Tagetes patula L. (Asteraceae): an evaluation of antihyperglycemic and antinociceptive activity of methanolic extract of leaves

Anjila Tabassum Zabin, Md. Ezazul Haque, Alok Kumar Paul, Shaiful Alam Bhuiyan, Md. Saiful Islam Roney, Mohammed Rahmatullah

Faculty of Life Sciences, University of Development Alternative, Dhanmondi, Dhaka-1205, Bangladesh.

ABSTRACT

The antihyperglycemic and antinociceptive activities of methanolic extract of Tagetes patula leaves were studied in Swiss albino mice, respectively, through oral glucose tolerance tests, and acetic acid-induced gastric pain with concomitant abdominal constrictions. In oral glucose tolerance tests, the extract at doses of 100, 200 and 400 mg per kg body weight reduced the increase in blood glucose concentrations in glucose-loaded mice by 27.9, 30.6, and 36.5%, respectively. The results were statistically significant compared to control animals at all the three afore-mentioned doses. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, reduced increases in blood glucose concentrations by 41.6%. Thus the extract demonstrated strong antihyperglycemic activity, which although not so potent as glibenclamide, at least merits further scientific studies towards isolation and identification of any antihyperglycemic constituent(s) present in leaves. In antinociceptive activity tests conducted with acetic acid-induced gastric pain in mice model, the extract, when administered at doses of 50, 100, 200 and 400 mg per kg body weight, reduced the number of gastric pain-induced abdominal constrictions in mice, respectively, by 50.0, 54.2, 62.5 and 66.7%. A standard antinociceptive drug, aspirin, when administered at a dose of 200 mg per kg body weight, reduced the number of abdominal constrictions in gastric pain induced mice by 37.5%. The results thus demonstrate that the leaf extract contains component(s) with more potent antinociceptive potential than that of aspirin. Overall, the results validate the folk medicinal uses in Bangladesh of the leaves for reduction of blood sugar in diabetic patients and for alleviation of pain.

Key words: Tagetes patula, antihyperglycemic, antinociceptive, Asteraceae

Introduction

Plants have since time immemorial proved to be a valuable resource to human beings for treatment of various ailments. This has continued even till present times when allopathic medicine is in vogue, for many important allopathic drugs have been and still are derived from plants. As such, documentation of traditional medicinal practices is important for this information can form the basis of for further scientific studies on any given plant species, and as such pave the way for discovery of better and more efficacious drugs. Most traditional medicinal practices in various countries of the world rely on medicinal plants as the main ingredient in formulations for treatment of various diseases. Folk medicine is widely prevalent in Bangladesh, and so can prove to be an excellent source for obtaining data on the basis of which pharmacological activity studies can be conducted on various plant species leading towards possible isolation and identification of lead compounds, which in turn can form the basis of newer drugs.

Ongoing studies by our research group have centered on ethnomedicinal surveys (Rahmatullah et al., 2009a-c; Rahmatullah et al., 2010a-g; Rahmatullah et al., 2011a,b; Rahmatullah et al., 2012a-d) followed by screening of the plants obtained for antihyperglycemic, antinociceptive and cytotoxic activities (Anwar et al., 2010; Jahan et al., 2010; Khan et al., 2010; Mannan et al., 2010; Rahman et al., 2010; Rahmatullah et al., 2010h; Shoha et al., 2010; Ali et al., 2011; Barman et al., 2011; Hossan et al., 2011; Jahan et al., 2011; Rahman et al., 2011; Sutradhar et al., 2011). As part of the screening process to locate plants with antihyperglycemic and antinociceptive properties, this study was conducted to evaluate the above two properties of methanolic extract

Corresponding Author: Dr. Mohammed Rahmatullah, Pro-Vice Chancellor and Dean, Faculty of Life Sciences University of Development Alternative House No. 78, Road No. 11A (new) Dhanmondi, Dhaka-1205 Bangladesh Tel: +88-01715032621; Fax: +88-02-815739; E-mail: rahamatm@hotmail.com
of leaves of *Tagetes patula* in Swiss albino mice. Notably, leaves and stems of this plant are used in folk medicines of Bangladesh for lowering of blood sugar in diabetic patients and for alleviation of pain.

**Materials and Methods**

Leaves of *Tagetes patula* were collected from Narayanganj in Dhaka district, Bangladesh during March 2013. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka. The sliced and air-dried leaves of *Tagetes patula* were grounded into a fine powder and 70g of the powder was extracted with methanol (1:5, w/v) for 48 hours. The extract was evaporated to dryness. The final weight of the extract was 4.66g.

**Chemicals:**

Glacial acetic acid was obtained from Sigma Chemicals, USA; aspirin, glibenclamide and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh.

**Animals:**

In the present study, Swiss albino mice (male), which weighed between 19-22 g were used. The animals were obtained from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). All animals were kept under ambient temperature with 12h light followed by a 12h dark cycle. The animals were acclimatized for three days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

**Antihyperglycemic activity:**

Glucose tolerance property of methanol extract of *Tagetes patula* leaves was determined as per the procedure previously described by Joy and Kuttan (1999) with minor modifications. In brief, fasted mice were grouped into six groups of five mice each. The various groups received different treatments like Group 1 received vehicle (1% Tween 80 in water, 10 ml/kg body weight) and served as control, group 2 received standard drug (glibenclamide, 10 mg/kg body weight). Groups 3-6 received methanol extract of *Tagetes patula* leaves at doses of 50, 100, 200 and 400 mg per kg body weight. Each mouse was weighed and doses adjusted accordingly prior to administration of vehicle, standard drug, and test samples. All substances were orally administered. Following a period of one hour, all mice were orally administered 2 g glucose/kg of body weight. Blood samples were collected 120 minutes after the glucose administration through puncturing heart. Blood glucose levels were measured by glucose oxidase method (Venkatesh *et al.*, 2004).

**Antinociceptive activity:**

Antinociceptive activity of the methanol extract of *Tagetes patula* leaves was examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005). Briefly, mice were divided into six groups of five mice each. Group 1 served as control and was administered vehicle only. Group 2 was orally administered the standard antinociceptive drug aspirin at a dose of 200 mg per kg body weight. Groups 3-6 were administered methanolic leaf extract of *Tagetes patula* at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or extract, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 15 minutes was given to each animal to ensure bio-availability of acetic acid, following which period, the number of writhings was counted for 10 min.

**Statistical analysis:**

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.

**Results and Discussion**

In oral glucose tolerance tests, the extract at doses of 100, 200 and 400 mg per kg body weight reduced the increase in blood glucose concentrations in glucose-loaded mice by 27.9, 30.6, and 36.5%, respectively. The results were statistically significant compared to control animals at all the three afore-mentioned doses. At a dose of 50 mg extract per kg body weight, a slight increase in blood glucose concentration was noted but the
result was not statistically significant when compared to control mice. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, reduced increases in blood glucose concentrations by 41.6%. The results are shown in Table 1. The present observations validate the folk medicinal uses of the plant to reduce blood sugar in diabetic patients, and further suggest that more studies may be carried out towards isolation and identification of the responsible antihyperglycemic phytochemical component(s) present within the methanolic extract of leaves.

In antinociceptive activity tests conducted with acetic acid-induced gastric pain in mice model, the extract, when administered at doses of 50, 100, 200 and 400 mg per kg body weight, reduced the number of gastric pain-induced abdominal constrictions in mice, respectively, by 50.0, 54.2, 62.5 and 66.7%. The results were both dose-dependent and statistically significant. A standard antinociceptive drug, aspirin, when administered at a dose of 200 mg per kg body weight, reduced the number of abdominal constrictions in gastric pain induced mice by 37.5%. The results are shown in Table 2. The results thus demonstrate that the leaf extract contains component(s) with more potent antinociceptive potential than that of aspirin. Overall, the results also validate the folk medicinal uses in Bangladesh of the leaves for alleviation of pain.

In the present study, the active components of the leaves responsible for the observed antihyperglycemic and antinociceptive effects were not isolated and identified. However, beta-caryophyllene and terpinolene has been reported from the plant, and which two components may be responsible for the observed antihyperglycemic and antinociceptive effects (Limem-Ben Amor et al., 2009; Siani et al., 1999).

### Table 1: Effect of methanol extract of Tagetes patula leaves on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Blood glucose level (mmol/l)</th>
<th>% lowering of blood glucose level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group 1)</td>
<td>10 ml</td>
<td>4.38 ± 0.12</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide (Group 2)</td>
<td>10 mg</td>
<td>2.56 ± 0.39</td>
<td>41.6*</td>
</tr>
<tr>
<td><em>Tagetes patula</em> (Group 3)</td>
<td>50 mg</td>
<td>4.92 ± 0.65</td>
<td>-</td>
</tr>
<tr>
<td><em>Tagetes patula</em> (Group 4)</td>
<td>100 mg</td>
<td>3.16 ± 0.29</td>
<td>27.9*</td>
</tr>
<tr>
<td><em>Tagetes patula</em> (Group 5)</td>
<td>200 mg</td>
<td>3.04 ± 0.20</td>
<td>30.6*</td>
</tr>
<tr>
<td><em>Tagetes patula</em> (Group 6)</td>
<td>400 mg</td>
<td>2.78 ± 0.15</td>
<td>36.5*</td>
</tr>
</tbody>
</table>

All administrations were made orally. Values represented as mean ± SEM, (n=5); *P < 0.05; significant compared to hyperglycemic control animals.

### Table 2: Antinociceptive effect of crude methanol extract of Tagetes patula leaves in the acetic acid-induced gastric pain model mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Mean number of writhings</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group 1)</td>
<td>10 ml</td>
<td>4.80 ± 0.20</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin (Group 2)</td>
<td>200 mg</td>
<td>3.00 ± 0.55</td>
<td>37.5*</td>
</tr>
<tr>
<td><em>Tagetes patula</em> (Group 3)</td>
<td>50 mg</td>
<td>2.40 ± 1.03</td>
<td>50.0*</td>
</tr>
<tr>
<td><em>Tagetes patula</em> (Group 4)</td>
<td>100 mg</td>
<td>2.20 ± 0.37</td>
<td>54.2*</td>
</tr>
<tr>
<td><em>Tagetes patula</em> (Group 5)</td>
<td>200 mg</td>
<td>1.80 ± 0.80</td>
<td>62.5*</td>
</tr>
<tr>
<td><em>Tagetes patula</em> (Group 6)</td>
<td>400 mg</td>
<td>1.60 ± 0.51</td>
<td>66.7*</td>
</tr>
</tbody>
</table>

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=5); *P < 0.05; significant compared to control.

### References


