A Preliminary Study Of The Antihyperglycemic And Antinociceptive Potential Of Tagetes Patula L. (Asteraceae) Stems


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ABSTRACT

The antihyperglycemic and antinociceptive potential of Tagetes patula stems, a plant used in folk medicines of Bangladesh for treatment of pain, inflammation, cuts and wounds, and lowering of blood sugar were investigated. In antihyperglycemic activity tests conducted with glucose-loaded Swiss albino mice, methanolic extract of stems significantly and dose-dependently reduced blood sugar concentrations. At extract doses of 50, 100, 200 and 400 mg per kg body weight mice, the percent lowering of blood sugar by the extract was, respectively, 47.11, 49.59, 50.62, and 60.02. The results were both dose-dependent and statistically significant. A standard antihyperglycemic drug, glibenclamide, when administered to glucose-loaded mice, reduced blood sugar level by 60.95%. The results demonstrate that the methanolic extract possesses considerable antihyperglycemic potential, and which is comparable to that of glibenclamide at the highest dose of the extract tested. In antinociceptive activity tests conducted with intraperitoneally administered acetic acid-induced gastric pain model in mice, the extract at the afore-mentioned four doses dose-dependently reduced the number of writhings in mice caused by the gastric pain, respectively, by 36.72, 40.88, 42.84, and 47.00%. The results were statistically significant at the three highest doses of the extract. A standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, reduced the number of writhings by 48.96 and 67.32%, respectively. The results thus demonstrate also significant antinociceptive potential of stems of the plant. Taken together, the results validate the folk medicinal uses of the plant for treatment of pain and for lowering blood sugar level in diabetic patients.

Key words: Tagetes patula, antihyperglycemic, antinociceptive, Asteraceae

Introduction

The French marigold (Tagetes patula L.) is a species in the daisy family (Asteraceae). In Bengali, the plant is known as Gada or Ganda. Leaves and stems of the plant are commonly used in folk medicine of Bangladesh for the treatment of pain, inflammation, and cuts and wounds (to stop bleeding). Juice obtained from macerated stems is also administered orally to diabetic people with high blood sugar levels to lower blood sugar concentrations. Various antioxidant agents like methyl protocatechuaceae along with patuleatin and patulitrin has been isolated from flowers of the plant; among the constituents, patuleatin has been reported to possess mild analgesic property (Faizi et al., 2011). Essential oil obtained from leaves and inflorescences have been shown to contain terpinolene, pipertitone, beta-caryophyllene, and cis-beta-oicinene (Armas et al., 2012). Thiophenes, triterpene and steroid has also been reported from roots, leaves and flowers of the plant (Bano et al., 2002).

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; Hossain 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 of stems of *Tagetes patula* in Swiss albino mice.

**Materials and Methods**

Stems of *Tagetes patula* were collected from Savar in Dhaka district, Bangladesh during April, 2011. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka (Accession Number 35,446). The sliced and air-dried stems of *Tagetes patula* were grounded into a fine powder and 100g of the powder was extracted with methanol (1:6, w/v) for 48 hours. The extract was evaporated to dryness. The final weight of the extract was 2.60g.

**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 15-22 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* stems 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 six 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* stems 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* stems was examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005). Briefly, mice were divided into seven groups of six mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard antinociceptive drug aspirin at a dose of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered methanolic stem 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**

Oral administration of the extract at doses of 50, 100, 200 and 400 mg per kg body weight to glucose-loaded mice resulted in dose-dependent and significant lowering of blood sugar in mice as compared to control mice, which were administered with vehicle only. At the afore-mentioned four doses, the percent reduction in blood sugar was, respectively, 47.11, 49.59, 50.62, and 60.02. A standard antihyperglycemic drug,
glibenclamide, used for lowering blood sugar in diabetic patients, when administered at a dose of 10 mg per kg body weight, reduced blood sugar by 60.95%. The highest dose of the extract, namely 400 mg, can therefore be favorably compared to glibenclamide. The results are shown in Table 1.

In antinociceptive activity test, the extract at the afore-mentioned four doses reduced the number of writhings in gastric pain model mice, respectively, by 36.72, 40.88, 42.84, and 47.00%. By comparison, a standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, reduced the number of writhings by 48.96 and 67.32%, respectively. Thus the extract, although not so potent as aspirin, still demonstrated significant antinociceptive potential. The results are shown in Table 2.

The observed reduction of blood sugar by the extract in glucose-loaded mice can be attributed to any of several possible mechanisms or a combination of the mechanisms. A compound or compounds may inhibit glucose absorption in gut, as observed with Mangifera indica L. (Anacardiaceae) stem-barks (Bhowmik et al., 2009). Alternatively, any bio-active compound or compounds present in the extract may lower blood sugar either by potentiating the pancreatic secretion of insulin or increasing the glucose uptake, as has been observed in studies with Artemisia extract and extract of Ageratum conyzoides L. (Asteraceae), respectively (Farjou et al., 1987; Nyunai et al., 2009). A further mechanism can possibly be increase of peripheral glucose consumption induced by the extract, as has been seen with ethanolic extract of Sapindus trifoliatus L. (Sapindaceae) (Sahoo et al., 2010). In either of these mechanisms or a combination of these mechanisms, the resultant effect will be reduction of sugar levels in the blood. Further experiments are necessary to elucidate the glucose lowering mechanism of the present extract.

Both central and peripheral analgesia can be detected with the test of acetic acid-induced gastric pain, followed by measurement of the number of writhings (Shanmugasundaram and Venkataraman, 2005), as has been done in the present study. Increased expression of prostaglandins [mainly prostacyclines (PGI2) and prostaglandin- (PG-E)] has been shown to be responsible for excitation of Adelta-nerve fibers, leading to the sensation of pain (Reynolds, 1982; Rang and Dale, 2003). As such, the antinociceptive activity exhibited by crude methanolic extract of the leaves may be due to the extract’s ability to block any further expression of prostaglandins, which may be mediated through inhibition of cyclooxygenase and/or lipooxygenase activities. It is to be noted that a similar mechanism has been proposed for antinociceptive activity of Ficus deltoidea Jack (Moraceae) aqueous extract in acetic acid-induced gastric pain model (Sulaiman et al., 2008), and this may also be the mechanism operating in the present study.

The results obtained in this study validate the folk medicinal use of the plant for lowering of blood sugar and alleviation of pain. In this preliminary study, neither the active components of the stem responsible for the observed antihyperglycemic and antinociceptive effects were isolated and identified, nor the exact mechanisms responsible for the observed effects elucidated. Further experiments are ongoing in our laboratory to identify the responsible components of the plant behind the observed pharmacological effects. 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 stems 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>9.68 ± 0.76</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide (Group 2)</td>
<td>10 mg</td>
<td>3.78 ± 0.28</td>
<td>60.95*</td>
</tr>
<tr>
<td>Tagetes patula (Group 3)</td>
<td>50 mg</td>
<td>5.12 ± 0.49</td>
<td>47.11*</td>
</tr>
<tr>
<td>Tagetes patula (Group 4)</td>
<td>100 mg</td>
<td>4.88 ± 0.67</td>
<td>49.59*</td>
</tr>
<tr>
<td>Tagetes patula (Group 5)</td>
<td>200 mg</td>
<td>4.78 ± 0.39</td>
<td>50.63*</td>
</tr>
<tr>
<td>Tagetes patula (Group 6)</td>
<td>400 mg</td>
<td>3.87 ± 0.26</td>
<td>60.02*</td>
</tr>
</tbody>
</table>

All administrations were made orally. Values represented as mean ± SEM, (n=6); *P < 0.05; significant compared to hyperglycemic control animals.

Table 2: Antinociceptive effect of crude methanol extract of Tagetes patula stems 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>8.17 ± 0.79</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin (Group 2)</td>
<td>200 mg</td>
<td>4.17 ± 0.65</td>
<td>48.96*</td>
</tr>
<tr>
<td>Aspirin (Group 3)</td>
<td>400 mg</td>
<td>2.67 ± 0.88</td>
<td>67.32*</td>
</tr>
<tr>
<td>Tagetes patula (Group 4)</td>
<td>50 mg</td>
<td>5.17 ± 1.68</td>
<td>36.72</td>
</tr>
<tr>
<td>Tagetes patula (Group 5)</td>
<td>100 mg</td>
<td>4.83 ± 1.08</td>
<td>40.88*</td>
</tr>
<tr>
<td>Tagetes patula (Group 6)</td>
<td>200 mg</td>
<td>4.67 ± 0.76</td>
<td>42.84*</td>
</tr>
<tr>
<td>Tagetes patula (Group 7)</td>
<td>400 mg</td>
<td>4.33 ± 0.49</td>
<td>47.00*</td>
</tr>
</tbody>
</table>

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=6); *P < 0.05; significant compared to control.
References


