Analgesic, Anti-Inflammatory and Antipyretic Effect of *Mentha Spicata* (Spearmint)

Patwary Md Hajjaj Yousuf[1], Nusrat Yousuf Noba[1], Mohammad Shohel[1], Rajib Bhattacherjee[1] and Biplab Kumar Das[2]*

[1]Department of Pharmacy, North South University, Bashundhara, Dhaka-1229, Bangladesh;
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.

ABSTRACT

Aim : *Mentha spicata* (L.) is popularly used as herbal remedy for various ailments. But the scientific basis for its medicinal use especially in pain and inflammation remains unknown. Therefore, the present study was aimed to investigate the analgesic, anti-inflammatory and antipyretic effects of whole plant of *Mentha spicata* in laboratory animals.
Materials and Method: The methanol extract of *Mentha spicata* (MEMS) was used to investigate the acute effect on analgesia by Hot-plate test and acetic acid induced writhing method (By acetic acid) in mice and on inflammation in rats by carrageen induced paw edema method. Subcutaneous injection of 20% aqueous suspension of Brewer's yeast in wistar rats leads to pyrexia.
Results: The extract showed a significant ($p<0.001$) dose dependent increase in reaction time in mice in the hot-plate test at the doses of 250 mg/kg and 500 mg/kg body weight. The extract showed a significant ($p<0.05$) dose dependent increase in reaction time in mice in writhing method at the doses of 250 and 500 mg/kg body weight. The extract also exhibited promising anti-inflammatory effect as demonstrated by statistically significant ($p<0.05$) inhibition of paw volume by 42.58% at the dose of 250 mg/kg body weight and 45.10% at the dose of 500 mg/kg body weight at the sixth hour of study. Intraperitoneal administration of MEMS showed dose dependent decrease in body temperature in brewer's yeast induced hyperthermia in rats at both doses. However, MEMS significantly decreased body temperature ($p<0.05$) at 500mg/kg compared to control.
Conclusion: This study suggests that the methanol extract of *Mentha spicata* have analgesic, anti-inflammatory and antipyretic activity in a dose dependent manner which supports its use as an analgesic, anti-inflammatory and antipyretic drug in folk medicine. ~~This plant may be a useful source of lead components in the treatment of pain, fever and inflammation.~~

Keywords: analgesic, antipyretic, anti-inflammatory, Mentha Spicata

* Tel No: +88-01720051994; Fax No: +88-2-8852016
E-mail:bkdas72@northsouth.edu; bkdas72@yahoo.com

1. INTRODUCTION

Mentha, a member of the Labiatae family is originated from Eastern Asia. Among the two major forms, namely *Mentha piperita* L. and *Mentha spicata* L. *Mentha spicata* is locally known as 'Pudina' in Bangladesh. Its English name is Spearmint which is 30–100 cm long and is characterized by its strong odor [1,2]. It has smooth or gray haired leaves and its flowers are pale blue and collected at the edges of the branches as a long and narrow spike. It contains volatile oil, carvone, limonene, *cis*-carveol, 1,8 cineol , *cis*-dihydrocarvone , carvyl acetate, *cis*-sabinene hydrate of which carvone is the most important constituent of *M. spicata* [3].

Indian and Eastern Asian people use spearmint as a common constituent in their diet. It is used with spices to give the food a special flavor and fragrance, also used for flavoring chewing gums, toothpaste, confectionery and pharmaceutical preparations [4]. Spearmint essential oil is a common constituent in hygiene and cosmetic products, and substantial amounts are used in the food and beverage industries [5]. The dry or fresh leaves of spearmint are added by the Middle East and African during the brewing of tea, where it provides a pleasant aroma and refreshing taste [6,7]. There was an investigation that confirmed that spearmint had significant inhibitory effects against the cooked meat heterocyclic amine mutagen both *in vitro* and *in vivo* [8].

Mentha spicata has high traditional medicinal value as it is one of the important constituents of Ayurveda, Homeopathy and Siddha systems of medicine. *Mentha* can be used for common cold, cough, sinusitis, fever, bronchitis, nausea, vomiting, indigestion, intestinal colic and loss of appetite [9]. It can have a calming effect when used for insomnia or massages. Essential oil of Spearmint was found to have some antimicrobial activity [10]. It is also a safe and effective therapeutic option for the treatment of chemotherapy-induced nausea and emesis in patients [11]. Spearmint (*Mentha spicata* L.) is widely used as a source of essential oils for flavouring agents, and more recently it has been used as a valuable source of the potent antioxidant rosmarinic acid for the neutraceutical and cosmetic industries [12]. Rosmarinic acid has earned the reputation as a molecule of interest owing to its multiple biological activities against inflammatory lung diseases, autoimmune arthritis, heart disease and suppression of autoimmune rejection in human skin transplant patients as well as its multipurpose activities against reverse transcriptase, integrase and RNase H in HIV infections [13-17]. Therefore interest in cultivating a quantifiable natural source of this potent and versatile antioxidant has become paramount.

Mint oil (oil obtained from *Mentha spicata*) also inhibits the inflammatory consequences of lipopolysaccharide (LPS), including inhibition of interleukin-1 (IL-1), prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4) production by LPS-stimulated human monocytes [18]. As these biological actions are considered to be related to the rosmarinic acid (RA) content of the plant, considerable effort has been invested in developing strategies to upregulate biosynthesis of RA by genetically modified plant tissues [19,20]. These efforts have successfully resulted in RA production of up to 45 mg/g plant tissue. Recently, selective breeding of *Mentha spicata* clones has generated plants which naturally over-produce RA, resulting in tissue concentrations of up to 122 mg/g [21,22]. The processed High-Rosmarinic-Acid of *M. spicata* resulting from these experiments has shown marked antioxidant activity *in vitro* [12,13] and may be an ideal candidate for nutritional intervention for inflammatory diseases [23]. Recent research has shown that spearmint tea may be used as a treatment for hirsutism in women, due to its anti-androgenic properties which reduces

* Tel No: +88-01720051994; Fax No: +88-2-8852016
E-mail:bkdas72@northsouth.edu; bkdas72@yahoo.com

the level of free testosterone in the blood and increase in LH and FSH levels, without affecting total testosterone and dehydroepiandrosterone (DHEA) [24,25]. In contrast, study revealed that the consumption of *Mentha longifolia* L. syrup decreased LH levels.

This present investigation was aimed to evaluate the analgesic (by writhing method and hot plate method), anti-inflammatory (carrageenan-induced rat paw edema method) and antipyretic effect (*yeast* induced pyrexia in rat method) of methanol extract of *Mentha spicata.*

2. MATERIAL AND METHODS

2.1 Plant material

The whole plant of *Mentha spicata* was collected from Amin bazar, Savar, Dhaka, Bangladesh, on 10th January 2012 when the plant is fully flowered. The plant was identified by the experts of Bangladesh National Herbarium (Accession No.37792).

2.2 Extraction

The collected plant were washed with water and separated from undesirable materials or plants or plant parts. They were partially dried by air and then heated in an oven at bellow 40ºC for two days to be fully dried. The fully dried leaves were then grinded to make them powder by the help of a suitable grinder. Then the powders were dissolved in methanol (80%) and kept for a period of 2 days accompanying occasional shaking and stirring. The whole mixture was then undergone a coarse filtration by a piece of clean, white cotton material followed by a second filtration through whatman filter paper. The filtrate obtained was evaporated by rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 5 to 6 rpm and 65ºC temperature. It rendered a gummy concentrate of chocolate black color that was designated as methanol extract of *Mentha Spicata* (MEMS). The crude methanol extract was finally dried by freeze drier and preserved.

2.3 Laboratory animals

Young Swiss-Albino mice aged about 4-5 weeks with average weight of 25-30 gm and adult Albino rats (Wistar strain) having average weight of 100-130 gm were used for this study. They were kept in standard environmental condition at 25°C for one week in the animal house of the Department of Pharmacy, North south University, Bangladesh for adaptation after their purchase. The animals were provided with standard laboratory food and tap water ad libitum and maintained at natural day night cycle. All the animals were used by the prior ethical approval of Institutional ethical approval committee and the certificate no. is NSUEACN-0340.

2.4 Drugs and chemicals

Ketorolac , paracetamol (Beximco Pharmaceutical Ltd., Bangladesh), acetic acid, Brewer's yeast (Merck Germany), carrageenan (Sigma Lambda, USA) were purchased.

2.5 Methods for the Evaluation of Analgesic Effect

2.5.1 Hot-plate test

* Tel No: +88-01720051994; Fax No: +88-2-8852016
E-mail:bkdas72@northsouth.edu; bkdas72@yahoo.com

The hot-plate test (Hot/Cold Plate Model-35100-001, UGO Basile, Italy) was employed for measurement of analgesic activity [26,27]. The temperature was regulated at 55° ± 1°C. Mice were divided into four groups consisting of five animals in each group. The mice of each group were placed in the beaker (on the hot plate) in order to obtain its response to electrical heat induced pain stimulus. Licking of the paws or jumping out of the beaker was taken as an indicator of the animal's response to heat-induced pain stimulus. The time for each mouse to lick its paws or jump out of the beaker was taken as reaction time (in second). Before treatment, the reaction time was taken once. The mean of this determination constituted initial reaction time before treatment of each group of mice. Each of the test mice was thereafter treated with either distilled water (DW), Ketorolac (2.5 mg/kg of body weight) or methanol extract of *M. spicata* at the doses of 250 and 500 mg/kg body weight orally. Thirty minutes after treatment, the reaction time of each group mice were again evaluated five times individually in one hour interval on this occasion.

Percent analgesic score was calculated as,

$$PAS = Tb\text{-}Ta/Tb \times 100$$

Where, Tb= Reaction time (in second) before drug administration;

Ta = Reaction time (in seconds) after drug administration.

2.5.2 Acetic acid induced writhing test

Acetic acid was administered intraperitoneally to the experimental animals to create pain sensation [28-30]. Ketorolac (10 mg/kg) was used as a positive control or a standard. The plant extract was administered orally in two different doses (250 and 500 mg/kg body weight) to the Swiss Albino mice after an overnight fast. Test samples and vehicle were administered orally 30 minutes prior to intraperitoneal administration of 0.7% v/v acetic acid solution at 10 ml/kg body weight. Animals were kept individually under glass jar for observation. Each mouse of all groups were observed individually for counting the number of writhing they made in 5minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. The number of writhes in each treated group was compared to that of a control group (Distilled water). % inhibition formula = [(C - T) / C] × 100%

Where, C = Mean of control

T = Mean of treated

2.6 Method for the Evaluation of Anti-inflammatory Effect

2.6.1 Carrageenan induced rat paw edema

Rats were randomly divided into four groups, each consisting of five animals, of which group I was kept as control giving only distilled water .Group II was given Ketorolac (10 mg/kg) as standard. Group III and group IV were given the test sample at the dose of 250 and 500 mg/kg body weight respectively. Half an hour after the oral administration of the test materials, 1% carrageenan was injected to the left hind paw of each animal. The volume of paw *edema* was measured at ½, 1, 2, 3 and 6 hours using plethysmometer after administration of carrageenan. The right hind paw served as a reference of non-inflamed paw for comparison [28].

The average percent increase in paw volume with time was calculated and compared against the control group. Percent inhibition was calculated using the formula-

$$\% \text{ Inhibition of paw } edema = V_c - V_t / V_c \times 100$$

* Tel No: +88-01720051994; Fax No: +88-2-8852016
E-mail:bkdas72@northsouth.edu; bkdas72@yahoo.com

Where V_c and V_t represent average paw volume of control and treated animal respectively.

2.7 Evaluation of Antipyretic Activity

The antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in rats [31-33]:. Wister albino rats were selected, weighed and divided in to three groups of five animals each. All these animals were fasted 18 h prior to commencement of experiment but water was provided *ad libitum*. Fever was induced by injecting 20 ml/kg (s.c.) of 20% aqueous suspension of Brewer's yeast in normal saline below the nape of the neck and rectal temperature was recorded by clinical thermometer immediately before (-18 h) and 18 h after (0 h) Brewer's yeast injection. Prior to the experiment, the rats were maintained in separate cages for 7 days and the animals with approximately constant rectal temperature were selected for the study. Paracetamol (100 mg/kg, p.o.) was used as standard drug for comparing the antipyretic action of extract. The extract at the doses of 500 mg/kg was administered intraperitoneally (i.p.), one group was administered with paracetamol (100 mg/kg) i.p. control group was given 0.5 ml normal saline. The rectal temperature was measured at 1, 2 and 3 h after drug administration by using digital thermometer. Percentage reduction in rectal temperature was calculated by considering the total fall in temperature to normal level.

2.8 Acute Toxicity

The acute toxicity test was carried out for MEMS to evaluate any possible toxicity. Mice (n = 6) of either sex were treated with different doses (500, 1000 and 2000mg/kg, p.o.), while the control group received saline (10ml/kg). All the groups were observed for any gross effect for first 4h and then mortality was observed after 24h [34].

2.9 Statistical Analysis

Results were expressed as Mean ± SEM (Standard Error Mean). The significance of difference between the control and treatment groups were determined using one way analysis of variance (ANOVA) and Dunnett's t-test. P value < 0.05 was considered as the minimum level of significance. SPSS statistical software was used.

3. RESULTS AND DISCUSSION

The methanol extract of *Mentha spicata* exhibited significant ($p < 0.001$) analgesic effect in hot plate test. The results were presented in Table 1 and Figure 1. The extract significantly increased the reaction time of mice in a dose-dependent manner. The maximum analgesic (40.38%, 250 mg/kg to 42.38%, 500 mg/kg) effect was observed at 3 hour post administration of the test material which was comparable to that of the standard drug Ketorolac (42.73%).

Table 1: Results of analgesic activity study of MEMS using the hot plate method

* Tel No: +88-01720051994; Fax No: +88-2-8852016
E-mail:bkdas72@northsouth.edu; bkdas72@yahoo.com

Group	Response time at different time intervals (in Sec)					
	0 Hour	½ Hour	1 Hour	2 Hour	3 Hour	4 Hour
Control	10.70±.846	9.660±.936	8.00±.814	6.580±.640	5.520±.549	5.0±.442
Standard	9.140± .524	11.02±1.001	12.60±.944**	14.160±1.076**	15.96±.676***	12.48±.698***
MEMS 250 mg/kg	9.020 ±.787	10.56±.773	11.680±.753*	13.10±.6841**	14.0±.501***	11.66±.186***
MEMS 500 mg/kg	8.980±.690	10.87±.639	11.80 ±.621*	13.820±.685**	14.52±.596***	11.89±.398***

Data are reported as mean ± S.E.M. The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *P < 0.05, **P < 0.01, ***P < 0.001

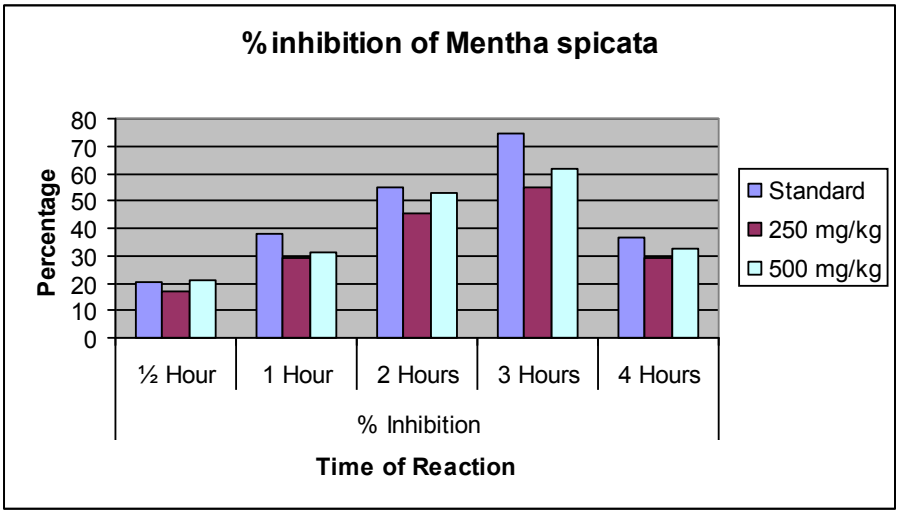

Fig 1: % of inhibition of analgesia of *MEMS*

3.3 Analgesic Activity by Acetic Acid Induced Writhing Method

In the acetic acid induced writhing test, the analgesic activity of MEMS was significantly ($p < 0.001$) revealed at the doses of both 250 and 500 mg/kg (Table 2). The percentage inhibition by MEMS at the dose of 500 mg/kg (60.30%) was comparable to that of the standard (66.66%).

Table 2: Results of analgesic activity study of MEMS using acetic acid induced writhing method

* Tel No: +88-01720051994; Fax No: +88-2-8852016
E-mail:bkdas72@northsouth.edu; bkdas72@yahoo.com

Group	Dose	Route	No. of writhing (Mean± SEM)	% Inhibition
Control	10 ml/kg	p.o	22.8000±3.006	
Standard	10 mg/kg	p.o	7.6000±0.812***	66.66%
MEMS	250 mg/kg	p.o	10.2000±0.969***	55.26%
	500 mg/kg	p.o	9.0500±1.363***	60.30%

Data are reported as mean ± S.E.M. The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *P < 0.05, **P < 0.01, ***P < 0.001

3.4 Anti-inflammatory activity:

The anti-inflammatory activity at test doses (250, 500 mg/kg) of MEMS is presented in Table 3, with the average volume of the paw edema. MEMS showed a significant dose dependent reduction of paw edema at both the doses of 250 and 500 mg/kg body weight. However, maximum (80.60%) inhibition of paw volume was found to be at three hour of study at the dose of 250 mg/kg body weight (Figure 2). The anti-inflammatory response of the extract was less than that of standard over a period of 6 hour in carrageenan-induced inflammation.

Table 3: Anti-inflammatory activity study of MEMS using carrageenan induced rat paw edema method

Treatment group	Dose	Paw volume at different time interval (in ml)				
		0 Hour	1 Hour	2 Hours	3 Hours	6 Hours
Control	10 ml/kg	.682±.048	.874±.059	1.080±.052	1.168±.011	1.212±.037
Standard	10 mg/kg	.666±.044	.850±.026	.992±.035	1.150±.029	.978±.056**
MEMS	250mg/kg	.526±.039	.666±.034	.834±.074	.954±.081	.750±.059***
	500mg/kg	.572±.043	.746±.051	.877±.153	1.00±.0.445	.830±.044***

Data are reported as mean ± S.E.M. The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *P < 0.05, **P < 0.01, ***P < 0.001

* Tel No: +88-01720051994; Fax No: +88-2-8852016
E-mail:bkdas72@northsouth.edu; bkdas72@yahoo.com

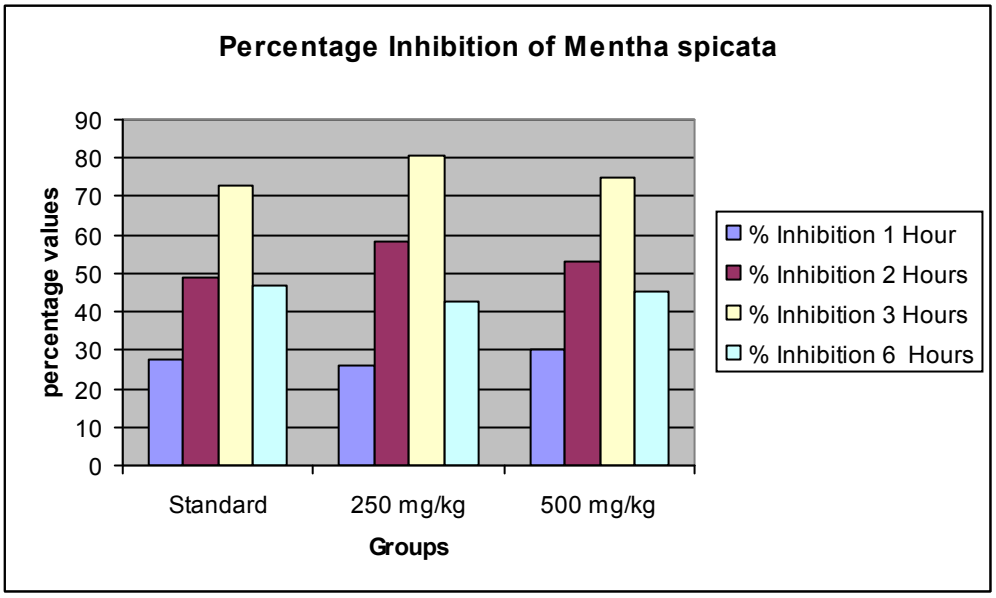

Fig 2: % of inhibition of inflammation of MEMS

3.5 Antipyretic activity by yeast induced pyrexia in rat method

The MEMS exhibited statistically highly significant ($p < 0.01$) antipyretic effect in yeast induced pyrexia in rat at the dose of 500 mg/kg at 3 hour (Table 4). Positive control paracetamol showed significant ($p < 0.05$) analgesic effect at the dose of 100 mg/kg at 2 hour and markedly ($p < 0.01$) at 3 hour.

Table 4: Antipyretic activity study of MEMS using yeast induced pyrexia in rat method

Data are reported as mean ± S.E.M. The data was analyzed by ANOVA followed by Dunnett's test. Asterisks

Group	Dose	Rectal temperature (°F)			
		0 Hour	1Hour	2 Hour	3 Hour
Control	0.5 ml/kg	92.00 ± 0.44	96.18 ± 0.44	96.38 ± 0.56	95.70 ± 0.66
Standard	100 mg/kg	91.90 ± 0.42	94.64 ± 0.68	93.56 ± 0.63*	91.98 ± 0.67**
MEMS	500 mg/kg	92.24± .21	94.82± 0.21	93.69± 0.20	92.14± 0.28**

indicated statistically significant values from control. *$P < 0.05$, **$P < 0.01$,

* Tel No: +88-01720051994; Fax No: +88-2-8852016
E-mail:bkdas72@northsouth.edu; bkdas72@yahoo.com

3.6 Acute toxicity

MEMS were found safe at all test doses (500, 1000 and 2000 mg/kgi.p.). During 24h assessment time, test animals were found normal.

4. DISCUSSION

Results of the present study showed that MEMS have marked antipyretic, analgesic and anti-inflammatory effects with a reasonable safety profile.

Hot plate method is a thermal nociception model which is the most common test for evaluating central analgesic efficacy of drugs/compounds. ~~The paws of mice are very sensitive to heat, at temperature which is not damaging to the skin. The responses are shaking, jumping, withdrawal of the paws and licking of the paws~~ [30].~~The time until these responses are prolonged after administration of centrally acting analgesics is measured as the indication of analgesic effect. MEMS showed significant ($P < 0.001$) prolongation of latency period in hot plate test that implicates spinal analgesic effect. In these pain paradigms ketorolac raised the pain threshold level within 30 min of administration. In contrast, MEMS showed maximum analgesic effect after 60 min of administration. This difference in the maximum analgesic point could be explained by difference in the metabolic rate of each drug or may be the potency of each drug as the analgesic potential of ketorolac is higher than MEMS (500 mg/kg). Moreover, MEMS showed a maximum effect after 60 min and remain up to 180 min in thermal tests.~~ The extract of the plant and ketorolac presented a longer latency time than the control group in the hot plate test in a dose dependant manner. Nociceptive pain inhibition was noticed higher at 180 minutes after administration of the extract and the response was comparable to standard drug ketorolac. As the hot plate method is considered to be selective for the centrally acting analgesics, the effect of the extract on this pain model indicates that it must have centrally acting antinociceptive activity.

The acetic acid-induced writhing is a sensitive method to evaluate peripherally acting analgesics~~. Methanolic extract of *Mentha spicata* possesses significant analgesic effects in the model of acetic acid-induced writhing test~~. Acetic acid induced writhing in mice finds much attention in the screening of analgesic drugs in acetic acid-induced abdominal writhing, the visceral pain model, released arachidonic acid via cyclooxygenase and prostaglandin biosynthesis which played a role in the nociceptive mechanism. This model of response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway. In other words, the acetic acid induced writhing has been associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products. The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability. The substance inhibiting the writhings will have analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [38]. Regarding the results of our extract in acetic acid-induced abdominal constriction assay, a prominent inhibition of writhing reflux was observed. These findings strongly recommend that MEMS has peripheral analgesic activity and their mechanisms of action may be mediated through inhibition of local peritoneal receptors which may be the involvement of cyclooxygenase inhibition potential. The profound analgesic activity of MEMS may be due to the interference of their active principle(s) with the release of pain mediators.

Carrageenan-induced paw edema is a well established animal model to assess the anti-inflammatory effect of natural products as well as synthetic chemical compounds. Edema

* Tel No: +88-01720051994; Fax No: +88-2-8852016
E-mail:bkdas72@northsouth.edu; bkdas72@yahoo.com

formation due to carrageenan in paw is a biphasic event, the initial phase (1h or 1.5h) is predominantly a non-phagocytic edema followed by a second phase (2–5 h) with increased edema formation that remained up to 5h [39]. The initial phase has been induced due to the action of mediators such as histamine, serotonin and bradykinin on vascular permeability. The late phase or second phase edema has been shown to be the result of overproduction of prostaglandins [35]. The result of pre-treatment of MEMS demonstrated that the extract is effective in the late phase of inflammation which is due to release of prostaglandins. The anti-inflammatory effect of the extract remains significant up to 6th h of the experiment.

Subcutaneous injection of Brewer's yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered as a useful test for the screening of plant materials as well as synthetic drugs for their antipyretic effect [40,41]. Yeast-induced pyrexia is called pathogenic fever and its etiology could be the production of prostaglandins. The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclooxygenase enzyme activity. There are several mediators for pyrexia and the inhibition of these mediators are responsible for the antipyretic effect [41]. The intraperitoneal administration of MEMS significantly attenuated rectal temperature of yeast induced febrile mice. Thus it can be postulated that MEMS contained pharmacologically active principle(s) that interfere with the release of prostaglandins.

5. CONCLUSION

In conclusion, ~~although *Mentha spicata* has not been evaluated in depth for its pharmacological properties but in our study~~, the methanol extract~~s~~ of *Mentha spicata* showed ~~highly~~ significant analgesic, anti-inflammatory and antipyretic properties. Further investigations are required to find the active component of the extract ~~and~~ in order to confirm the mechanism of action in the development of a potent analgesic, anti-inflammatory and antipyretic agent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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* Tel No: +88-01720051994; Fax No: +88-2-8852016
E-mail:bkdas72@northsouth.edu; bkdas72@yahoo.com

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* Tel No: +88-01720051994; Fax No: +88-2-8852016
E-mail:bkdas72@northsouth.edu; bkdas72@yahoo.com

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* Tel No: +88-01720051994; Fax No: +88-2-8852016
E-mail:bkdas72@northsouth.edu; bkdas72@yahoo.com