“ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF STEM BARK OF *MYRICA NAGI* (T.)”

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**ABSTRACT**

*Aim of the study:* *M. nagi* is traditionally used in the treatment of various inflammatory conditions like chronic bronchitis and asthma and in relief of dental pain; however there is a scarcity of scientific data to support the ethnobotanical claim. The aim of the present study was to evaluate the analgesic and anti-inflammatory activity of MNSBEE in laboratory animals. **Materials and methods:** Analgesic activity was carried out by acetic acid-induced abdominal writhing and hot plate test in mice at the dose level of 100, 200 and 400 mg/kg. Anti-inflammatory activity was carried out by carrageenan (acute) and cotton pellet granuloma (chronic) tests and in combination with MNSBEE (400 mg/kg) and diclofenac (5 mg/kg) in rats. **Results:** The pharmacological screening revealed that MNSBEE significantly increased pain latency in acetic acid-induced writhing and hot plate in mice and significantly inhibited paw edema volume in carrageenan-induced paw edema and significantly inhibited granuloma weight in cotton pellet granuloma model at the doses of 200 and 400 mg/kg, p.o. The combination of MNSBEE and diclofenac has been studied and found to be most significant anti-inflammatory effect without toxic effect. **Conclusion:** The observed results suggested the MNSBEE showed significant analgesic and anti-inflammatory activity in acute as well as chronic conditions which support its folk medicine use. Thus, the combination of herbal product (ethanolic extract of *Myrica nagi*) with modern medicine will produce the best anti-inflammatory action and will be useful for long-term treatment of chronic conditions.

**KEYWORDS:** *Myrica nagi*, Analgesic, Anti-inflammatory, Carrageenan, Hot Plate.
1. INTRODUCTION

Plants have been used in the traditional health care system from time particularly among the local and indigenous communities (Slomon J et al., 2011). *Myrica nagi* (Thunb.) is a subtropical shrub commonly known as box berry, *M. esulanta* ‘Katphala’ (Hindi) member of Myricaceae. It is an evergreen, sun temperate tree growing to 12 m height (Alam A et al., 2000). The medicinal uses and chemical constituents of *Myrica nagi* have been widely studied (Malterud KE et al., 1996). Ethanolic extract (50%) of *M. esulanta* stem bark showed antiprotozoal activity against *Ent. histolytica* strain and hypotensive effect in dog/cat. It showed antispasmodic activity on isolated guinea pig ileum (Dhar ML et al., 1968). The dried water extract of *M. esulanta* stem bark in a dose of 250 mg/kg i.p showed analgesic action in rats by tail flickering method (Gupta RA et al., 1982). The ethyl acetate extract of bark was investigated for mast cell stabilizing activity (Patel T et al., 2011). The chloroform and ethyl acetate extract of *Myrica nagi* was established for anti-neoplastic activity (Masud Rana AYKM et al., 2004) the other activity which is reported as, anti-inflammatory (Nadkarni KM, 1954), chronic bronchitis (Slomon J et al., 2011; Satyavati GV et al., 1987), antibacterial (Normand P et al., 1996), antihelmintic (Jain VK et al., 2010), anxiolytic and antidepressant (Md.Khan Y et al., 2008), antioxidant activity (Tapan S, 2011).

Phytochemicals reported in *M. nagi* are tannins, saponins, gallic acid, flavanoids, flavanols, alkaloids, phenolic compounds, triterpenes, β- sitosterols (Sun D et al., 1988).

However the analgesic and anti-inflammatory activity of the ethanolic extract of stem bark of *Myrica nagi* (called as MNSBEE) has not been reported. The objective of the present investigation was to evaluate the analgesic and anti-inflammatory activity MNSBEE.

2. MATERIALS AND METHODS

2.1. Collection and authentification of plant

*Myrica nagi*. Bark was collected from Shimla. The plant was identified and authenticated at National Botanical Research Institute (NBRI), Lucknow, India and voucher specimen was deposited at that Institute.

2.2. Drugs and Chemicals

Carrageenan, acetyl salicylic acid, pentazocin and diclofenac were purchased from Sigma-Aldrich, MO, USA. Acetic acid, ethanol and tween 80 were purchased from Research lab, India.
2.3. Preparation of Ethanolic extract
Air-dried and powdered bark of *myrica nagi* was exhaustively extracted with ethanol in soxlet apparatus. The obtained extracts were filtered and evaporated under reduced pressure, on a rotary evaporator at 40–45 °C (yield 13.3% w/w).

2.4 Experimental animals
Wistar rats (150–180 g) and Swiss albino mice (25–30 g) of either sex were purchased from National Toxicology Centre, Pune, India. They were maintained at a temperature of 25 ± 1 °C and relative humidity of 45 to 55% under 12-h light: 12-h dark cycle. The animals had free access to food pellets (Nutrivet Pvt. Ltd, Pune, India) and water was *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India.

2.5 Toxicity studies
Toxicity studies of the ethanol extract were carried out in Swiss Albino mice of either sex weighing between 20 and 25 g. The LD$_{50}$ of the MNSBEE was found to be more than 2000 mg/kg (p.o).

2.6 Analgesic activity
2.6.1. Acetic acid-induced abdominal writhing
Mice were treated according to the method described by Collier et al (Collier HO et al., 1968). The mice were divided into five groups (n = 6) viz; group I- vehicle as a control (1% tween 80 suspended in distilled water, 1 ml/kg), group II-acetyl salicylic acid (100 mg/kg), group III-MNSBEE (100 mg/kg), group IV-MNSBEE (200 mg/kg) and group V-MNSBEE (400 mg/kg). The mice were pretreated orally with MNSBEE and acetylsalicylic acid, 60 min before administration of acetic acid (0.6%, i.p.). The number of abdominal constrictions (full extension of both hind paws) were cumulatively counted over a period of 15 min. The analgesic activities were expressed as mean number of writhes and percent number of writhes (Table 1) and calculated by following formula:

\[
\% \text{ Inhibition} = \frac{W_c - W_t}{W_c} \times 100
\]

Where, $W_c$ and $W_t$ are mean number of writhes observed in vehicle control group and treatment group respectively.
2.6.2 Hot plate test

Eddy and Leimback described method for analgesic activity (Eddy NB et al., 1953). The mice were divided into five groups (n = 6):

- Group I: Vehicle as a control (1% tween 80 suspended in distilled water, 1 ml/kg);
- Group II: Pentazocin (30 mg/kg);
- Group III: MNSBEE (100 mg/kg);
- Group IV: MNSBEE (200 mg/kg);
- Group V: MNSBEE (400 mg/kg).

Mice were placed individually on a thermostatically controlled hot plate (Ugo basil, Italy) maintained at 55 ± 0.5 °C. The pain threshold was considered to be reached when the animals lifted and licked their paws or attempted to jump off. The time taken for the mice to react in this test was obtained using a stopwatch. The animals were first tested for the paw-lick or jump response and only those that reacted after 4 s were used for the experiment. A cut-off time of 15 s was used to avoid harm to the animals. The results are expressed as mean latencies (Table 2).

2.7 Anti-inflammatory activity

2.7.1. Carrageenan-induced rat hind paw edema (acute study)

The method described by Winter et al. was used for the effect of MNSBEE on acute inflammation (Winter et al., 1962). The rats were divided into five groups (n = 6):

- Group I: vehicle as a control (1% tween 80 suspended in distilled water, 1 ml/kg);
- Group II: Diclofenac (5 mg/kg, p.o.);
- Group III: MNSBEE (100 mg/kg);
- Group IV: MNSBEE (200 mg/kg);
- Group V: MNSBEE (400 mg/kg).

The hind paw edema was produced by injecting 0.1 ml of carrageenan (prepared as 0.9% suspension in sterile normal saline) in the right hind paw of each rat under the subplantar region. Rats were pretreated with orally administered MNSBEE and diclofenac 1 h before carrageenan injection. The rat pedal volume up to the ankle joint was measured using plethysmometer (Ugo Basile, Italy) at 1, 2 and 3 h after the carrageenan injection (time 0 considered).
Increase in the paw edema volume was considered as the difference between 1, 2 and 3 h and expressed as the mean difference in paw volume (ml). Percent inhibition of edema volume between treated and a control group was calculated as following formula and result presented in Table 3.

\[ \% \text{ Inhibition} = \left( \frac{V_c - V_t}{V_c} \right) \times 100 \]

Where, \( V_c \) and \( V_t \) represent mean increase in paw volume in control and treated groups, respectively.

2.7.2. Anti-inflammatory effect of the MNSBEE and diclofenac in combination

It was noticed from the above experiment that the dose of 400 mg/kg (p.o.) of the MNSBEE produced significant anti-inflammatory effect. Paw edema was produced as before. Both the MNSBEE (400 mg/kg, p.o.) and diclofenac (5 mg/kg, p.o.) were given 30 min before carrageenan injection. Eighteen wistar albino rats of either sex were grouped (each group consisted of six rats) as follows:

- Group I: received the control 1% tween 80 (1 ml/kg).
- Group II: received the diclofenace (5 mg/kg, p.o., standard).
- Group III: received the MNSBEE (400 mg/kg, p.o.) and diclofenac (5 mg/kg, p.o.).

Percentage inhibition of edema of combined effect of the MNBEE and diclofenac was calculated as before (Table 4).

2.7.3. Cotton pellet granuloma in rats (chronic study)

The effect of MNSBEE on chronic or proliferative phase of inflammation was assessed in cotton pellet granuloma rat model as described by Meier et al (Meier R et al., 1950). The rats were divided into five groups (n = 6):

- Group I: vehicle as a control (1% tween 80 suspended in distilled water, 1 ml/kg);
- Group II: Diclofenac (5 mg/kg);
- Group III: MNSBEE (100 mg/kg);
- Group IV: MNSBEE (200 mg/kg);
- Group V: MNSBEE (400 mg/kg).

Autoclaved cotton pellets weighing 35 ± 1 mg each were implanted subcutaneously through small incision made along the axilla or flank region of the rats anesthetized with ether. MNSBEE and diclofenac once daily for seven consecutive days from the day of cotton pellet insertion. On the eighth day, blood was withdrawn by retro-orbital puncture technique and
serum was separated by centrifugation. All rats were sacrificed and the cotton pellets covered by the granulomatous tissue were excised and dried in hot air oven at 60 °C till a constant weight was achieved. Granuloma weight was obtained by subtracting the weight of cotton pellet on 0 day (before start of experiment) from the weight of the cotton pellet on eighth day (at the end of experiment). The results are summarized in Table 5.

2.8 Statistical analysis
Data was expressed as mean ± SEM and statistical analysis was carried out by one-way ANOVA with post hoc Dunnet’s test performed using GraphPad InStat version 5.00 for Windows 7®, GraphPad Software, San Diego California USA, www.graphpad.com. P value was considered significant when p < 0.05.

3. RESULTS
3.1. Analgesic activity
3.1.1. Acetic acid-induced writhing
The MNSBEE showed marked reduction in the number of abdominal constriction induced by the injection of aqueous solution of acetic acid (0.6%) in a dose dependent manner. MNSBEE (100, 200 and 400 mg/kg) pretreatment significantly (p < 0.001) inhibited writhing in mice by 21.71, 54.37 and 70.21% respectively.

Pretreatment of acetyl salicylic acid significantly (p < 0.001) inhibited writhing by 61.86% in mice. The analgesic effect was observed in group treated with 200 mg/kg and 400 mg/kg (Table 1).

3.1.2. Hot Plate
The results of this test are shown in Table 2. Dose dependent increase in time of response to thermal stimulation was observed. Pretreatment of pentazocin (30 mg/ kg, p.o.) showed significantly (p < 0.01) increase in pain latency while pretreatment of the animals with 100, 200 and 400 mg/kg MNSBEE showed significant (p < 0.001) increase in pain latency by 9.89, 10.12 and 10.73 sec respectively.

3.2. Anti-inflammatory activity
3.2.1. Carrageenan-induced rat hind paw oedema (acute study)
MNSBEE (200 and 400 mg/kg, p.o.) showed a significant (P < 0.001) inhibition in carrageenin-induced paw edema volume as compared to the vehicle treated (control) animals
Dose dependent percent inhibition was observed (28.85, 42.31 and 48.08% at the
doses of 100, 200 and 400 mg/kg, p.o. respectively) at 3 h readings. Diclofenac (5 mg/kg)
significantly reduced paw edema volume by 62.5% at 3 h reading. MNSBEE was found to be
less active than diclofenac.

3.2.2. Anti-inflammatory effect of combined administration of MNSBBE and diclofenac
The anti-inflammatory activity was studied in combination with diclofenac to find out the
presence of any synergistic action (Table 4). The combination reduced the inflammatory
response in paw edema volume to 0.17, 0.12 and 0.08 ml at 1, 2 and 3 h, respectively. Percent
inhibition was found 80.9, 87.37 and 92.31 respectively for 1, 2 and 3 h at dose of MNSBEE
400 mg/kg.

3.2.3. Cotton pellet granuloma in rats (chronic study)
MNSBEE (100, 200 and 400 mg/kg, p.o.) significantly decreased the granuloma weight as
compared to the vehicle treated (control) animals. Dose dependent percent inhibition was
recorded (19.31, 33.81 and 64.71% at the doses of 100, 200 and 400 mg/kg, p.o. respectively)
whereas diclofenac (5 mg/kg) also showed percent inhibition was 75.2% (Table 5).

Table 1: Effect of stem bark of ethanolic extract of Myrica nagi (MNSBEE) in acetic acid
induced writhing in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Writhings</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>51.26 ± 5.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetyl salicylic acid</td>
<td>100</td>
<td>19.55 ± 2.26***</td>
<td>61.86</td>
</tr>
<tr>
<td>MNSBEE</td>
<td>100</td>
<td>40.13 ± 4.12</td>
<td>21.71</td>
</tr>
<tr>
<td>MNSBEE</td>
<td>200</td>
<td>23.39 ± 2.56***</td>
<td>54.37</td>
</tr>
<tr>
<td>MNSBEE</td>
<td>400</td>
<td>15.27 ± 2.26***</td>
<td>70.21</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., n=6 in each group; statistical analysis by one-way ANOVA
followed by Dunnet’s test using Graphpad Instant software.
* p < 0.05.
** p < 0.01.
*** p value < 0.001 compared to vehicle.

Table 2: Effect of stem bark of ethanolic extract of Myrica nagi (MNSBEE) in hot plate
test in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Mean latency (sec.) ± S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>5.32 ± 0.65</td>
<td></td>
</tr>
<tr>
<td>Pentazocin</td>
<td>30</td>
<td>9.56 ± 0.54**</td>
</tr>
<tr>
<td>MNSBEE</td>
<td>100</td>
<td>9.89 ± 0.46***</td>
</tr>
</tbody>
</table>
Table 3: Effect of stem bark of ethanolic extract of *Myrica nagi* (MNSBEE) on carrageenan-induced paw edema.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>1 h</th>
<th>Percent inhibition</th>
<th>2 h</th>
<th>Percent inhibition</th>
<th>3 h</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td>0.89 ± 0.10</td>
<td></td>
<td>0.95 ± 0.13</td>
<td></td>
<td>1.04 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>5</td>
<td>0.64 ± 0.08</td>
<td>28.09</td>
<td>0.47 ± 0.03**</td>
<td>50.53</td>
<td>0.39 ± 0.05***</td>
<td>62.5</td>
</tr>
<tr>
<td>MNSBEE</td>
<td>100</td>
<td>0.82 ± 0.08</td>
<td>7.87</td>
<td>0.81 ± 0.06</td>
<td>14.74</td>
<td>0.74 ± 0.10</td>
<td>28.85</td>
</tr>
<tr>
<td>MNSBEE</td>
<td>200</td>
<td>0.80 ± 0.05</td>
<td>10.12</td>
<td>0.76 ± 0.08</td>
<td>20</td>
<td>0.60 ± 0.05***</td>
<td>42.31</td>
</tr>
<tr>
<td>MNSBEE</td>
<td>400</td>
<td>0.70 ± 0.07</td>
<td>21.35</td>
<td>0.64 ± 0.05</td>
<td>32.64</td>
<td>0.54 ± 0.02***</td>
<td>48.08</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., n=6 in each group; statistical analysis by one-way ANOVA followed by Dunnet’s test using Graphpad Instant software.

* p < 0.05.

** p < 0.01.

*** p value < 0.001 compared to vehicle.

Table 4: Effect of stem bark of ethanolic extract of *Myrica nagi* (MNSBEE) and diclofenac in combination on carrageenan-induced paw edema.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>1 h</th>
<th>Percent inhibition</th>
<th>2 h</th>
<th>Percent inhibition</th>
<th>3 h</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td>0.89 ± 0.10</td>
<td></td>
<td>0.95 ± 0.13</td>
<td></td>
<td>1.04 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>5</td>
<td>0.64 ± 0.08</td>
<td>28.09</td>
<td>0.47 ± 0.03**</td>
<td>50.53</td>
<td>0.39 ± 0.05***</td>
<td>62.5</td>
</tr>
<tr>
<td>MNSBEE + Diclofenac</td>
<td>400 + 5</td>
<td>0.17 ± 0.02***</td>
<td>80.9</td>
<td>0.12 ± 0.02***</td>
<td>87.37</td>
<td>0.08 ± 0.03***</td>
<td>92.31</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., n=6 in each group; statistical analysis by one-way ANOVA followed by Dunnet’s test using Graphpad Instant software.

* p < 0.05.

** p < 0.01.

*** p value < 0.001 compared to vehicle.
Table 5: Effect of stem bark of ethanolic extract of *Myrica nagi* (MNSBEE) on cotton pellet granuloma in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Cotton granuloma pellet weight (mg)</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td>99.89 ± 5.56</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>5</td>
<td>24.78 ± 3.65***</td>
<td>75.2</td>
</tr>
<tr>
<td>MNSBEE</td>
<td>100</td>
<td>80.61 ± 4.26*</td>
<td>19.31</td>
</tr>
<tr>
<td>MNSBEE</td>
<td>200</td>
<td>66.12 ± 5.98***</td>
<td>33.81</td>
</tr>
<tr>
<td>MNSBEE</td>
<td>400</td>
<td>35.26 ± 4.23***</td>
<td>64.71</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., n=6 in each group; statistical analysis by one-way ANOVA followed by Dunnet’s test using Graphpad Instant software.

* p < 0.05.

** p < 0.01.

*** p value < 0.001 compared to vehicle.

4. DISCUSSION

The central aim of current study was to investigate the scientific basis for the traditional use of MNSBEE had an analgesic and anti-inflammatory activity. Acute oral toxicity of MNSBEE was found to be more than 2000 mg/kg. The MNSBEE exhibited analgesic activity. It significantly inhibited the abdominal constriction induced by acetic acid in the mice. Acetic acid causes an increase in peritoneal fluids of PGE2 and PGF2α (Deraedt R et al., 1980) and is a very sensitive method of screening antinociceptive effect of extracts (Collier HO et al., 1968). From the effect of MNSBEE it may be concluded that prostaglandins may be involved in the action of the extract. At 200 and 400 mg/kg the MNSBEE exhibited highly significant results and showed to have analgesic activity. If the herbal products as it is and modern medicine are combined, it will produce best drug in the treatment of various chronic conditions like rheumatoid arthritis, bronchial asthma, peptic ulcer, hypertension and other immune disorders.

Hence, the current study on the MNSBEE in combination with modern medicine (diclofenac, NSAID) produced the most potent anti-inflammatory agent without any toxic effect. Both are acting through different mechanism of action so when combined found to have enhanced anti-inflammatory action. Prostaglandin and Platelet Activation Factor (PAF) are two major factors involved in carrageenan-induced paw edema in rats. The carrageenan test is highly sensitive to non-steroidal anti-inflammatory drugs and has long been accepted as an important tool for investigating new anti-inflammatory drugs (Just MJ et al., 1998). For
determination of orally active anti-inflammatory agents carrageenan-induced inflammation
model in rat is widely used (Willoughby DA et al., 1972) and therefore has a significant
predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute
inflammation (Mossa JS et al., 1995).

Carrageenan induced edema formation is a biphasic event in nature. When the first 3-h
segment of the curve is analyzed, a biphasic response has been observed. It has been also
established that during first 2-h edema formation was due to histamine, serotonin and PAF
which is a powerful mediator of inflammation. Plateau phase was maintained by a kinin like
substances and then late phase of edema formation was mainly due to release of
prostaglandins, protease and lysosome (Levy L, 1969; Henriques et al., 1987; Nantel F et al.,
1999; Vinger et al., 1987; Posadas et al., 2004; Di Rosa et al., 1971; Rocha et al., 2006).
Treatment with COX-1 inhibitor reduced the early phase of paw edema (Siqueira et al., 2003)
whereas COX-2 is involved in second phase (Posadas et al., 2004). Conversely, TNF-α and
nitric oxide are also implicated in carrageenan induced paw edema ((Posadas et al., 2004;
Rocha et al., 2006). As per the results of our study, MNSBEE was able to inhibit the edema
effectively in the late phases, suggesting that MNSBEE inhibits different chemical mediator
of inflammation such as prostaglandins, proteases. Thus MNSBEE probably acts by up-
regulating COX-2 in late phase of inflammation thereby inhibits prostaglandins. Therefore, it
is possible that a relative long lasting anti-inflammatory action of MNSBEE. This delayed
anti-inflammatory action is due to its dependence on the modulation of transcriptional factors
and de novo synthesis of proteins involved in the inflammatory response.

The inflammatory granuloma is a best method for testing the proliferation phases like
granuloma formation, provoked by subcutaneous implantation of cotton pellets. During the
repair process of inflammation, there is proliferation of macrophages, neutrophils, fibroblast
and multiplication of small blood vessels, which are the main sources of highly vascularised
reddish mass, termed as granulomatous tissue. The amount of granulomatous tissue is directly
proportional to the dry weight of the pellets (Swingle et al., 1972). In the current study
MNSBEE exhibited significant activity against cotton pellet granuloma in rats and so it was
observed that the ability of MNSBEE in reducing the synthesis of collagen, number of
fibroblast and mucopolysaccharides which are involved in granuloma tissue formation.

The preliminary phytochemical analysis of MNSBEE showed the presence of alkaloids,
flavonoids, tannins, and phenolic compounds (Sun D et al., 1988). Previously, alkaloids,
triterpenes and flavonoids were reported to possess anti-inflammatory and analgesic potential (Calixto et al., 2000). Flavonoid glycosides have been shown to possess analgesic and anti-inflammatory potential in various in vivo and in vitro animal models of inflammation (Ojewole JA, 2005; Backhouse et al., 2002; Moreira et al., 2000). Flavonoids were also reported to have antioxidant activity (Pietta PG, 2000). Moreover, phytochemicals with antioxidant potentials are shown to possess analgesic and anti-inflammatory activity in case of many plant extracts and phytochemicals (Sur et al., 2001; Middleton E, 1998). Flavonoids were shown to possess anti-inflammatory property via inhibition of prostaglandin E2 and leukotriens C4 and by improvement in immunity (Middleton E, 1998). Alkaloids have also reported anti-inflammatory potential (Murayama et al., 1991). Triterpenes especially saponins reported to have anti-inflammatory activity (Liu J, 1995).

Therefore, analgesic and anti-inflammatory activity of MNSBEE can be attributed to its phytochemicals like flavanoids, alkaloids, tannins and triterpenes. Thus taken together, the results presented herein strongly suggest that Myrica nagi possesses analgesic and anti-inflammatory effects, supporting the use of this plant species in folk medicine. Furthermore, the acute toxicity does not show any symptoms or mortality at 2 g/kg doses that indicate a therapeutic safety for the doses pharmacologically active. The precise mechanisms through which MNSBEE exerts its action are currently under investigation but possibly it could be related to arachidonic acid cascade or inhibition of prostaglandins inhibition via COX-2.

**Conflict of interest**

No conflict of interest declared.

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