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Evaluation of Analgesic and Antipyretic activity of Marsilea trifolia Blanco

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Abstract— The main aim of this study was to find out the Antipyretic and analgesic effects of ***Marsilea trifolia*** **Blanco** (Family: Marsileaceae) fresh leaf aqueous extract, a Bangladeshi medicinal plant. The extract exhibited marked analgesic effect by reduction of writhings inducted by acetic acid at dose of 100, 200 and 300mg/kg (p.o) in mice. Analgesic effect was also observed with the hot plate device maintained at 55°C. Moreover, the extract produced a significant inhibition (P<0,01) in yeast induced pyrexia in rats. This study confirms traditional use of ***Marsilea trifolia*** **Blanco** as remedy for fever.

Index Terms— Marsilea trifolia Blanco, Marsileaceae, aqueous extract, analgesic, antipyretic, writhings, hot plates test.

1 INTRODUCTION

In Bangladesh, ***Marsilea trifolia*** **Blanco**, commonly known as "Aamrul", is traditionally used for the treatment of fever and gastro-intestinal disturbance. Several studies have reported biological effects of ***Marsilea trifolia*** **Blanco** such as antimicrobial, antioxidant and cytotoxic. In this study, I examined the effect of the fresh leaves aqueous extract of ***Marsilea trifolia*** **Blanco** for analgesic and antipyretic activities in experimental animals.

2 MATERIALS AND METHODS

2.1 Plant Material

The plant, ***M. trifolia*** was collected from Noakhali district in the month of March, 2011 and identified by Dr. M.A. Razzaque Shah, Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Bangladesh. The Voucher specimen is deposited at the national herbarium of Bangladesh.

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2.2 Extraction and Phytochemical Screening

The aqueous extract was prepared according to the method used in Bangladeshi traditional medicine. 20g of fresh leaves was crushed in blender in 500ml of distilled water. The extract obtained was filtered, evaporated, lyophilized and stored at 4°C until further use..

2.3 Animal

Male Swiss Albino mice (20-25g) and male Sprague Dawley rats (140-240g) were housed with free access to food and water on 12h light/12h dark cycle. The temperature was maintained at 25±2°C. The animals were deprived of food for 24h before the test, but they have free access to water.

2.4 Analgesic Activity

a) Writhing Test

The different concentrations of leaf aqueous extract of *M. trifolia* (100, 200 and 300mg/kg) was administered orally to three different groups of six mice each. The reference drug (acetylsalicylic acid 100mg/kg orally) was administered orally and used as positive control. After one hour, 0.6% acetic acid solution (10ml/kg) was injected Intraperitonneally.

Nociception was evaluated 15min after acetic acid injection by counting the number of abdominal constrictions.

A significant reduction in the number of abdominal contractions (treated animals) compared to the control group (received only distilled water) was considered as analgesic response.

b) Hot Plates Test

Three different groups of mice received orally 100, 200 and 300mg/kg of the extract. Acetylsalicylic acid (100mg/kg) was administered orally to positive control. Distilled water (10ml/kg) was given to control group. One hour after treatment, the animals were placed on a hot plate maintained at 55±2°C. The time taken by the mice to start licking the paw or jump out of the hot plate was considered as the reaction time. The test was carried before the treatment and at 60, 90, 120, 150 and 180 min after administration.

2.5 Antipyretic Activity

Initial rectal temperature of rats was recorded using an Ellab thermometer. Hyperthermia was induced by subcutaneous injection of 10ml/kg of 20% aqueous suspen-

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sion of brewer's yeast. After 18h of yeast injection, the animals showing a rise in temperature inferior to 0.7°C was discarded. Aqueous extract was given orally (300, 500 and 800mg/kg). Acetylsalicylic acid (100mg/kg orally) was used as reference drug. Control group received only distilled water (10ml/kg). The temperature was recorded at 1h, 2h, 3h and 4h after treatment.

3 STATISTICAL ANALYSIS

The result of the experiments was expressed as mean±S.E.M. Statistical significance was determined by the Student's test. Values with P<0,05 were considered significant.

4 RESULT AND DISCUSSION

The present study is evaluation of new analgesic and antipyretic active principles from Bangladeshi medicinal plants. The analgesic effect with chemical and thermal stimuli has been investigated. The aqueous extract of ***M. trifolia*** (100, 200 and 300mg/kg orally) presented a potent antinociceptive activity in acetic acid-induced writhings response (Table 1). This activity was dose dependant, so the percentages of inhibition were respectively 65.96%; 79.52% and 9.77%. The maximum of inhibition (89.77%) was obtained with 300mg/kg of ***M. trifolia*** extract and was comparable to that obtained by acetylsalicylic acid 100mg/kg orally).

Table 1: Effect of the aqueous extract of *M. trifolia* leaves on acetic acid-induced writhing in mice.

Groups	Dose (mg/kg)	Number of writhings	Inhibition (%)
Control (distilled water)	-	55.33 ± 0,42	-
Acetylsalicylic acid	100	4.33 ± 0,55	92.17 *
Aqueous extract	100	18.83 ± 4,02	65.96 *
	200	11.33 ± 1,74	79.52 *
	300	5.66 ± 0,21	89.77 *
Each value represents the mean ± S.E.M. (n=6) * P < 0,001 compared with control.			

This activity has been confirmed by the results of hot plate method (Table 2). However, the extract (300mg/kg) exhibited significant analgesic effect 90min after administration. Acetic acid and hot plate tests are used to study the peripheral and central analgesic effects. So the results obtained may be supported the ability of the aqueous extract to have peripheral and central pain inhibition mechanisms.

Other property exhibited by ***M. trifolia*** is antipyretic effect.

A dose dependant antipyretic effect observed with different doses (300-800mg/kg orally) (Table 3). The aqueous extract showed significant degree of antipyretic activity at dose of 500 and 800mg/kg. The reduction of hyperthermia was pronounced 60 min after administration and was prolonged for three hours. Subcutaneous injection of yeast induces pyrexia by synthesis of prostaglandin. Since, the inhibition of prostaglandin may be responsible for antipyretic effect of aqueous extract of ***M. trifolia.***

Table 2: Effect of the aqueous extract of *M. trifolia* leaves on thermal induced pain responses in mice.

		Reaction time (sec.)					
Groups	**Dose (mg/kg)**	**0 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**
Control(distilled water)	-	7,33± 0,61	7,55± 0,48	7,03± 0,50	7,16± 0,24	7,11± 0,37	7,30±1,05
Acetylsalicylic acid	100	6,73± 0,41	11±0,51**	8,71± 1,41	8,78± 1,04	7,13± 1,13	6,7 ±0,65
Aqueous extract	100	7,73± 1,35	7,66± 0,29	7,76± 0,41	7,66± 0,29	7,48± 0,69	7,73±0,63
	200	6,86± 0,57	7,21± 0,45	7,6±0,43	7,33± 0,42	7,33± 0,3	7,61±0,91
	300	7,1 ± 1,05	8,51± 0,43	10,3± 1,25*	7,85± 0,63	8,95± 1,70	8±0,89
Each value represents the mean ± S.E.M. (n=6) ** P < 0, 01 compared with control. * P < 0, 05							

Table 3: Effect of the aqueous extract of *M. trifolia* on yeast-induced pyrexia in rats.

Groups	Dose (mg/kg)	Rectal temperature °C before yeast injection	Rectal temperature °C after yeast injection				
			0h	1h	2h	3h	4h
Control (distilled water)	-	38,37± 0,09	39,33±0,08	39,57±0,14	39,58±0,11	39,50±0,12	39,62±0,12
Acetylsalicylic acid	100	37,85± 0,15	38,92±0,09	38,12±0,07**	37,78±0,17**	37,55±0,06**	37,97±0,16**
Aqueous extract	300	37,45± 0,15	38,40±0,21	38,38±0,26*	38,27±0,26*	38,08±0,19**	38,00±0,16**
	500	37,40± 0,12	38,58±0,16	38,26±0,20**	38,15±0,18**	37,88±0,15**	37,95±0,20**
	800	37,20± 0,11	38,70±0,12	37,72±0,33**	37,40±0,35**	37,33±0,25**	37,65±0,34**

Each value represents the mean ± S.E.M. (n=6)
** P < 0,001 compared with control.
*P < 0,01

5 CONCLUSION

The present study indicated that the aqueous extract of the aerial part of M. trifolia have got profound analgesic and antipyretic effect and may have potential use in medicine.

In comparison with the positive control (acetylsalicylic acid), the analgesic and antipyretic activities exhibited by the aqueous extract of the plant showed potent activity. This clearly indicates the presence of potent bioactive principles in this aqueous extract which might be very useful as analgesic and antipyretic agent.

Further studies comprising of phytochemical investigations of the used plant and evaluation for analgesic and antipyretic activities using other methods (e.g. various biochemical assays both in vivo and in vitro) are essential to characterize them. It may be concluded from this study that M. trifolia is active in the tested animals. In addition, the results confirm the use of the plant in traditional medicine. The results of the investigation do not reveal that which chemical compound is responsible for aforementioned activity. Now my next aim is to explore the lead compound liable for aforementioned activity from this plant.

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