 µl/ml v/v it was 0.84 ± 0.7 ml mean value volume (displace volume of plethysmomete). But at conc. of 5 µl/100ml v/v its seems to be little variable comparatively with 4 µl/ml v/v conc. After 5 µl/ml i.e, 6- 10 µl/ml there is no variation in the volume . These means that 4 µl/ml v/v conc. will only required for inflammation activity.

Then on getting max. inflammatory conc. of 4 µl/ml graph was plotted in F*igure 3* and Table 5 where the conc. of venom is given to 10 rats from 1 - 10 µl/ml v/v post administered with 10mg/ml with conc. of plant extract in 0.5 ml. In 1 - 7 µl/ml shows no inflammation and in the 8 µl/ml gives 0.01ml and 9 µl/ml gives 0.19 ml and finally 10 µl/ml shows 0.21 ml of inflammation. This means due to the plant extract the inflammation is shifted from point of concentration 4 µl/ml to 8 µl/ml (higher conc.). Also the volume of the inflammation retards from 0.84 ml to 0.2 ml volume in plethysmometer.

Conclusion

After going through these extracts the effective dose were investigated for the inflammation and its antagonists. The post- administrated rats with plant extract, it was observed that the venom extract of H.fossilis is not only shows the reduction in the volume of inflammation compared to the control administered venom extract but only the shifting of the conc. margin to 8 µl/ml instead of 4 µl/ml conc. of venom effectivity.

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