Evaluation of Analgesic, Anti-Inflammatory and CNS Activities of the Methanolic Extract of *Syzygium samarangense* Leave

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**Abstract:** *Syzygium Samarangense* is a potential medicinal drug. We aimed to evaluate the analgesic, anti-inflammatory and CNS activities of the methanolic extract of *Syzygium Samarangense* leave in mice. The analgesic activity was examined by acetic acid induced writhing and formalin tests. The anti-inflammatory activity was studied using carrageenan induced hind paw edema model. The analgesic activity of the methanolic extract of *Syzygium Samarangense* leaves was evaluated by acetic acid induced writhing and formalin tests at the dose of 100 mg/kg and 200 mg/kg, significantly (*p*<0.05) reduced the writhing caused by acetic acid and the number of licks induced by formalin in a dose dependent manner. The extract of *Syzygium Samarangense* leaves caused significant (*p*<0.05) inhibition of carrageenan induced paw edema after 4 hrs in a dose dependent manner. The CNS depressant activity was evaluated by observing the reduction of locomotor and exploratory activities in the open field and hole cross tests at a dose of 100 mg/kg and 200 mg/kg body weight. The findings of the study suggested that the methanolic extract of *Syzygium Samarangense* leave has remarkable analgesic, moderate effect against inflammation and significant CNS effects, conforming the traditional use of this plant for inflammatory pain alleviation.

**Key words:** Analgesic • Anti-Inflammatory • CNS Activities • Leave • Pain • *Syzygium Samarangense*

**INTRODUCTION**

Plants are an essential component of the universe. Human beings have used plants as medicine from the very beginning of time. After various observations and experiments medicinal plants were identified as a source of important medicine, therefore, treatment through this medicinal plants, began in the early stages of human civilization [1]. The use of these medicinal plants is increasing in many countries where 35% of drugs contain natural products. At present, thousands of plant metabolites are being successfully used for the treatment of variety of diseases [2]. In Bangladesh thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. The beneficial medicinal effects of plant materials typically results from combinations of secondary product present in plant such as alkaloids, steroids, tannins, phenol compounds, resins, gums, flavonoids and fatty acids which are capable of producing definite physiological action on body [3].

*Syzgium samarangense* belongs to the Myrtaceae family and is among the fruits of economic importance in Asia and Taiwan. Jamrul (Zamrul) is a local name at my country Bangladesh. The name is varied at different countries. Common names of *Syzygium samarangense* are wax apple, love apple, java apple, chomphu (In Thai), Man (In Vietnam), bellfruit (In Taiwan), Jamaican Apple, Otaheti Apple (In Jamaica), jambu air (In Indonesian), water apple, mountain apple, cloud apple, jambu air (“water guava” in Malay), wax jambu, rose apple, bell offruit, makopa, tambis (Philippines) and chambekka in Malayalam, jamrul (In Bengali) and jambu (Sri Lanka).
Jamrul or wax apple doesn’t taste like an apple. This is a juicy fruit like watermelon. The taste of the jamrul is not too much sweet, but it has a nice smell. Also the leaves of the tree has the same smell. In Bangladesh, usually two different types of jamrul have been seen—one is pink to red or purple color and another one is pale with a bit greenish shade. To carry out this research we have selected red jamrul plant. The plant of red Jamrul is bush type. Red Jamrul fruit is small, compare with natural jamrul. Syzygium samarangense is a tropical tree growing to 12 m tall, with evergreen leaves 10-25 cm long and 5-10 cm broad. It’s leaves are a bit larger compare to other common fruit of Bangladesh. The flowers are white, 2.5 cm diameter, with four petals and numerous stamens. The fruit is a bell-shaped, edible berry, with colors ranging from white to red or green to red, purple, or crimson, to deep purple 4-6 cm long in wild plants. Here the season of the fruit is May and June. The flowers and resulting fruit are not limited to the axils of the leaves and can appear on nearly any point on the surface of the trunk and branches. When mature, the tree is considered a heavy bearer, yielding a crop of up to 700 fruits [4]. Its leaves, roots, bark, flowers and fruits all have potential medical applications. Some Myrtaceae plants have reportedly been used as medicinal herbs for the treatment of bronchitis, asthma, diabetic mellitus and inflammation syndromes [5]. They exert potent free radical scavenging, antioxidant, antitumor, and anticancer activities [6]. The leaves of wax apple also contain abundant phytochemicals including ellagitannins, flavanones, flavonol glycosides, proanthocyanidins, anthocyanidins, triterpenoids, chalcones and volatile terpenoids [7-12]. Wax apple fruit has reportedly demonstrated antihyperglycemic activity in alloxan-induced (Type 1 DM) diabetic mice [11]. Syzygium samarangense invites great attention for researchers worldwide due to its various pharmacological activities such as anti-diabetic, antimicrobial activity, antiviral activity, spasmylocytic activity, protease inhibitory/intiammesiac activity, immunomodulatory activity, antihyperglycemic activity, analgesic and anti-inflammatory activities [Edible Medicinal And Non-Medicinal Plants: Volume 3, Fruits] and wound healing. The red fruits are juicier and more flavorful and suitable for eating out-of-hand. In Malaya, the greenish fruits are eaten raw with salt or may be cooked as a sauce. They are also stewed with true apples. In Indonesia, flowers eaten as salads. The flowers are astringent and used in Taiwan to treat fever and halt diarrhea. Malayans use powdered dry leaves for cracked tongues. Juice of leaves used for baths and lotion. The phytochemicals in the Java apple tree show some antibiotic action against staphylococcus aureus, candida albicans and mycobacterium smegmatis. Investigators have found the flowers principal constituent to be tannin. The red, hard wood is used for constructing huts in the Andaman and Nicobar Islands.

The fruits are used in traditional medicine to cure diabetes. Several Syzygium species are reported to possess antibacterial [13][14], antifungal and anti-inflammatory activities [15]. The flavonoids isolated from S. samarangense were reported to possess antihyperglycemic activity [16], spasmylocytic activity and immunomodulatory activity fractionation of the methanolic extracts of the pulp and seeds of the fruits of Syzygium samarangense led to the identification of four cytotoxic chalcones and eight antioxidants. Investigators have found the flowers principal constituent to be tannin. Leave oil largely composed of monoterprenenes (30% sesquerpenenes, 9% caryophyllene). Prolyl endopeptidase inhibitors were isolated from the hexane extract of the leaves of S.samarangense. A new triterpene methyl-3-epi-betulinate in its native form and 4’, 6’-dihydroxy-2’-methoxy-3’, 5’-dimethyl chalcone along with ursolic acid, jacoumaric acid and arjunolic acid have been isolated from the aerial parts of Syzygium samarangense.

MATERIALS AND METHODS

Animal Selection: Swiss albino mice (25-30g) were used for assessing biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water ad libitum. The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. The animals were divided into different groups, each consisting of five animals which were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee, Atish Dipankar University of Science and Technology, Dhaka, Bangladesh. Animal treatment and maintenance for acute toxicity and analgesic effects were conducted in accordance with the Principle of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Animal Care and Use Guidelines of Atish Dipankar University of Science and Technology, Dhaka, Bangladesh.

Collection of Plant Materials: The leaves of the Syzygium Samarangense (red jamrul) were collected from the adjoining area of Jahangirnagar University Campus, Bangladesh during Aril 2013. The plant material was taxonomically identified by the National Herbarium of Bangladesh-Dhaka.
Chemicals: Diclofenac Na, Ibuprofen and Diazepam were leave (100 mg/kg and 200 mg/kg, per oral) and 30 min after administration of Diclofenac Na (10 mg/kg, i.p./intraperitoneal). The mice were observed for 30 min after the injection of formalin and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is referred to as the early phase and the period between 15 and 30 min as the late phase. The total time spent licking or biting the injured paw (pain behavior) was measured with a stop watch.

Preparation of Plant Extract: The plant material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve #40 and stored in a tight container. The dried powder material (1.2 kg) was refluxed with methanol for three hours. The total filtrate was concentrated to dryness, in vacuo at 40°C to render the methanol extract (310 g). The obtained methanolic extract was concentrated deep green colored. The extract was preserved in a air-tight container as a crude extract sample.

Acute Toxicity Study: Acute oral toxicity assay was performed in healthy nulliparous and non pregnant adult female albino Swiss mice (25-30g) divided into different groups. The test was performed using increasing oral dose of the methanolic extract of Syzygium Samarangense leaves in water (50, 100, 200, 500, 1000 mg/kg body weight), in 20 ml/kg volume to different test groups. Normal group received water. The mice were allowed to feed ad libitum, kept under regular observation for 48 hrs, for any mortality or behavioral change [17]

Analgesic Activity
Acetic Acid-induced Writhing Test: The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice. The animals were divided into six groups with five mice in each group. Group I animals received vehicle (Water), Group II received Diclofenac Na at 10 mg/kg body weight while animals of groups-III and IV were treated with 100 mg/kg and 200 mg/kg body weight (p.o./per oral) of the methanolic extract of Syzygium Samarangense leaves. Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 1.0% v/v acetic acid but Diclofenac-Na was administered intraperitonially 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as ‘writhing’ for the next 10 min [18].

Formalin Test: The antinociceptive activity of the drugs was determined using the formalin test [19]. Control group received 5% formalin. 20 µl of 5% formalin was injected into the dorsal surface of the right hind paw 60 min after administration of the extract of Syzygium Samarangense leave (100 mg/kg and 200 mg/kg, per oral) and 30 min after administration of Diclofenac Na (10 mg/kg, i.p./intraperitoneal). The mice were observed for 30 min after the injection of formalin and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is referred to as the early phase and the period between 15 and 30 min as the late phase. The total time spent licking or biting the injured paw (pain behavior) was measured with a stop watch.

Anti-inflammatory Activity
Carrageenan Induced Paw Edema Test in Mice: Swiss albino mice (25-30g) were divided into six groups of five animals each. The test groups received 100 mg/kg and 200 mg/kg body weight,( p.o./per oral) of the methanolic extract of Syzygium Samarangense leave. The reference group received Ibuprofen (10 mg/kg body weight, per oral) while the control group received 1 ml/kg body weight normal saline. After 1 h, 0.1 ml 1% carrageenan suspension in normal saline was injected into the subplanatar tissue of the right hind paw. The paw volume was measured at 1, 2, 3 and 4 hrs after carrageenan injection using a micrometer screw gauge. The percentage inhibition of the inflammation was calculated from the formula:

\[
% \text{ inhibition} = (1-\frac{D_t}{D_c}) \times 100
\]

Where, \(D_o\) was the average inflammation (hind paw edema) of the control group of mice at a given time, \(D_c\) was the average inflammation of the drug treated mice at the same time [20].

Statistical Analysis: All values were expressed as the mean ± SEM of three replicate experiments. The analysis was performed by using SPSS statistical package for WINDOWS (version 16.0; SPSS Inc, Chicago). Results related to the reducing power activities were statistically analyzed by applying the Student t-test and \(p<0.001\) were considered to be statistically significant. All data are subjected to ANOVA followed by Dunnett’s test and \(p<0.05\) were considered to be statistically significant.

RESULTS

Acute Toxicity Studies: The acute toxicity studies mainly aim at establishing the therapeutic index, i.e., the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species. The methanolic extract of Syzygium Samarangense leaves was safe up to a dose of 1000 mg/kg (p.o.) body weight.
Behavior of the animals was closely observed for the first 3hrs then at an interval of every 4h during the next 48hrs. The methanolic extract did not cause mortality in mice during 48h observation but little behavioral changes, locomotor ataxia, diarrhea and weight loss were observed. Food and water intake had no significant difference among the group studied.

*In vivo* Analgesic Activity

Acetic Acid-induced Writhing Test: Table 1 shows the effects of both extract of on acetic acid-induced writhing in mice. The oral administration of the methanolic extract of *Syzygium Samarangense* leaves significantly \( (p<0.05) \) inhibited writhing response induced by acetic acid in a dose dependent manner.

Formalin Test: The methanolic extract of *Syzygium Samarangense* leaves (100 mg/kg and 200 mg/kg, p.o.) significantly \( (P<0.05) \) suppressed the licking activity in either phase of the formalin-induced pain in mice in a dose dependent manner (Table 2). The methanolic extract of *Syzygium Samarangense* leaves at the dose of 200 mg/kg body weight, showed the almost similar licking activity against both phases of formalin-induced pain than that of the standard drug diclofenac Na.

### Table 1: Effects of the methanolic extract of *Syzygium samarangense* (red jamrul) leave on acetic acid-induced writhing in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>No. of writhing</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Vehicle</td>
<td>35.60</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>10</td>
<td>10.10</td>
<td>71.62*</td>
</tr>
<tr>
<td>Group III</td>
<td>100</td>
<td>19.40</td>
<td>45.50*</td>
</tr>
<tr>
<td>Group IV</td>
<td>200</td>
<td>12.14</td>
<td>65.89*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, \( (n = 5) \); *\( p<0.05 \) as compared to vehicle control (One way ANOVA followed by Dunnet test). Group I animals received vehicle (water); group II received Diclofenac Na (10 mg/kg body weight); group III, IV were treated with 100 mg/kg and 200 mg/kg body weight (p.o.) of the methanolic extract of *Syzygium samarangense* leave.

### Table 2: Effects of the methanolic extract of *Syzygium samarangense* (red jamrul) leave hindpaw licking in the formalin test in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Early phase (Sec)</th>
<th>% protection</th>
<th>Late phase (Sec)</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Vehicle</td>
<td>36.66 ± 1.38</td>
<td>-</td>
<td>42.18 ± 1.03</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>10</td>
<td>16.03 ± 0.90*</td>
<td>56.27</td>
<td>17.23 ± 0.70*</td>
<td>59.15</td>
</tr>
<tr>
<td>Group III</td>
<td>100</td>
<td>22.0 ± 0.84*</td>
<td>34.64</td>
<td>16.60 ± 0.74*</td>
<td>50.68</td>
</tr>
<tr>
<td>Group IV</td>
<td>200</td>
<td>17.60 ± 0.95*</td>
<td>47.71</td>
<td>12.00 ± 0.84*</td>
<td>64.35</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, \( (n = 5) \); *\( p<0.05 \) as compared to vehicle control (One way ANOVA followed by Dunnet test). Group I animals received vehicle (water); group II received Diclofenac Na (10 mg/kg body weight); group III, IV were treated with 100 mg/kg and 200 mg/kg body weight (p.o.) of the methanolic extract of *Syzygium samarangense* leave.

### Table 3: Effect of methanolic extract of *Syzygium samarangense* (red jamrul) leaves in carrageenan induced inflammation

<table>
<thead>
<tr>
<th>Oedema diameter (mm)</th>
<th>Inhibition (%)</th>
<th>1h</th>
<th>2 hrs</th>
<th>3 hrs</th>
<th>4 hrs</th>
<th>1h</th>
<th>2 hrs</th>
<th>3 hrs</th>
<th>4 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Vehicle</td>
<td>5.4±0.20</td>
<td>6.35±0.49</td>
<td>6.06±0.58</td>
<td>6.3±±0.84</td>
<td>55.33</td>
<td>70.72</td>
<td>76.25</td>
<td>84.18</td>
</tr>
<tr>
<td>Group II</td>
<td>10</td>
<td>2.4±0.47*</td>
<td>1.86±0.46*</td>
<td>1.44±0.3*</td>
<td>1.0±0.32*</td>
<td>25.06</td>
<td>41.44</td>
<td>44.57</td>
<td>50.02</td>
</tr>
<tr>
<td>Group III</td>
<td>100</td>
<td>4.06±0.30*</td>
<td>3.72±0.29*</td>
<td>3.36±0.34*</td>
<td>3.16±0.49*</td>
<td>32.45</td>
<td>43.95</td>
<td>50.18</td>
<td>65.20</td>
</tr>
<tr>
<td>Group IV</td>
<td>200</td>
<td>3.66±0.34*</td>
<td>3.56±0.30*</td>
<td>3.02±0.29*</td>
<td>2.2±0.29*</td>
<td>32.45</td>
<td>43.95</td>
<td>50.18</td>
<td>65.20</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, \( (n = 5) \); *\( p<0.05 \), Dunnet test as compared to vehicle control. Group I animals received vehicle (water); group II received Ibuprofen 10 mg/kg body weight; group III and group IV were treated with 100 mg/kg and 200 mg/kg body weight (p.o.) of the methanolic extract of *Syzygium samarangense* leave.
**DISCUSSION**

Acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics and represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from the tissue phospholipid [21]. The response is thought to be mediated by peritoneal mast cells [22], acid sensing ion channels [23] and the prostaglandin pathways [24]. The organic acid has also been postulated to act indirectly by inducing the release of endogenous mediators, which stimulates the nociceptive neurons that are sensitive to NSAIDs and narcotics [25]. It is well known that non-steroidal anti-inflammatory and analgesic drugs mitigate the inflammatory pain by inhibiting the formation of pain mediators at the peripheral target sites where prostaglandins and bradykinin are proposed to play a significant role in the pain process [26]. Therefore, it is likely that the methanolic extract of *Syzygium samarangens* leaves might have exerted its peripheral antinociceptive action by interfering with the local reaction caused by the irritant or by inhibiting the synthesis, release and/or antagonizing the action of pain mediators at the target sites. The above findings clearly demonstrated that both central and peripheral mechanisms are involved in the antinociceptive action of the methanolic extract of *Syzygium samarangens* leave. Interestingly, compounds like flavonoids and steroids, triterpenes in part, have been shown to possess anti-inflammatory and analgesic activity [27].

The formalin model normally postulates the site and the mechanism of action of the analgesic. This biphasic model is represented by neurogenic (0-5 min) and inflammatory pain (15-30 min), respectively [28]. Drugs that act primarily on the central nervous system such as narcotics inhibit both as steroids and NSAIDs suppress mainly the late phase [25]. The suppression of neurogenic and inflammatory pains by the extract might imply that it contains active analgesic principle that may be acting both centrally and peripherally. This is an indication that the extract can be used to manage acute as well as chronic pain. The mechanism by which formalin triggers C-fibers activation remained unknown for a relatively long time. Recently, however, McNamara [29] demonstrated that formalin activates primary afferent neurons through a specific and direct on TRPA1, a member of the transient receptor potential family of cation channels, expressed by a subset of C-fiber nociceptors and this effect is accompanied by increased influx of *Ca*\(^{2+}\) ions. TRPA1 cation channels at primary sensory terminals were also reported to mediate noxious mechanical stimuli [30]. These experiments suggest that *Ca*\(^{2+}\) mobilization through TRPA1 cation channels is concomitant with noxious chemicals and mechanical stimuli as they produce their analgesic action. It is likely that the inhibitory effect methanolic extract of *Syzygium samarangens* leave to pain response is due to inhibit the increase of the intracellular *Ca*\(^{2+}\) through TRPA1, presumably evoked by formalin. So, the extract of *Syzygium Samarangense* leave may contain substances that affect the metabolism of *Ca*\(^{2+}\).

Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages [31-33]. Since the extract significantly inhibited paw edema induced by carrageenan in the second phase and this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract and this effect is similar to that produced by non-steroidal anti-inflammatory drugs such as ibuprofen, whose mechanism of action is inhibition of the cyclooxygenase enzyme. Flavonoids and saponins are well known for their ability to inhibit pain perception as well as anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation. This hypothesis is strongly supported by the previous study, which has shown that methanolic extract of *Syzygium samarangens* leave possess anti-inflammatory activity due to the presence of flavonoid content [34,35].

**CNS Depressant Activity**

**Hole Cross Test:** The method used was done as described by Takagi et al. [36]. The animals were divided into control, standard and test groups (n = 5 per group). The control group received vehicle (water) whereas the test group received methanolic extract of *Syzygium samarangens* leaves (at the doses of 100 mg/kg and 200 mg/kg p.o.) and standard group received diazepam at the dose of 1 mg/kg body weight orally. Each animal was then placed on one side of the chamber and the number of passages of each animal through the hole from one chamber to the other was recorded for 30 min on 0, 30, 60 and 90 mins. during the study period.
Table 4: Effect of methanolic extract of *Syzygium samarangens* (red jamrul) leaves on hole cross test in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>10ml/kg</td>
<td>118.4 ± 1.20</td>
<td>118 ± 1.30</td>
<td>115.4 ± 0.50</td>
<td>117.4 ± 1.16</td>
<td>-</td>
</tr>
<tr>
<td>Group-II</td>
<td>1mg/kg</td>
<td>117.2 ± 1.15*</td>
<td>64.6± 0.43*</td>
<td>38.8± 0.58*</td>
<td>15.8± 0.86</td>
<td>-</td>
</tr>
<tr>
<td>Group-III</td>
<td>100 mg/kg</td>
<td>1.80 ± 0.67*</td>
<td>1.60 ± 0.95*</td>
<td>3.8±1.89</td>
<td>1.4± 0.74*</td>
<td>-</td>
</tr>
<tr>
<td>Group-IV</td>
<td>200 mg/kg</td>
<td>2.40 ± 0.95*</td>
<td>3.8 ± 1.43*</td>
<td>3.4±1.72</td>
<td>2±1.19*</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, (n = 6); *p<0.05, Dunnet test as compared to vehicle control. Group I animals received vehicle (water), group II received diazepam 1 mg/kg body weight, group III and group IV were treated with 100 mg/kg and 200 mg/kg body weight (p.o.) of the methanolic extract of *Syzygium samarangens* leave.

Table 5: Effect of methanolic extract of *Syzygium samarangens* (red jamrul) leave on Open Field test in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>10ml/kg</td>
<td>12.8 ± 1.15</td>
<td>13 ± 1.41</td>
<td>13.6 ± 0.92</td>
<td>14.2 ± 0.86</td>
<td>-</td>
</tr>
<tr>
<td>Group-II</td>
<td>1mg/kg</td>
<td>11.2 ± 0.58</td>
<td>6 ± 0.70*</td>
<td>4.0 ± 0.83*</td>
<td>2.4±0.81*</td>
<td>-</td>
</tr>
<tr>
<td>Group-III</td>
<td>100 mg/kg</td>
<td>169.8± 0.63*</td>
<td>168± 1.76*</td>
<td>63.8±1.97*</td>
<td>61.4±1.48*</td>
<td>-</td>
</tr>
<tr>
<td>Group-IV</td>
<td>200 mg/kg</td>
<td>274 ± 2.05</td>
<td>148.4±0.35*</td>
<td>52.4±0.35*</td>
<td>44.6±1.70*</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, (n = 5); *p<0.05, Dunnet test as compared to vehicle control. Group I animals received vehicle (water), group II received diazepam 1 mg/kg body weight; group III and group IV were treated with 100 mg/kg and 200 mg/kg body weight (p.o./per oral) of the methanolic extract of *Syzygium samarangens* leave.

**Open Field Test:** This experiment was carried out as described by Gupta *et al.* [37]. The animals were divided into control, standard and test groups (n = 5 per group). The control group received vehicle (water), the test group received the crude extract (at the doses of 100 mg/kg and 200 mg/kg p.o.) and standard group received diazepam at the dose of 1mg/kg body weight orally. The animals were placed on the floor of an open field (100 cm×100 cm×40 cm h) divided into a series of squares. The number of squares visited by each animal was counted for 3 min on 0, 30, 60 and 90 mins during the study period.

**Result of CNS Depressant Activity**

**Open-field Test:** In the open-field test the methanolic extract of *Syzygium Samarangense* leave extract exhibited a decrease in the movements of the test animals at all dose levels tested. The results were statistically significant for all doses and followed a dose-dependent response (Table 5).

**Hole-Cross Test:** Results of the hole-cross test followed a similar trend to the ones observed in the open-field test. They were statistically significant for almost all doses levels and followed a dose-dependent response. The depressing effect was most intense during the fourth (90 mins) observation periods (Table 4).

**CONCLUSION**

The results of the experiments suggest that the methanolic extract of *Syzygium Samarangense* leave may be used as an alternative or supplementary herbal remedy for the treatment of analgesic, inflammatory and depressant disease. Because of its analgesic, anti-inflammatory and antidepressant effects, the methanolic extract of *Syzygium Samarangense* leave have beneficial effects together with drugs known for a remarkable analgesic, moderate anti-inflammatory as well as antidepressant effects. Thus the present study warrants further investigation involving components of the methanolic extract of *Syzygium Samarangense* leave for possible development of new class of analgesic and anti-inflammatory drugs.

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