

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

3 **Patwary Md Hajjaj Yousuf¹, Nusrat Yousuf Noba¹, Mohammad Shohel¹,**
4 **Rajib Bhattacharjee¹ and Biplab Kumar Das^{2*}**

5 ¹Department of Pharmacy, North South University, Bashundhara, Dhaka-1229, Bangladesh;

6 ²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka,
7 Dhaka-1000, Bangladesh.

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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.

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

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1. INTRODUCTION

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

33

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

43

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

59 Mint oil (oil obtained from *Mentha spicata*) also inhibits the inflammatory consequences of
60 lipopolysaccharide (LPS), including inhibition of interleukin-1 (IL-1), prostaglandin E₂ (PGE₂),
61 leukotriene B₄ (LTB₄) production by LPS-stimulated human monocytes [18]. As these
62 biological actions are considered to be related to the rosmarinic acid (RA) content of the
63 plant, considerable effort has been invested in developing strategies to upregulate
64 biosynthesis of RA by genetically modified plant tissues [19,20]. These efforts have
65 successfully resulted in RA production of up to 45 mg/g plant tissue. Recently, selective
66 breeding of *Mentha spicata* clones has generated plants which naturally over-produce RA,
67 resulting in tissue concentrations of up to 122 mg/g [21,22]. The processed High-
68 Rosmarinic-Acid of *M. spicata* resulting from these experiments has shown marked
69 antioxidant activity *in vitro* [12,13] and may be an ideal candidate for nutritional intervention
70 for inflammatory diseases [23]. Recent research has shown that spearmint tea may be used
71 as a treatment for hirsutism in women, due to its anti-androgenic properties which reduces

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

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

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80 2. MATERIAL AND METHODS

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82 2.1 Plant material

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

87

88 2.2 Extraction

89

90 The collected plant were washed with water and separated from undesirable materials or
91 plants or plant parts. They were partially dried by air and then heated in an oven at below
92 40°C for two days to be fully dried. The fully dried leaves were then grinded to make them
93 powder by the help of a suitable grinder. Then the powders were dissolved in methanol
94 (80%) and kept for a period of 2 days accompanying occasional shaking and stirring. The
95 whole mixture was then undergone a coarse filtration by a piece of clean, white cotton
96 material followed by a second filtration through whatman filter paper. The filtrate obtained
97 was evaporated by rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 5 to 6 rpm and
98 65°C temperature. It rendered a gummy concentrate of chocolate black color that was
99 designated as methanol extract of *Mentha Spicata* (MEMS). The crude methanol extract was
100 finally dried by freeze drier and preserved.

101

102 2.3 Laboratory animals

103

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

112

113 2.4 Drugs and chemicals

114

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

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118 2.5 Methods for the Evaluation of Analgesic Effect

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120 2.5.1 Hot-plate test

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

$$136 \qquad \qquad \qquad \text{PAS} = \text{Tb} - \text{Ta} / \text{Tb} \times 100$$

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

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

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141 **2.5.2 Acetic acid induced writhing test**

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143 Acetic acid was administered intraperitoneally to the experimental animals to create pain
144 sensation [28-30]. Ketorolac (10 mg/kg) was used as a positive control or a standard. The
145 plant extract was administered orally in two different doses (250 and 500 mg/kg body weight)
146 to the Swiss Albino mice after an overnight fast. Test samples and vehicle were
147 administered orally 30 minutes prior to intraperitoneal administration of 0.7% v/v acetic acid
148 solution at 10 ml/kg body weight. Animals were kept individually under glass jar for
149 observation. Each mouse of all groups were observed individually for counting the number of
150 writhing they made in 5 minutes commencing just 5 minutes after the intraperitoneal
151 administration of acetic acid solution. The number of writhes in each treated group was
152 compared to that of a control group (Distilled water). % inhibition formula = $[(C - T) / C] \times$
153 100%

154 Where, C = Mean of control

155 T = Mean of treated

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157 **2.6 Method for the Evaluation of Anti-inflammatory Effect**

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159 **2.6.1 Carrageenan induced rat paw edema**

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

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

$$171 \qquad \qquad \qquad \% \text{ Inhibition of paw edema} = \frac{V_c - V_t}{V_c} \times 100$$

172 Where V_c and V_t represent average paw volume of control and treated animal respectively.
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2.7 Evaluation of Antipyretic Activity

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177 The antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in rats [31-33].
178 Wistar albino rats were selected, weighed and divided in to three groups of five animals
179 each. All these animals were fasted 18 h prior to commencement of experiment but water
180 was provided *ad libitum*. Fever was induced by injecting 20 ml/kg (s.c.) of 20% aqueous
181 suspension of Brewer's yeast in normal saline below the nape of the neck and rectal
182 temperature was recorded by clinical thermometer immediately before (-18 h) and 18 h after
183 (0 h) Brewer's yeast injection. Prior to the experiment, the rats were maintained in separate
184 cages for 7 days and the animals with approximately constant rectal temperature were
185 selected for the study. Paracetamol (100 mg/kg, p.o.) was used as standard drug for
186 comparing the antipyretic action of extract. The extract at the doses of 500 mg/kg was
187 administered intraperitoneally (i.p.), one group was administered with paracetamol (100
188 mg/kg) i.p. control group was given 0.5 ml normal saline. The rectal temperature was
189 measured at 1, 2 and 3 h after drug administration by using digital thermometer. Percentage
190 reduction in rectal temperature was calculated by considering the total fall in temperature to
191 normal level.

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2.8 Acute Toxicity

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

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

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209 The methanol extract of *Mentha spicata* exhibited significant ($p < 0.001$) analgesic effect in
210 hot plate test. The results were presented in Table 1 and Figure 1. The extract significantly
211 increased the reaction time of mice in a dose-dependent manner. The maximum analgesic
212 (40.38%, 250 mg/kg to 42.38%, 500 mg/kg) effect was observed at 3 hour post
213 administration of the test material which was comparable to that of the standard drug
214 Ketorolac (42.73%).
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216 Table 1: Results of analgesic activity study of MEMS using the hot plate method
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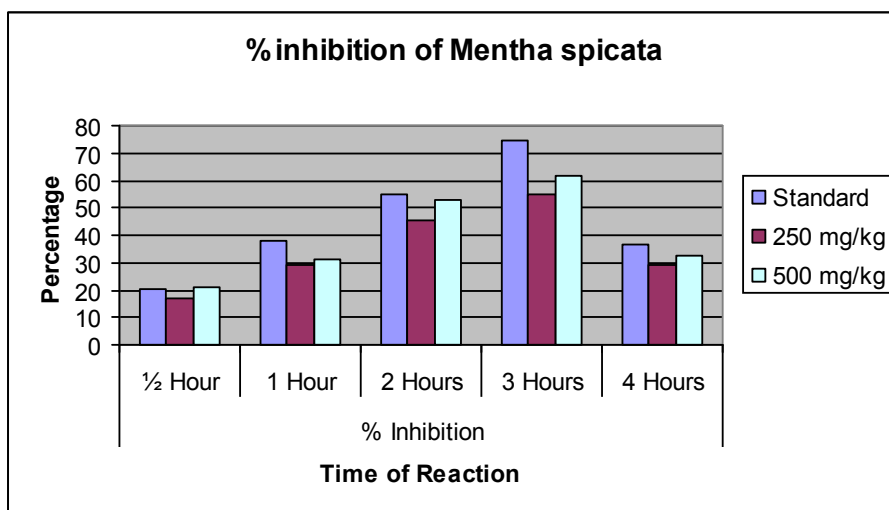
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Group	Response time at different time intervals (in Sec)					
	0 Hour	½ Hour	1 Hour	2 Hour	3 Hour	4 Hour
Control	10.70±.846	9.660±.936	8.00±.814	6.580±.640	5.520±.549	5.0±.442
Standard	9.140±.524	11.02±1.001	12.60±.944**	14.160±1.076**	15.96±.676***	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***

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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. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$



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Fig 1: % of inhibition of analgesia of MEMS

3.3 Analgesic Activity by Acetic Acid Induced Writhing Method

240 In the acetic acid induced writhing test, the analgesic activity of MEMS was significantly ($p <$
241 0.001) revealed at the doses of both 250 and 500 mg/kg (Table 2). The percentage inhibition
242 by MEMS at the dose of 500 mg/kg (60.30%) was comparable to that of the standard
243 (66.66%).

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

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%

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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. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

259 3.4 Anti-inflammatory activity:

260 The anti-inflammatory activity at test doses (250, 500 mg/kg) of MEMS is presented in Table
261 3, with the average volume of the paw edema. MEMS showed a significant dose dependent
262 reduction of paw edema at both the doses of 250 and 500 mg/kg body weight. However,
263 maximum (80.60%) inhibition of paw volume was found to be at three hour of study at the
264 dose of 250 mg/kg body weight (Figure 2). The anti-inflammatory response of the extract
265 was less than that of standard over a period of 6 hour in carrageenan-induced inflammation.

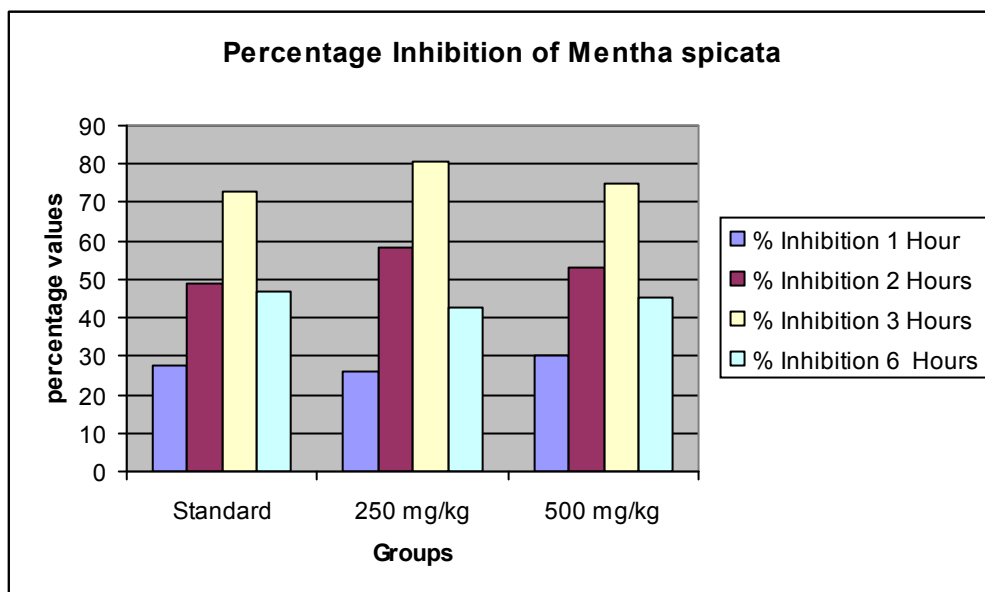
266 **Table 3: Anti-inflammatory activity study of MEMS using carrageenan induced rat paw**
267 **edema method**

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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***

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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. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$



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276 **Fig 2: % of inhibition of inflammation of MEMS**

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278 **3.5 Antipyretic activity by yeast induced pyrexia in rat method**

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

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

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

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

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

297 **3.6 Acute toxicity**

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

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302

303 **4. DISCUSSION**

304

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

307

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

326

327 The acetic acid-induced writhing is a sensitive method to evaluate peripherally acting
328 analgesics. Methanolic extract of *Mentha spicata* possesses significant analgesic effects in
329 the model of acetic acid induced writhing test. Acetic acid induced writhing in mice finds
330 much attention in the screening of analgesic drugs in acetic acid-induced abdominal
331 writhing, the visceral pain model, released arachidonic acid via cyclooxygenase and
332 prostaglandin biosynthesis which played a role in the nociceptive mechanism. This model of
333 response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and
334 the prostaglandin pathway. In other words, the acetic acid induced writhing has been
335 associated with increased level of PGE2 and PGF2 α in peritoneal fluids as well as
336 lipoxygenase products. The increase in prostaglandin levels within the peritoneal cavity then
337 enhances inflammatory pain by increasing capillary permeability. The substance inhibiting
338 the writhings will have analgesic effect preferably by inhibition of prostaglandin synthesis, a
339 peripheral mechanism of pain inhibition [38]. Regarding the results of our extract in acetic
340 acid-induced abdominal constriction assay, a prominent inhibition of writhing reflex was
341 observed. These findings strongly recommend that MEMS has peripheral analgesic activity
342 and their mechanisms of action may be mediated through inhibition of local peritoneal
343 receptors which may be the involvement of cyclooxygenase inhibition potential. The
344 profound analgesic activity of MEMS may be due to the interference of their active
345 principle(s) with the release of pain mediators.

346

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

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

357

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

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370

371 5. CONCLUSION

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

379

380 COMPETING INTERESTS

381

382 Authors have declared that no competing interests exist.

383

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