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ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF*AMORPHOPHALLUS PAEONIIFOLIUS* AND ITS POSSIBLE MECHANISM

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ABSTRACT

Inflammation is a tissue reaction to infection, irritation or foreign substances. The tubers of *Amorphophallus paeoniifolius* are anti-inflammatory in nature and traditionally used in inflammations. The tubers were dried under shade and made to fine powder and extracted successively with petroleum ether, chloroform, methanol and water by using soxhlet extractor. For the assessment of the anti-inflammatory activity of the extracts at the dose of 200 and 400 mg/ kg, we used carrageenan induced paw edema model in rats. The standard drug diclofenac sodium at the dose of 5 and 10 mg/ kg were administered. For the assessment of antihistaminic effect, we measured triple responses in guinea pigs and used isolated guinea pig ileum preparation for bio assay. Results show that only the methanol extracts have got prominent anti-inflammatory activity. The results of the bio assay and triple response results also show that the methanol extract has got anti-histaminic activity.

KEY WORDS

Amorphophallus paeoniifolius, carrageenan, anti-inflammatory, anti-histaminic.

INTRODUCTION

Inflammation is a tissue reaction to infection, irritation or foreign substances. There are several tissue factors or mechanisms that are known to be involved in the inflammatory reaction such as release of histamine, bradykinin and prostaglandins. In addition to local changes in an inflammatory area, there are often various responses such as rise in temperature, an increase in blood leucocytes etc. There are also increases in certain plasma proteins termed acute phase proteins[1]. Histamine has been implicated as a mediator of vasodilatation and other changes that occur during inflammation. It promotes adhesion of leukocytes to vascular endothelium by expressing adhesion molecule P- selectin on endothelial cell surface, sequestrating leukocytes at the inflammatory site. It may also regulate microcirculation according to the local needs [2]. Carrageenan – induced paw edema is

the most commonly used method in experimental pharmacology. Carrageenan is a sulphated polysaccharide obtained from seaweed (Rhodophyceae), and by causing the release of histamine, 5-HT, bradykinin and prostaglandins it produces inflammation and edema [3]. Guinea pig ileum preparations are very commonly used for isolated tissue works. It is sensitive to histamine in nanogram/ ml concentrations. When histamine injected intradermally, it elicit the triple responses consisting of red spot, wheal and flare. Cetrizine hydrochloride has marked affinity for peripheral H_1 receptors and also inhibits release of histamine [4].

Dietary measures and traditional plant therapies as prescribed by ayurvedic and other indigenous systems of medicine are used commonly in India [5, 6] .*Amorphophallus paeoniifolius* known as Elephant foot yam is basically a crop of south East Asian origin. In India, it is commonly known as "*Suran*" or "*Jimmikand*". This tuber is consumed by many people as a food and widely used in many Ayurvedic preparations [7].The tubers are anodyne, anti-inflammatory, anti-haemorrhoidal, and expectorant. They are traditionally used in arthralgia, elephantiasis, tumors, inflammations, hemorrhoids, hemorrhages, cough, bronchitis, asthma etc [8]. The tuber is reported to have CNS depressant [9], cytotoxic activity [10], analgesic activity[11], and antiprotease activity [12]. In this research, we compare the anti-inflammatory activity of different extracts of *amorphophallus paeoniifolius* and try to clarify possible mechanism of action of methanolic extract for its anti-inflammatory activity by using specific experimental pharmacological models.

Animals

For the anti-inflammatory activity 55 male Wister rats (125–175 g) and for the antihistaminic activity 20 guinea pigs (500-750 g) were supplied by animal house of Gupta College of Technological Sciences. The Animal Ethics Committee of Gupta College of Technological Sciences approved the experimental protocol [955/A/06/CPCSEA].

Preparation of the extracts

The tubers of the plant were dried under shade and made to a fine powder using a laboratory mill and extracted successively with petroleum ether (Merck, India), chloroform (Merck, India), methanol (Merck, India) and water by using soxhlet extractor. The phytochemical screening of the successive extracts was done.

Anti-inflammatory activity on rats

For the assessment of the anti-inflammatory activity of the extracts at dose of 200 and 400 mg/ kg, we used carrageenan induced paw edema model on rats[13, 14]. The standard drug Diclofenac sodium (Voveran, Novartis, India) in dose of 5 and 10 mg/ kg were administered in the form of suspension in 5% v/v Tween 80 (Burgoyne Burbidges and company, India) solution as vehicle. The extracts were also administered in the form of suspension in the same vehicle. The animals were divided into 11 groups each comprises of 5 animals. All the groups received intraperitoneal injection.

In group I, Control animals received 5% Tween 80 at the dose of 10 ml/kg, in group II and III animals received standard Diclofenac sodium at the dose of 5 and 10 mg/kg respectively, and the remaining 8 groups received petroleum ether extract, chloroform extract, methanol extract and aqueous extract at the dose of 200 and 400 mg/kg respectively.

Initially the left paw of each rat was marked just beyond tibio-tarsal junction and volume of the paw up to the mark was measured by using Plethysmometer. After thirty minutes of drug administration in the groups, 0.1 ml of 1% carrageenan solution was injected in the plantar region of the left hind paw of the rats. Left paw volumes were measured ater1, 2, 3 and 5 h. after carrageenan injection. The edema was expressed as an increase in the volume of the paw and the percentage of inhibition for each group was obtained as follows-

$$\% \text{ of inhibition} = \frac{(V_t - V_0) \text{ control} - (V_t - V_0) \text{ treated}}{(V_t - V_0) \text{ control}} \times 100\ \%$$

The determination of swelling index (the percentage of swelling after administration of drugs) helps in ascertaining anti- inflammatory activity of a drug. The swelling indexes were calculated at 1, 2, 3 and 5th h of drug administration.

Assessment of antihistaminic effect in guinea pig by measuring triple response

For the assessment, we used the modified method described by Jacob *et al*[15]. 4 groups of sensitized guinea pigs comprising 4 in each were taken. The group I received 0.3 ml saline, group II received 0.3 ml of 40 mg/ml cetrizine hydrochloride and group III, IV received 0.3 ml of methanol extract at dose of 200 mg/kg and 400 mg/kg intraperitopnially. After 30 min, intradermal injection of histamine (100 µg in 0.1 ml aqueous solution) was given to every animal. The initial zero time volume (base value) of the injecting sites were measured. The volume of the red spots was measured for every 15 min intervals until the red spots were disappeared.

Bioassay of methanol extract by using guinea pig intestine [16]

Overnight fasted animals were used for better responses of drug on intestinal smooth muscle. Drugs, Histamine stock solution (1mg/ml), cetrizine hydrochloride (Cetzine, Glaxo, India) stock solution (1 mg/ml), methanol extract stock solution (5 mg/ml) were prepared. 2-3 cm long pieces of ileum was taken from sacrificed guinea pigs and mounted in the organ bath containing tyrode solution maintained at 32-35^0 C and bubbled with air. Tension of 0.5 gm was applied for each tissue and the tissues were allowed to equilibrate for 30 min the experiment starts. Contact time for recording response was 30 sec and 10 min time cycle was maintained for proper recording of the responses.

A dose response curve of standard histamine was obtained by adding 0.1 ml, 0.2 ml, 0.4 ml, 0.8 ml solution of histamine stock solution and the sealing dose was determined. Now responses of sealing dose histamine along with 0.2 ml, 0.4 ml, 0.8ml methanol extract and 0.2ml, 0.4 ml, 0.8 ml cetrizine hydrochloride from their respective stock solutions were taken.

Statistical analysis

Data obtained from pharmacological experiments was expressed as Mean ± SEM. Difference between the control and the treatments in these experiments were tested for significance using ANOVA. Values of $P<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

From the data (Table-1) it is evident that the methanol extract of *Amorphophallus paeoniifolius* has prominent anti-inflammatory activity while the chloroform extract has milder activity. The other two extracts show insignificant results. 3 hours later of the carrageenan injection, the methanol extract at the dose of 200 and 400 mg/kg give 37.5 % and 45.83% inhibition when compared to the control group. (Table-2) represents the index of swelling. In case of control group where the maximum swelling index reaches up to 59.2 at 5th hour where as in case of groups received diclofenac sodium 10 mg/kg and methanol extract 400 mg/kg the maximum swelling index were 16.02 and 23.29 at 2nd and 3rd hour respectively.

Table 1

Assessment of Anti-inflammatory Activity of Extracts of A. paeoniifolius by Using Carrageenan Induced Paw Edema Model in Rats.

Group (n= 5)	Dose (mg/ml)	Paw volume in ml					Percentage inhibition			
		0 h	1st h	2nd	3rd h	5th	1st h	2nd h	3rd h	5th h
Control	–	0.57± 0.024	0.7± 0.037	0.73± 0.04	0.81± 0.036	0.91 ± 0.039	–	–	–	–
Diclofenac sodium	5	0.60± 0.029*	0.69± 0.033*	0.71+ 0.023*	0.73± 0.026*	0.72 ± 0.029*	30.76	31.25	54.26	64.7
Diclofenac sodium	10	0.56 ± 0.021*	0.64 ± 0.02*	0.65 ± 0.034*	0.64 ± 0.029*	0.62 ± 0.041*	38.46	43.75	66.66	82.35
Methanol extract	200	0.59± 0.026*	0.70± 0.038*	0.71± 0.034*	0.74± 0.036*	0.73± 0.035*	15.38	25	37.5	58.82
Methanol extract	400	0.56± 0.034	0.65± 0.023	0.66± 0.032	0.69 ± 0.026	0.69 ± 0.018	30.76	37.5	45.83	67.64
Chloroform extract	200	0.55 ± 0.029	0.67 ± 0.038	0.7 ± 0.039	0.76 ± 0.038	0.77 ± 0.028	7.69	6.25	12.5	35.29
Chloroform extract	400	0.55 ± 0.025	0.65 ± 0.21	0.67 ± 0.02	0.76 ± 0.02	0.77 ± 0.02	23.07	25	12.5	35.29
Petroleum ether extract	200	0.53 ± 0.021	0.65 ± 0.026	0.72 ± 0.029	0.76 ± 0.017	0.82 ± 0.13	7.69	-18.8	4.16	14.7
Petroleum ether extract	400	0.55 ± 0.02	0.68± 0.019	0.72± 0.018	0.78± 0.031	0.83± 0.041	0	-6.25	4.16	17.64
Aqueous extract	200	0.57 ± 0.029	0.68 ± 0.04	0.75 ± 0.043	0.8 ± 0.032	0.86 ± 0.022	15.38	-12.5	4.16	14.7
Aqueous extract	400	0.59± 0.037	0.71± 0.024	0.75± 0.021	0.85± 0.09	0.9± 0.013	7.69	6.25	-8.33	8.82

*All the data were expressed as MEAN ± SEM. Comparison were made between different groups verses control, * p<0.05, n=5.*

Table 2

Assessment of anti-inflammatory activity of Ethanol Extract of Amorphophallus paeoniifolius Tuber by using Carrageenan Induced Paw Edema Model in Rats- Swelling Index Comparison.

Group (n=5)	Dose (mg/kg)	Swelling Index			
		1st hour	2nd hour	3rd hour	5th hour
Control	-	22.85 ± 0.19	28.12 ± 0.54	41.98 ± 0.41	59 .2 ± 0.72
Diclofenac Sodium	5	14.77 ± 0.66	18.21 ± 0.52	21.41 ± 0.42	20.0 ± 0.1 2
Diclofenac Sodium	10	14.04 ± 0.51	16.02 ± 0.39	14.22 ± 0.27	10.72 ± 0 .11
Methanol extract	200	18.59 ± 0.12	20.54 ± 0.29	25.49 ± 0.51	23.52 ± 0.83
Methanol extract	400	16.09 ± 1.01	17.86 ± 0.29	23.29 ± 0.81	23.01 ± 0.71

All the data were expressed as MEAN ± SEM, n=5.

Table 3

Evaluation of Anti-histaminic Activity of Methanol Extract of Amorphophallus paeoniifolius Tuber on Guinea Pig by Measuring Triple Response.

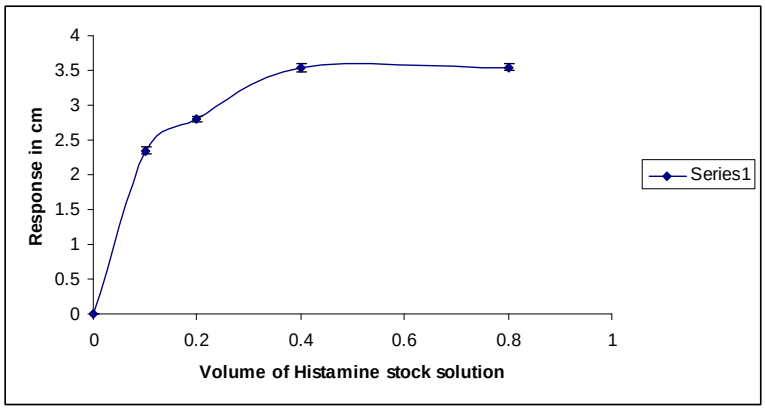
Time (min)	Control group	Standard group	Group received methanol extract	
			200 mg / kg	400 mg / kg
0	0.37 ± 0.15	0.51 ± 0.16	0.4 ± 0.04	0.43 ± 0.12
5	0.48 ± 0.103	0.56 ± 0.14	0.45 ± 0.15	0.5 ± 0.267
15	0.82 ± 0.17	0.4 ± 0.13	0.46 ± 0.28	0.53 ± 0.16
30	0.95 ± 0.238	0.43 ± 0.11	0.432 ± 0.43	0.38 ± 0.0 9
45	1.06 ± 0.449	0.38 ± 0.21	0.42 ± 0.32	0.32 ± 0.14
60	1.07 ± 0.45	0.35 ± 0.12	0.37 ± 0.36	0.3 ± 0.19
75	1.1 ± 0.494	0.26 ± 0.11	0.37 ± 0.53	0.25 ± 0.15
90	1.15 ± 0.49	0.25 ± 0.12	0.35 ± 0.53	0.26 ± 0.12
105	1.2 ± 0.49	0.2 ± 0.15	0.3 ± 0.22	0.21 ± 0.12
120	1.22 ± 0.53	0.17± 0.13	0.26 ± 0.45	0.17 ± 0.25
135	1.18 ± 0.53	0.13 ± 0.14	0.23 ± 0.51	0.12 ± 0.41
150	1.01 ± 0.53	0.05 ± 0.1	0.17 ± 0.06	0.07± 0.22
165	0.88 ± 0.45	0 ± 0	0.12 ±0.36	0.02± 0.26
180	0.62 ± 0.25	-	0.08 ± 0.332	0 ± 0
195	0.57 ± 0.23	-	0.02± .0489	-
210	0.45 ± 0.17	-	0.02± 0.3	-
225	0.4 ± 0.204	-	0 ± 0	-
240	0.28 ± 0.143	-	-	
255	0.18 ± 0.230	-	-	-
270	0.06± 0.125	-	-	-
285	0 ± 0	-	-	-

All the data were expressed as MEAN ± SEM, n=4.

From the (Table-3) it is evident that the methanol extract of *Amorphophallus paeoniifolius* has anti histaminic activity. In control group, the wheal formed due to the local release of mediators subsided after 285 min of histamine injection. In case of standard group, the time is only 165 min. The wheals disappeared in 225 and 180 min respectively after the histamine injection in the test groups which received 200 and 400 mg/kg methanol extract of *Amorphophallus paeoniifolius* respectively. The diameters of the red wheal also indicated the anti histaminic activity. In the control group, the average diameter reaches up to 1.22 cm from its base value 0.37 cm. In the standard group the base value 0.51cm was increased up to 0.56 cm only. In case of the test groups (methanol extract at 200 and 400 mg/kg), the values reached up to 0.46 and 0.53 cm respectively when their base values were 0.4 and 0.43 cm respectively.

Figure 1

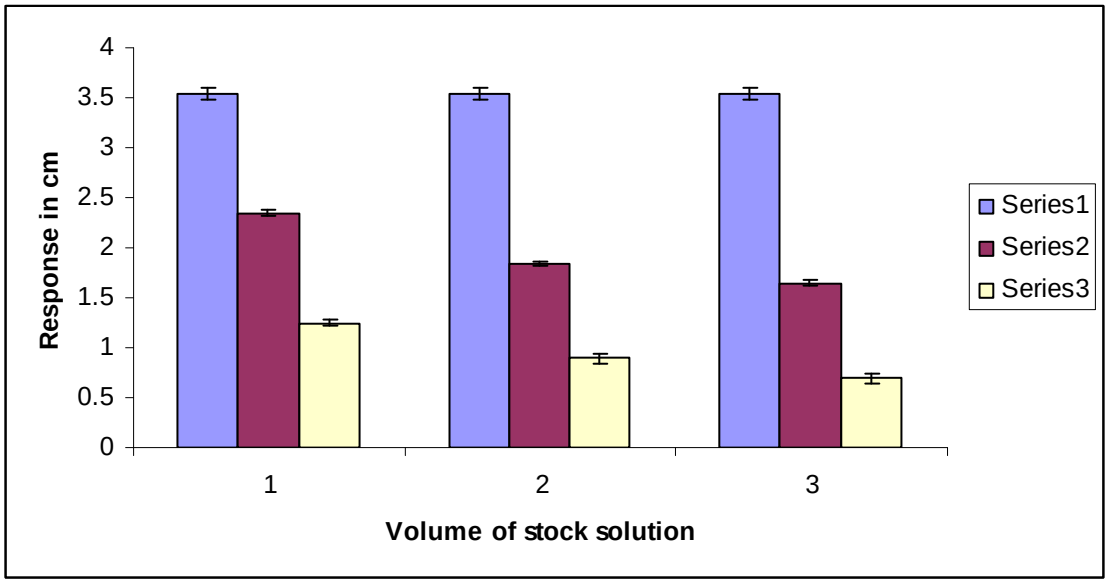
Dose Response Curve of Histamine on Guinea Pig Ileum.



All All the data were expressed as MEAN ± SEM , n=4.

Figure 2

Evaluation of Anti-histaminic Activity of Methanol Extract of Amorphophallus paeoniifolius Tuber on Guinea Pig Ileum.



All the data were expressed as MEAN ± SEM, n=4. Series 1 represents responses of 0.4 ml histamine stock solution; Series 2 represents responses of 0.4 ml histamine stock solution and 0.2 ml, 0.4 ml and 0.8 ml methanol extract stock solutions respectively, Series 3 represents responses of 0.4 ml histamine stock solution and 0.2 ml, 0.4 ml and 0.8 ml cetrizine stock solutions respectively.

(Figure-1) shows the dose response curve of histamine on isolated guinea pig ileum. From the figure it was found that at the 0.4 ml stock solution at concentration 1 mg/ml give the sealing effect. (Figure-2) shows comparative dose responses of pure histamine at 0.4 ml stock solution, histamine along with methanol extract and cetrizine at different stock volume.

From the figure it was obvious that the both of the methanol extract and cetrizine antagonizes the activity of histamine. So, we can say the methanol extract has got antihistaminic activity.

CONCLUSION

Inflammation induced by carrageenan involves 3 distinct phases of releases of mediators, including serotonin and histamine in the first phase (0 -2 h), kinins in the second phase (3 h) and prostaglandin in the third phase (>4 h) [17]. As the methanol extract at the dose of 200 and 400 mg/kg significantly inhibit paw edema induced by carrageenan in the first phase, so the methanol extract may inhibit the release of histamine or serotonins. The bio assay or the triple response effects on guinea pigs says that the methanol extract of the *Amorphophallus paeoniifolius* has got prominent anti histaminic activity. So the anti-inflammatory activities of the methanol extract of the *Amorphophallus paeoniifolius* might be due to its anti histaminic activity. The phytochemical investigations suggest that the methanol extract of the *Amorphophallus paeoniifolius* contain alkaloids and flavonoids. So the activities may be due to the alkaloids and flavonoids. Further investigations are needed for better understanding of molecular mechanism of action and signal transduction of the components present in methanol extract of *Amorphophallus paeoniifolius* regarding anti-inflammatory activity.

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