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Analgesic and antipyretic activities of two medicinal plants - *Salvinia minima* and *Dactyloctenium australe* in experimental animal models

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ABSTRACT

The present study was conducted to evaluate the possible analgesic and antipyretic effects of aqueous extracts of leaves of Salvinia minima and Dactyloctenium australe. For investigating the analgesic activity, hot plate method maintained at 55°C was used. The extracts of both the plants were found to have significant ($p<0.001$) analgesic activity at the oral dose of 100, 200 & 200 mg/kg b. wt., in the tested models. In hot plate test, both plants showed increased latency period which are significant ($p<0.001$) compared to control. Besides, yeast-induced hyperpyrexia was used to evaluate the antipyretic activity. A significant ($P < 0.01$) reduction in hyperpyrexia in the tested models was observed by the aqueous extract of D. australe. Antipyretic effect of S. minima was also observed but no significant effect was found.

Keywords: Analgesic, Antipyretic, *Dactyloctenium australe* Steud., *Salvinia minima* Baker.

INTRODUCTION

Nature acts as a good source of salvation for human being by providing different remedies from its plants, animals, and other sources to cure all ailments of mankind[1]. Among the natural sources, medicinal plants are one of the most important contributors to the medicinal preparations as raw plant materials, refined crude extracts and mixtures etc. Several thousands of plants containing medicinal values have been identified and used to treat different ailments [2]. Even in this recent time, majority of the people are still depending on the traditional medicine for their primary health care [3]. So, medicinal plants are an essential element of indigenous medical systems. Besides report shows that almost 80% of the world population still uses plants for various medical purposes [4, 5].

Salvinia minima Baker belongs to the family Salviniaceae is usually referred to as common salvinia or water spangles [6]. Salviniaceae is a family of heterosporous ferns and this family contains the two genera *Azolla* and *Salvinia*. [7,8]. Common salvinia (*Salvinia minima* Baker) is a free-floating aquatic fern occuring in nature as a sporophyte. It contains a horizontal rhizome lying just below the surface of the water with a pair of floating leaves [9]. *S. minima* can have adverse affects on crawfish farming, rice farming, and other commercial activities that occur in waterways where it is present [10]. *Dactyloctenium australe* Steud. is a species which belongs to the family of the Poaceae (*Gräser*). The Poaceae (also called Gramineae or true grasses) are a large family of flowering plants. Poaceae is the the fifth-largest plant family, following the Orchidaceae, Asteraceae, Fabaceae, and Rubiaceae [11].

These two plants are new in recearch field, *Dactyloctenium australe* is totally new. There were no research work concerning this plant. Morever, inadequate literatures concerning the analgesic and antipyretic properties of *Salvinia*

minima initiated the present study to find out the analgesic and antipyretic potentials of extract of *Salvinia minima* and *Dactyloctenium australe* .

Analgesic compounds selectively relieve pain as a symptom by acting in the CNS or on the peripheral pain mechanism without affecting its cause [12]. Pyrexia or fever is usually caused as a secondary impact of infection, tissue damage, inflammation, graft rejection and malignancy or other diseased states. The body by it's natural defense mechanism create an environment where infectious agent or damaged tissue cannot survive. Normally the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediator's such as cytokines like interleukin 1β, α, β and TNF-α, which generally increase the synthesis of prostaglandin E2 (PGE2) near preoptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature [13]. Most of the antipyretic drugs normally prevent or inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE2 biosynthesis [14]. Moreover, these synthetic agents irreversibly inhibit COX-2 with high selectivity which are toxic to the hepatic cells, glomeruli, cortex of brain and heart muscles, whereas natural COX-2 inhibitors usually have lower selectivity with fewer side effects [14]. Therefore, a natural antipyretic agent with reduced or no toxicity is essential for our own benefit.

MATERIALS AND METHODS

Plant materials collection

Leaves of two plants named *Salvinia minima* Baker and *Dactyloctenium australe* Steud. were collected by the authors from the surrounding area of Noakhali, a coastal region of Bangladesh, in May, 2011. *Salvinia minima* was collected from the pond of Noakhali Govt. College and *Dactyloctenium australe* was from the premises of Noakhali Govt. College. The plants were identified and authenticated by expert taxonomist Prof. Bikash Ranjan Deb, department of Botany, Noakhali Govt. College, where the voucher specimen has been deposited for future reference.

Preparation of Plant Extract

The aqueous extract of each plant was prepared according to the method used in Bangladeshi traditional medicine. 20g of fresh leaves of each plant was crushed in blender in 500 ml of distilled water, separately. The extract of each plant obtained was filtered, evaporated by water bath, lyophilised and stored at 4°C until further use [15].

Test animals

Young male Swiss-albino mice aged 4-5 weeks, average weight 20-25gm were collected from Animal Resource Branch of ICDDR,B (International Centre for Diarrhoeal Disease and Research, Bangladesh). They were kept in suitable condition for one week for adaptation and fed rodent food and water *ad libitum* formulated by ICDDR, B. The animals were maintained consciously under standard environmental conditions (temperature: 25.0±1.0°C, relative humidity: 55-65% and 12 h light/ dark cycle) and had free access to feed and water *ad libitum*. All protocols for animal experiment were approved by the animal ethical committee of NSTU (Noakhali Science and Technology University). For each plant extract animals were divided into ten groups each of six animals (n=6) for analgesic and antipyretic studies.

Analgesic activity of each plant

The analgesic activity was investigated by hot plate (Eddy's Hot Plate; Techno, India) method [16]. Three different groups of mice received orally 100, 200 and 300mg/kg of the extract of plant. Aceclofenac (20mg/kg) was given orally to positive control and distilled water (10ml/kg) was administered to control group. One hour after treatment, the animals were placed on Eddy's hot plate kept at a temperature of 55±0.5 ^{0}C [17]. A cut off period of 15 s [18] was observed usually to avoid damage to the paw. The time taken by the mice to start licking the paw or jump out of the hot plate was recorded as the reaction time. The test was carried carefully before the treatment and at 60, 90, 120, 150 and 180 min after administration.

Antipyretic activity of each plant

The antipyretic activity was evaluated by Brewer's yeast induced pyrexia in experimental animal [19]. Hyperpyrexia was induced by subcutaneous administration of 10 ml/kg body weight 20% aqueous suspension of brewer's yeast. The selected animals were fasted overnight with water *ad libitum* before the experiments. Initial rectal temperature of animals was recorded using an Ellab thermometer. After 18 hrs of subcutaneous administration the animals that showed an increase of 0.3–0.5 °C in rectal temperature were selected for the antipyretic activity. Aqueous extract of plant was given orally (100, 200 and 300mg/kg). Paracetamol (100mg/kg orally) was used orally as reference drug. Control group received distilled water (10ml/kg) only. The rectal temperature was recorded at 1 h intervals for 4 h after treatment [20].

Statistical Analysis

Results of the experiments were expressed as mean±SEM. Statistical significance was determined using the Student's t – test and values with P<0.01 were considered significant [21]

RESULTS AND DISCUSSION

Analgesic activity

Hot plate method

Results of hot plate test are presented in Table 1 and Table 2 for the aqueous extract of *Salvinia minima* and *Dactyloctenium australe* respectively. The extracts of both the plants were found to exhibit a dose dependent increase in latency time when compared with control. The aqueous extract of *Salvinia minima* (100, 200 and 300mg/kg orally) presented a potent antinociceptive activity in hot plates method (Table 1). However, the extract (200 and 300mg/kg) showed significant analgesic effect 60 and 90min after administration. On the other hand, the aqueous extract of *D. australe* (100, 200 and 300mg/kg orally) presented a potent antinociceptive activity in hot plate method (Table 2). However, the extract (300mg/kg) exhibited significant analgesic effect 90min after administration. Hot plate test is used to study the peripheral analgesic effects. So the results obtained may be supported the ability of the aqueous extracts to have peripheral pain inhibition mechanisms.

Table 1: Effect of the aqueous extract of *Salvinia minima* 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)	-	11.27±0.23	11.07±0.14	11.36±0.15	11.28±0.19	11.24±0.23	11.29±0.18
Aceclofenac	20	11.43±0.39	13.13±0.23**	13.14±0.04**	12.63±0.06*	12.17±0.19*	11.68±0.28
Aqueous extract	100	11.24±0.11	11.85±0.13*	12.54±0.05*	11.63±0.26	11.19±0.2	11.13±0.56
	200	11.68±0.4	12.8±0.2**	12.58±0.1**	11.88±0.12*	11.55±0.17	11.49±0.1
	300	11.04±0.23	12.95±0.23**	13.15±0.24**	12.86±0.33*	12.55±0.09*	12.17±0.18*

Each value represents the mean ±S.E.M. (n=6)
*** P < 0.001 compared with control.*
** P < 0.01*

Table 2: Effect of the aqueous extract of *D. australe* 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)	-	11.25±0.42	11.05±0.17	11.53±0.29	11.11±0.32	11.5±0.57	11.41±0.36
Aceclofenac	20	11.88±0.13	13.09±0.22**	13.81±0.12**	13.05*±0.17	12.55±0.32	11.73±0.27
Aqueous extract	100	11.36±0.23	11.78±0.13*	11.32±0.23	10.88±0.13	11.25±0.73	11.18±0.53
	200	11.78±0.53	12.18±0.13	12.32±0.23*	11.11±0.12	11.75±0.13	11.72±0.33
	300	11.28±0.34	12.67±0.73	13.48±0.36*	12.71±0.43*	12.09±0.13	11.88±0.43

Each value represents the mean ±S.E.M. (n=6)
*** P < 0.001 compared with control.*
** P < 0.01*

Table 3: Effect of the aqueous extract of *Salvini minima* on yeast-induced pyrexia in mice

Groups	**Dose (mg/kg)**	**Rectal temperature °C before yeast injection**	**Rectal temperature °C after yeast injection**				
			0h	**1h**	**2h**	**3h**	**4h**
Control (distilled water)	-	35.9±0.18	37.5±0.23	37.82±0.13	37.76±0.08	37.11±0.24	37.4±0.11
Paracetamol	100	36.2±0.16	37.43±0.02	36.44±0.09**	36.18±0.19**	36.29±0.12*	36.85±0.31
Aqueous extract	100	35.9±0.28	37.56±0.05	37.54±0.1	37.72±0.04	37.22±0.1	37.51±0.34
	200	36.5±0.55	37.51±0.05	37.26±0.57	37.08±0.09**	37.58±0.14	37.49±0.43
	300	36.4±0.6	36.39±0.16	37.54±0.12	37.48±0.2	37.42±0.27	37.37±0.15

Each value represents the mean ±S.E.M. (n=6)
*** P < 0.001 compared with control.*
**P < 0.01*

Antipyretic activity

Antipyretic effect is not significantly exhibited by *Salvinia minima*. The aqueous extract has not shown significant degree of antipyretic activity. The slight reduction of hyperthermia was pronounced 120 min after administration (200mg/kg orally) and was not prolonged for next two hours.(Table 3). But the aqueous extract of *D. australe* showed significant degree of antipyretic activity at dose of 200 and 300mg/kg.(Table 4). The reduction of hyperthermia was pronounced 60 min after administration and was prolonged for three hours. Subcutaneous

administration of yeast induces pyrexia by synthesis of prostaglandin [22] Since, the inhibition of prostaglandin may be responsible for antipyretic effect of aqueous extract of *D. australe.*

Table 4: Effect of the aqueous extract of *D. australe* on yeast-induced pyrexia in mice

Groups	Dose (mg/kg)	Rectal temperature °C before yeast injection	Rectal temperature °C after yeast injection				
			0h	1h	2h	3h	4h
Control (distilled water)	-	35.9±0.19	37.5±0.23	37.82±0.13	37.76±0.08	37.11±0.24	37.4±0.11
Paracetamol	100	36.2±0.15	37.43±0.02	36.44±0.09**	36.18±0.19**	36.29±0.12*	36.85±0.31
Aqueous extract	100	35.9±0.21	37.56±0.25	37.42±0.07*	37.75±0.01	37.22±0.16	37.22±0.22
	200	36.1±0.35	37.44±0.03	36.67±0.12**	37.08±0.09**	36.94±0.11	36.90±0.27
	300	36.0±0.05	36.56±0.5	36.31±0.23**	36.98±0.32	36.16±0.17**	36.98±0.08

Each value represents the mean ± S.E.M. (n=6)
*** P < 0.001 compared with control.*
**P < 0.01*

DISCUSSION

It is well known that pharmaceutical companies throughout the world are interested in developing safer and more effective drugs to treat pain, fever and so on. [23]. The present study evaluated the analgesic and antipyretic effect of aqueous extract *Salvinia minima* and *Dactyloctenium australe* in experimental animal models.

In hot plate method, the aqueous extracts of both plants showed significant analgesic effect compared to the reference drug. The significant pain reduction of both plant extracts might be due to the presence of analgesic principles acting with the prostaglandin pathways. This test indicates that the extracts of both plants may be centrally acting. [17].

Subcutaneous administration of Brewer's yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered as a useful experiment for the screening of plants materials as well as synthetic drugs for their antipyretic effect [24,25]. Yeast-induced pyrexia is known as pathogenic fever. Its etiology could be the production of prostaglandins [26]. The inhibition of prostaglandin production could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin production can be achieved by blocking the cyclo-oxygenase enzyme activity. There are several mediators in our body for pyrexia and the proper inhibition of these mediators are more responsible for the antipyretic effect [13,14]. The intraperitoneal administration of *Dactyloctenium australe* leaves extract significantly attenuated rectal temperature of yeast induced febrile mice. Thus it can be postulated that this extract contained pharmacologically active principle(s) that interfere with the release of prostaglandins. Thus this result supports the use of *D. australe* as an antipyretic for the treatment of fever. But our another plant named *Salvinia minima* has failed to exhibit a significant antipyretic effect.

CONCLUSION

In conclusion, we can confirm that the aqueous extracts of both plants are endowed significant analgesic properties. However, further study is needed in order to understand the precise mechanism. *Dactyloctenium australe* leaves extract significantly reduces rectal temperature of yeast induced febrile mice. So it can be used as an antipyretic for the treatment of fever. But another plant named *Salvinia minima* has failed to exhibit a significant antipyretic effect.

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