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RESEARCH ARTICLE

STUDY OF ANALGESIC ACTIVITY OF THE METHANOLIC EXTRACT OF *ACORUS CALAMUS L.* AND *OROXYLUM INDICUM VENT* BY ACETIC ACID INDUCED WRITHING METHOD

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The present research was conducted to investigate the analgesic activity of methanol extracts of plants of *Acorus calamus (Family: Acoraceae) and Oroxylum indicum (Family: Bignoniaceae).* The analgesic activity of the methanolic extract of the *Acorus calamus and Oroxylum indicum* at the dose of 250 and 500 mg/kg body weight was evaluated against the standard drug - Diclofenac sodium, at a dose of 25 mg/kg body weight. Adult swiss albino mice of either sex of five numbers in each group, was undertaken for study and evaluated by acetic acid induced writhing method. The methanol extract of *Acorus calamus* inhibited writhing reflex by 30.77% and 39.86% at the dose of 250 and 500 mg/kg body weight, respectively while the methanolic extract of *Oroxylum indicum* inhibited writhing reflex by 26.22% and 36.36% at the dose of 250 and 500 mg/kg body weight. The methanolic extract of *Acorus calamus* was found to be more active than *Oroxylum indicum* as a pain killer.

Key words: *Acorus calamus, Oroxylum indicum,* Analgesic activity, Writhing reflex.

INTRODUCTION

Literature is enriched with several findings proving ability of microorganisms, higher plants and marine sponges to produce a wide spectrum of natural products with different pharmacological activities (Almeida *et al* 2001; Malairajan *et al* 2006; Ebaba *et al* 2010; Dahiya and Gautam, 2011; Jain *et al* 2011). Acetic acid is a pain stimulus. Intraperitoneal administration of acetic acid (0.7%) causes the release of free arachidonic acid from tissue phospholipid by the action of phospholipase A_2 and other acyl hydrolases. There are three major pathways in the synthesis of the eicosanoids from arachidonic acid. All the eicosanoids with ring structures *i.e.* the prostaglandins, thromboxanes and prostacyclines are synthesized *via* the cyclooxygenase pathway (Hossain *et al* 2009). The leucotrienes, HETE (hydroxy eicosatetraenoic acids) and HPETE (hydroperoxy eicosatetraenoic acids) are hydroxylated derivatives of straight-chain fatty acids and are synthesized *via* the lipooxygenase pathway (Adedapo *et al* 2009). The released prostaglandins, mainly prostacyclin (PGI_2) and prostaglandin E have been reported to be responsible for pain sensation by exciting the Aδ-fibres. Activity in the Aδ-fibres cause a sensation of sharp well localized pain (Yerima *et al* 2009).

The acetic acid induced writhing method is an analgesic behavioral observation assessment that demonstrates a noxious stimulation in mice (Whittle, 1964). The test consists of injecting 0.7% acetic acid solution intraperitoneally and then, observing the animal for specific contraction of body referred as 'writhing'. A comparison of writhing is made between

positive control (Diclofenac sodium) and test sample given orally 30 min prior to acetic acid injection. If the sample possesses analgesic activity, the animal that received the sample, will give lower number of writhing than the control, *i.e.* the sample having analgesic activity will inhibit writhing. Diclofenac sodium is used as reference standard drug (Kouadio *et al* 2000) which has analgesic, antipyretic and anti inflammatory actions. In the light of the given information, the present investigation was undertaken which deals with the studies of the analgesic activity of the methanolic extract of the leaves of *Acorus calamus L.* and *Oroxylum indicum Vent* (**Figure 1**) by acetic acid induced writhing method (Whittle, 1964).

a) *A. calamus* b) *O. indicum*

Fig. 1. Photographs of medicinal plants

MATERIAL AND METHODS

Experimental animal

Young swiss albino mice (Age: 4-5 weeks, Avg. weight: 18-25 g) were used for the experiment. The mice were purchased from the animal research branch of the International Centre for Diarrhoeal Disease and Research (ICDDR), Bangladesh. They were kept under standard environmental condition for one week for adaptation after their purchase and fed ICDDR formulated rodent food and water.

Study design

Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III and group-IV consisting of 5 mice in each group. Each group received a particular treatment *i.e.* control, positive control and the two doses of the extract. Each mouse was weighed properly and dose of samples and control were adjusted accordingly (**Table 1**).

Preparation of different solution

Preparation of sample solution

To prepare suspension of the test sample at the dose 500 and 250 mg/kg body weight, 0.5 and 0.25 mg of the sample was measured, respectively. The extracts were triturated separately in unidirectional manner by the addition of small amount of tween 80. After proper mixing of extract and tween 80, the distilled water was slowly added and final volume of the suspension was made up to 10 ml.

Preparation of standard solution

For the preparation of standard at the dose of 25 mg/kg body weight, 0.025 g of Diclofenac sodium (Clofenac 25 mg, from Square Pharmaceuticals Ltd.) was taken and a suspension of 10 ml was made.

Preparation of acetic acid solution

For preparation of 0.7% acetic acid solution, 0.7 ml glacial acetic acid was mixed with distilled water and volume was made up to 100 ml.

Preparation of control solution

For the preparation of control, 2 drops of tween 80 was added in distilled water and volume was made up to 10 ml.

Table 1. Comparative experimental profiles of crude extracts of *Acorus calamus* and *Oroxylum indicum*

Animal group	Treatment	Dose (ml/kg body weight)	Route of administration
I (Control) n = 5	1% Tween 80 solution in distilled water	15 ml	Oral
II (Positive control) n = 5	Diclofenac sodium	25 mg	Oral
III (Test group-I) n = 5	Methanol extract of *Acorus calamus/Oroxylum indicum*	500 mg	Oral
IV (Test group-II) n = 5	Methanol extract of *Acorus calamus/Oroxylum indicum*	250 mg	Oral

n = number of mice

Methodology

Test samples, control and standard were given orally by means of a feeding needle. A 30 min interval was given to ensure proper absorption of the administered substances. Then, the writhing inducing chemical, acetic acid solution (0.7%, 15 ml/kg) was administered intraperitoneally to each of the animals of a group. After an interval of 5 min, which was given for absorption of acetic acid, number of squirms (writhing) was counted for 15 min (**Figure 2**).

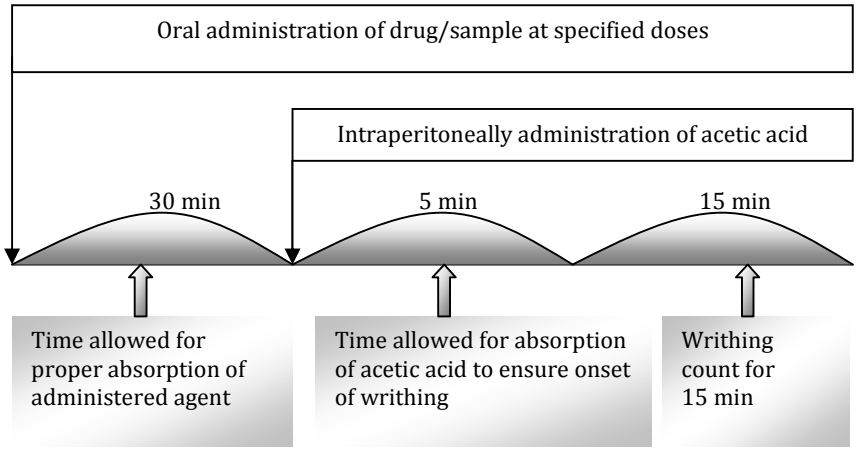

Fig. 2. Schematic representation of acetic acid induced writhing of mice for investigation of analgesic activity

Tabulation of writhing

Each mouse of all groups was observed carefully to count the number of writhing that they had made in 15 min. Two half writhing was counted as a full writhing.

Determination of analgesic activity

Analgesic activity was determined by comparing the percent writhing inhibition by the crude methanolic extracts in comparison to the control and the positive control groups. The more the writhing inhibition by the test groups, the more the positive activity (Tambe *et al* 2010).

RESULT AND DISCUSSION

The present study was undertaken to investigate the analgesic activities of methanol extracts of plants of *Acorus calamus* and *Oroxylum indicum*. The methanol extract of *A. calamus* inhibited writhing reflex by 30.77% (P>0.10) and 39.86% (P>0.10), at dose of 250 and 500 mg/kg body weight respectively (**Table 2**, **Figure 3**).

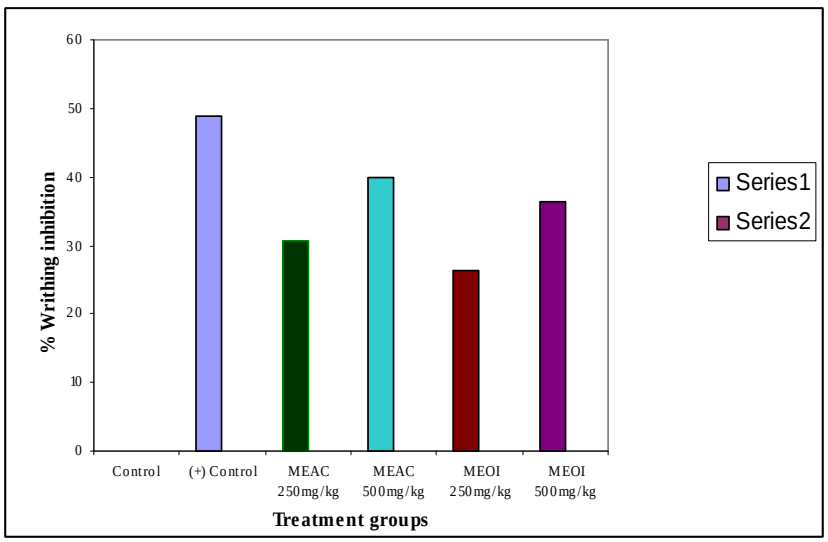

Fig. 3. Writhing inhibition by the extracts, control and positive control

Table 2. Writhing inhibition of methanol extract of *Acorus calamus*

Clinical groups	No. of mice	BW (g)	Writhing count	TW	MW	% Writhing	% WI	No. of writhing	SD	SEM	t- test
Control (1% tween 80)	1	18	65	286	57.2	100	0	57.2±7.495	14.99	7.495	-
	2	19	42								
	3	22	57								
	4	22	44								
	5	23	78								
Positive control Diclofenac sodium (25 mg/kg)	1	19	30	146	29.2	51.04	48.96	29.2±4.307	8.614	4.307	3.07
	2	22	33								
	3	24	23								
	4	24	41								
	5	25	19								
MEAC* (250 mg/kg)	1	18	51	198	39.6	69.23	30.77	39.6±4.944	9.889	4.944	2.58
	2	20	35								
	3	20	26								
	4	23	47								
	5	25	39								
MEAC (500 mg/kg)	1	21	43	172	34.4	60.14	39.86	34.4±5.74	11.48	5.74	2.45
	2	21	47								
	3	22	19								
	4	25	27								
	5	25	36								

*MEAC=Methanol extract of *Acorus calamus*; BW = Body weight; TW = Total writhing; MW = Mean writhing; WI = Writhing inhibition; SD = Standard deviation; SEM = Standard error mean

while the methanol extract of *Oroxylum indicum* inhibited writhing reflex by 26.22% (P>0.10) and 36.36% (P>0.10) at the dose of 250 and 500 mg/kg body weight, respectively (**Table 3**) where the standard drug was Diclofenac sodium at a dose of 25 mg/kg body weight. The methanol extract of *Acorus calamus L.* was found to be more significant action than the *Oroxylum indicum Vent* as a pain killer.

Table 3. Writhing inhibition of methanol extract of *Oroxylum indicum*

Clinical groups	No. of mice	BW (g)	Writhing Count	TW	MW	% Writhing	% WI	No. of writhing	SD	SEM	t- test
Control (1% tween 80)	1	18	65	286	57.2	100	0	57.2±7.495	14.99	7.495	-
	2	19	42								
	3	22	57								
	4	22	44								
	5	23	78								
Positive Control Diclofenac sodium (25 mg/kg)	1	19	30	146	29.2	51.04	48.96	29.2±4.307	8.614	4.307	3.07
	2	22	33								
	3	24	23								
	4	24	41								
	5	25	19								

MEAC (250 mg/kg)	1	18	53	211	42.2	73.78	26.22	42.22±4.463	8.927	4.463	2.71
	2	20	47								
	3	20	42								
	4	23	40								
	5	25	29								
MEAC (500 mg/kg)	1	21	51	182	36.4	63.64	36.36	36.4±5.826	11.653	5.826	2.80
	2	21	21								
	3	22	42								
	4	25	39								
	5	25	29								

*MEOI = Methanol extract of *Oroxylum indicum*; BW = Body weight; TW = Total writhing; MW = Mean writhing; WI = Writhing inhibition; SD = Standard deviation; SEM = Standard error mean

CONCLUSION

Acorus calamus (Family: Acoraceae) and *Oroxylum indicum* (Family: Bignoniaceae) are traditional medicinal plants which are used locally in our country. These plant extracts contain many useful constituents which possess promising analgesic activity. However, further research is required to establish the therapeutic usefulness of plants like *Acorus calamus* and *Oroxylum indicum*.

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