ORIGINAL ARTICLES

In Vivo Evaluation of CNS Depressant and Antinociceptive Activities of Methanol Extract of Hibiscus sabdariffa Fruits


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ABSTRACT

The present study was designed to investigate the CNS depressant and antinociceptive activity of methanol (85%) extracts of Hibiscus sabdariffa Linn fruit (MEHS). The CNS depressant was evaluated by observing the reduction of locomotor and exploratory activities in the open field and hole cross tests at doses of 250 and 500 mg/kg body weight while analgesic activity was examined using acetic acid-induced writhing test and formalin test in rat model at 100 and 200 mg/kg body weight. The results of the statistical analysis showed that the plant extract had significant (p<0.01) dose dependent CNS depressant and antinociceptive activities. In the acetic acid-induced writhing model, the extract showed better analgesic effects at higher doses characterized by reduction in the number of writhes when compared to the control. The extract, at the dose of 200 mg/kg, exerted a maximum of 66.464% inhibition of writhing response which is more potent to the reference drug Indomethacin (58.8%). Altogether, these results suggest that the fruit extract of H. sabdariffa possesses remarkable CNS depressant and analgesic properties.

Key words: Antidepressant, analgesic, Hibiscus sabdariffa, writhing, locomotor activity.

Introduction

Use of plant products is increasing in many segment of the population (Eisenberg et al., 1993). At present, thousands of plant metabolites are being successfully used for the treatment of variety of diseases. According to an estimate, 80% of the world's population relied upon plants for their medication (Rakh and Chaudhari, 2010). The use of the medicinal plants is increasing in many countries where 35% of drugs contain natural products. As a result of adverse effects such as gastric lesions caused by non-steroidal anti-inflammatory drugs (NSAID), tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all cases (Dharmasiri et al., 2003, Park et al., 2004). Moreover, synthetic drugs are very expensive to develop. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs (Hossain et al., 2011). 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 (Joshi et al., 2009).

Hibiscus sabdariffa L. (Malvaceae), commonly referred to in English as ‘Roselle’, and in Bangladesh, it is known as Chukair or mesta (Ghani, 1998). The plant is native to tropical central or West Africa but well cultivated in tropical Asia, northern Australia and many other tropical countries. It is an annual shrub and commonly used to make beverages. The calyces have been used in folk medicines and claimed effective as diuretics, stomachic, aphrodisiac, antiseptic, astringent, cholagogue, digestive, sedative, laxative, antimicrobial or as remedy for pyrexia, abscesses, heart ailments and hypertension (Perry, 1980). H. sabdariffa has been reported to possess biological activities like antihypertensive, anticancer, antihyperlipidemic, antioxidant,
anticonvulsant, anxiogenic, CNS-depressant, serotonergic activities, reducing oxidative liver damage, anti-inflammatory, antimicrobial and hypoglycemic activity (Mahadevan et al., 2009, Chen et al., 2003 and Gosain et al., 2010). Some active compounds isolated from H. sabdariffa calyces and their pharmacological activities have been reported (Iyare and Adegoke, 2011). Protocatechuic acid, a phenolic compound isolated from the plant has protective activities against tert-butyl hydroperoxide and lipopolysaccharide induced hepatic damage in rats (Tseng et al., 1997; Liu et al., 2002; Lin et al., 2003). It also inhibits tumor in mouse skin and acts as an apoptosis inducer in human leukemia cells (Tseng et al., 2000). The antipyretic, analgesic and anti-inflammatory activity of ethanol and aqueous extract of red calyces of H. sabdariffa has also been reported (Reanmongkol and Itharat, 2007). Despite the various pharmacological activities of this plant are reported, no studies combining the CNS depressant and analgesic action of this plant have so far been undertaken. Besides this, medicinal value of the plant extract profoundly affected by seasonal and geographical variations. Taking this in view and as a part of our ongoing research (Habibur Rahman et al., 2011) on biological investigations of Bangladeshi medicinal plants, the present study was aim to evaluate the CNS depressant and analgesic activity of MEHS in animal model.

Materials and methods

Plant Material:

For this present investigation, fruits of Hibiscus sabdariffa were collected from Mirpur, Dhaka, Bangladesh in October, 2010 and were identified by the experts of Bangladesh National Herbarium, Dhaka, where a voucher specimen has been retained. The collected plant parts were dried for one week and pulverized into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Preparation of the Extract:

About 150 gm of powdered material was taken in a clean, flat bottomed glass container and soaked in 200 ml of 85% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate (methanol extract) obtained was evaporated using rotary evaporator. It rendered a gummy concentrate of reddish black color. The gummy concentrate was designated as crude extract of methanol. The extract was transferred to a closed container for further use and protection.

Animals:

Young Long-Evans rats of either sex weighing about 80-120gm were used for the experiment. The rats were purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B). They were kept in standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hours light/dark cycle) for one week for acclimation after their purchase and fed ICDDR,B formulated rodent food and water ad libitum. The set of rules followed for animal experiment were approved by the institutional animal ethical committee (Zimmermann, 1983).

Drugs and chemicals:

Acetic acid was obtained from Merck, Germany. Tween-80 was obtained from BDH Chemicals, UK. Formalin was purchased from CDH, India. Normal saline solution was purchased from Beximco Infusion Ltd., and indomethacin and diazepam were obtained from Square Pharmaceuticals Ltd., Bangladesh. All chemicals used were of analytical reagent grade.

Acute toxicity:

The 50% lethal dose (LD50) of the extract of H. sabdariffa in rats was estimated by the up and down method (Bruce, 1985). Doses were adjusted up or down by a constant multiplicative factor (1.5) depending on the previous outcome.
CNS Depressant Activity:

**Hole cross test:**

The method was carried out as described by Takagi et al., (1971). A steel partition was fixed in the middle of a cage having a size of 30×20×14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. Twenty animals were divided into four groups with five rats in each group. Group I animals received vehicle (1% Tween 80 in water, 10 ml kg⁻¹ p.o.), animals of Group II received diazepam at 1 mg kg⁻¹ body weight (p.o.) while animals of Group III and Group IV were treated with 250 and 500 mg kg⁻¹ body weight (p.o.) of the MEHS. The number of passages of a rat through the hole from one chamber to other was counted for a period of 3 min on 0, 30, 60, 90 and 120 min after oral administration of test drugs.

**Open field test:**

The animals were treated as discussed above. The experiment was carried out according to the methods described by Gupta et al., (1971). The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had 40 cm height a wall. The number of squares visited by the animals was counted for 3 min, on 0, 30, 60, 90 and 120 min after oral administration of test drugs.

**Analgesic Activity:**

**Acetic Acid Induced Writhing Method:**

The analgesic activity of the samples was also studied using acetic acid-induced writhing model in rats (Sharma et al., 2010). Test samples (100 and 200 mg/kg body weight), vehicle (1% tween 80 in water) and indomethacin (10mg/kg) were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid. Then the rats were observed for specific contraction of body referred to as ‘writhing’ for the next 20 min (Sharma et al., 2010). Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group while indomethacin (10mg/kg) was used as a reference substance (positive control). The percent inhibition (% analgesic activity) was calculated by

\[
\% \text{ inhibition} = \left\{\frac{\text{A}-\text{B}}{\text{A}}\right\} \times 100
\]

Where, \( \text{A} \) = Average number of writhing of the control group; \( \text{B} \) = Average number of writhing of the test group.

**Formalin test:**

The antinociceptive activity of the drugs was determined using the formalin test described by Sharma et al. (2010). 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 MEPH (100 and 200 mg/kg, p.o.) and Indomethacin (10 mg/kg, p.o.). The rats 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.

**Statistical Analysis:**

Data are expressed as mean ± SEM and were analyzed statistically by one-way ANOVA's procedures, followed by using Dunnett's test. A difference was considered significant at \( p<0.01 \).

**Results and discussions**

**Acute toxicity:**

In the acute toxicity test, no any toxicity was observed within 7 days after oral administration at the high dose of 15 g/kg methanol extract of \( H. \ sabdariffa \) fruits in rats.
CNS Depressant Activity:

Hole cross test:

In the hole cross test, the extracts showed a decrease in locomotion in the test animals. The number of hole crossed from one chamber to another by rats of the control group is increased from 0 minutes to 120 minutes (Table 1). Hole cross test of the extract of *H. sabdariffa* fruits at 250 mg/kg & 500 mg/kg dose showed significant decrease of movement from its initial value at 0 to 120 minutes. The extract showed dose dependent activity and maximum depressive effect was observed at third observation period.

Open field test:

Open field test was carried out to determine the depressive action of the test drugs on CNS in rats (Gupta et al., 1971). MEHS significantly decreased the locomotor activity. The locomotor activity lowering effect of the extract was evident at the 2nd observation (30 min) period and continued up to 5th observation period (120 min). The results were also dose dependent and statistically significant (Table 2).

Analgesic Activity:

Acetic Acid-induced Writhing Test:

Table 3 shows the effect of the methanol extract of fruits of *H. sabdariffa* on acetic acid-induced writhing in rats. Both doses of extract of *H. sabdariffa* showed significant reduction (p<0.01) of writhing induced by the acetic acid after oral administration in a dose dependant manner. After oral administration of two different doses (100 and 200 mg/kg body weight) of the extract and standard drug indomethacin (10 mg/kg body weight), the percent inhibitions were 54.425%, 66.464% & 58.87% respectively. The plant extract at the dose 200 mg/kg body weight was found more potent than the standard drug indomethacin.

Formalin test:

The methanol extract of fruits of *H. sabdariffa* (100 and 200 mg/kg, p.o.) significantly suppressed the licking activity in either phase of the formalin-induced pain in rats (Table 4) in a dose dependant manner. The reference antinociceptive drug Indomethacin (10 mg/kg) also significantly inhibited the licking activity against both phases of formalin-induced nociception. But the extract of *H. sabdariffa* at the dose of 200 mg/kg body weight showed the more licking activity against late phase of formalin-induced pain than that of the standard drug Indomethacin.

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**Table 1:** CNS depressant activity of the methanol extract of fruits of *H. sabdariffa* on hole cross test in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (p.o.)</th>
<th>Number of Movements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Group-I</td>
<td>1% tween 80 in water</td>
<td>10ml/kg, p.o</td>
<td>12.8±2.588</td>
</tr>
<tr>
<td>Group-II</td>
<td>Diazepam</td>
<td>1mg/kg, p.o</td>
<td>11.2±1.304</td>
</tr>
<tr>
<td>Group-III</td>
<td>MEHS</td>
<td>250 mg/kg, p.o</td>
<td>12±1.581</td>
</tr>
<tr>
<td>Group-V</td>
<td>MEHS</td>
<td>500 mg/kg, p.o</td>
<td>12.2±1.483</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± STD (n=6); One way Analysis of Variance (ANOVA) followed by Dunnet’s test. *P<0.01, significant compared to control.

**Table 2:** CNS depressant activity of the methanol extract of fruits of *H. sabdariffa* on open field test in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (p.o.)</th>
<th>Number of Movements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Group-I</td>
<td>1% tween 80 in water</td>
<td>10mg/kg</td>
<td>118.4±2.70</td>
</tr>
<tr>
<td>Group-II</td>
<td>Diazepam</td>
<td>1mg/kg</td>
<td>117.2±2.59</td>
</tr>
<tr>
<td>Group-III</td>
<td>MEHS</td>
<td>250 mg/kg</td>
<td>118.2±1.92</td>
</tr>
<tr>
<td>Group-V</td>
<td>MEHS</td>
<td>500 mg/kg</td>
<td>117.2±2.39</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± STD (n=6); One way Analysis of Variance (ANOVA) followed by Dunnet’s test. *P<0.01, significant compared to control.

**Table 3:** Analgesic activity of the extract of *H. sabdariffa* on acetic acid induced writhing method in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose, route</th>
<th>No. of writhing</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I (Control)</td>
<td>1% Tween 80 in water</td>
<td>0.1 ml/10gm body weight</td>
<td>26.33±1.366</td>
<td>-</td>
</tr>
<tr>
<td>Group-II (Standard)</td>
<td>Indomethacin</td>
<td>10mg/kg, p.o</td>
<td>10.83±2.994*</td>
<td>58.87</td>
</tr>
<tr>
<td>Group-III (Extract)</td>
<td>MEHS</td>
<td>100mg/kg, p.o</td>
<td>12±4.817&quot;</td>
<td>54.425</td>
</tr>
<tr>
<td>Group-IV (Extract)</td>
<td>MEHS</td>
<td>200mg/kg, p.o</td>
<td>8.83±3.488*</td>
<td>66.464</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± STD (n=6); One way Analysis of Variance (ANOVA) followed by Dunnet’s test. *P<0.01, significant compared to control.
has been associated with increased level of PGE2 and PGF2α products (Derardt et al, 1980). The increase in prostaglandin levels within the peritoneal cavity then enhances the inflammation process through GABA A, therefore it is possible that extracts of H. sabdariffa might be responsible for the observed analgesic effect. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins (Rajnarayana et al, 2003). Phytochemical studies include the presence of alkaloids, flavonoids, saponins and steroids in the plant (Mungole and Chaturvedi, 2011). So might be this phytoconstituents are responsible for its CNS depressant activity.

Acetic acid induced writhing in rats attributed viscer al pain finds much attention of screening analgesic drugs (Hasan et al., 2010). The two different doses (100 & 200 mg/kg b. wt.) of crude extract of the plant showed significant analgesic activity compared to the reference drug Indomethacin but higher dose (200 mg/kg) was found to exhibit higher analgesic activity against acetic acid induced pain in rats. These results showed that MEHS have higher analgesic activity than earlier investigation (Reanmongkol and Itharat, 2007). Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid (Ahmed et al., 2008) via cyclooxygenase (COX), and prostaglandin biosynthesis (Duarte et al., 1988). 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 (Derardt et al., 1980). The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability (Zakaria et al., 2008). The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Ferdous et al., 2008). The significant pain reduction of the plant extract might be due to the presence of analgesic principles acting with the prostaglandin pathways. The abdominal writhing induced by acetic acid was also reported to be less selective (Collier et al., 1968) and proposed to act indirectly by releasing endogenous mediators stimulating neurons that are sensitive to other drugs such as narcotics and centrally acting agents (Toma et al., 2003). Phytochemical studies include the presence of alkaloids, flavonoids, saponins and steroids in H. sabdariffa (Mungole and Chaturvedi, 2011). Therefore, it is assumed that these compounds may be responsible for the observed analgesic activity. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins (Rajnarayana et al., 2001). Besides, alkaloids are well known for their ability to inhibit pain perception (Uche et al., 2008).

The pain in the early phase of formalin test was due to the direct stimulation of the sensory nerve fibres by formalin while the pain in the late phase was due to inflammatory mediators, like histamine, prostaglandins, serotonin and bradykinins (Dharmasiri et al., 2003). This test is believed to be a more valid analgesic model which is better correlated with clinical pain (Tjolsen et al., 1992 and Ghannadi et al., 2005). In this study, the extract caused a dose-dependent decrease in licking time (Table 4) by the rats injected with formalin signifying the analgesic effect of the extract. But no significant licking activity of ethanol and aqueous activity were found against both phases in formalin test in previous studies (Reanmongkol and Itharat, 2007).

Conclusion:

On the basis of results obtained from the present study, it can be concluded that the plant extract possesses remarkable CNS depressant and analgesic activities, thereby lends support to the traditional use of the plant in painful and inflammatory disorders. Present work was a preliminary effort which will require further detailed...
investigation including characterization of active compounds and requires preformulation studies for development of a potential dosage form.

References


Ghani, A., 1998 Medicinal Plants of Bangladesh: Chemical constituents and uses; Asiatic Society of Bangladesh., 84-85.


