ORIGINAL ARTICLE

**Antihyperglycemic and antinociceptive properties of methanolic extract of whole plants of *Amaranthus viridis* L. (Amaranthaceae)**

Farhana Rahman, Sohana Afroz, Sharmin Jahan, Mobasser Hosain, Dalia Flowrin Khondoker, Sk. Mizanur Rahman, Joyonta Banik, Ishtiaq Ahmad, Mohammed Rahmatullah

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


**ABSTRACT**

*Amaranthus viridis* is traditionally eaten as a vegetable in Bangladesh, India and many other countries of the world. In the Indian Ayurvedic medicinal system, the leaves are considered diuretic and purgative and are used fresh or in the powdered form for treatment of inflammations, boils, gonorrhea, orchitis, and hemorrhoids. The objective of the present study was to evaluate the antihyperglycemic and antinociceptive potential of methanol extract of whole plants in oral glucose tolerance tests and acetic acid-induced gastric pain model in Swiss albino mice, respectively. In oral glucose tolerance tests, conducted in glucose-loaded mice, the extract was found to demonstrate dose-dependent significant antihyperglycemic activity. At extract doses of 50, 100, 200 and 400 mg per kg body weight of mice, serum glucose level was lowered, respectively, by 23.7, 27.1, 42.7 and 49.2%. The results were statistically significant at the three higher doses of 100, 200 and 400 mg extract administered per kg body weight. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, decreased serum glucose level by 46.1%, suggesting that whole plants of *Amaranthus viridis* possess considerable antihyperglycemic potential and may be used in cases of diabetic patients with high blood glucose levels. In antinociceptive activity tests, the extract when administered at doses of 50, 100, 200 and 400 mg per kg body weight in mice, reduced the number of acetic acid-induced writhings by 24.2, 30.4, 51.5 and 60.5%, when compared to control animals. The results were significant at the two higher doses of extract at 200 and 400 mg per kg body weight. A standard antinociceptive drug, aspirin, when administered at a dose of 400 mg per kg body weight reduced the number of writhings by 60.5%. The results suggest that the plant may be used for further scientific studies leading to isolation of active phytochemical constituents, which can prove useful as novel and more efficacious anti-diabetic and pain-killing drugs.

Key words: *Amaranthus viridis*, antihyperglycemic, antinociceptive, Amaranthaceae.

**Introduction**

*Amaranthus viridis* L. (Amaranthaceae) is known locally in Bangladesh as ‘data shak’ and in English as Slender Amaranth or Green Amaranth. The plant is traditionally eaten as a vegetable in Bangladesh and in South India, especially in Kerala, where it is known as ‘kuppacheera’. In Greece, the plant is known as ‘vlita’ and is traditionally eaten following boiling and is served with olive oil and lemon. The plant is also eaten in parts of Africa, Sri Lanka, Fiji, and Jamaica. The Sanskrit name of the plant is ‘tanduliya’, and it is considered in Ayurvedic medicine as a medicinal plant. In Ayurveda, the leaves are considered as diuretic and purgative and are used for the treatment of inflammations, boils, gonorrhea, orchitis, and hemorrhoids. The leaves are also believed to have febrifugal properties. The ash of the plant is rich in soda and is sometimes used to make soap.

A novel antiproliferative and antifungal lectin has been reported from the seeds of the plant (Kaur et al., 2006). Antidiabetic, antihyperlipidemic and antioxidant activities of methanolic extract of the plant has been reported in alloxan-induced diabetic rats (Ashok Kumar et al., 2012). Antihyperglycemic and antihyperlipidemic activity of methanolic extract of leaves has been observed in experimental diabetes (Krishnamurthy et al., 2011). Antihyperglycemic and hypolipidemic activity of methanol extract of leaves has been shown (Girija et al., 2011). Extract of the plant has been reported to modulate C-reactive protein, protein profile, ceruloplasmin and glycprotein in experimentally induced myocardial infracted rats (Saravanan and Ponnurugan, 2012).
Methanolic extract of whole plant reportedly demonstrated hepatoprotective and antioxidant activities (Ashok Kumar et al., 2011). Antiinflammatory activity of various solvent extracts of leaves has been reported in carrageenan-induced paw edema and cotton pellet granuloma in rats (Macharla et al., 2011). Methanolic extract of whole plant reportedly demonstrated antinociceptive and antipyretic activity using acetic acid-induced writhing test, hot plate test and tail immersion test in mice (Ashok Kumar et al., 2009a).

Any discovery of a new drug from medicinal plants usually involves an initial gathering of information from traditional practitioners of medicine followed by screening of the plant for relevant pharmacological activity. We had been conducting such surveys for a number of years to document the use of various plants of Bangladesh for treatment of various ailments (Rahmatullah et al., 2009a-c; Rahmatullah et al., 2010a-g; Rahmatullah et al., 2011a,b; Rahmatullah et al., 2012a-d). Furthermore, on the basis of our ethnomedicinal surveys, we had been screening medicinal plants for their anti-hyperglycemic and antinociceptive effects (Anwar et al., 2010; Jahan et al., 2010; Khan et al., 2010; Mannan et al., 2010; Rahman et al., 2010; 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), because diabetes and pain are two of the most common diseases affecting millions of people throughout the world. As part of this ongoing screening of medicinal plants of Bangladesh for possible antinociceptive effects, the objective of the present study was to evaluate the methanolic extract of whole plants of *Amaranthus viridis* for antihyperglycemic activity using the oral glucose tolerance test (OGTT), and antinociceptive activity through the acetic acid-induced gastric pain model in Swiss albino mice.

**Materials and Methods**

Whole plants of *Amaranthus viridis* were collected from Savar in Dhaka district, Bangladesh during May, 2011. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka (Voucher specimen No. 35,578). The sliced and air-dried whole plants of *Amaranthus viridis* 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 6.35g.

**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 18-22 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 *Amaranthus viridis* whole plants 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 *Amaranthus viridis* whole plants 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. Serum glucose levels were measured by glucose oxidase method (Venkatesh et al., 2004).

**Antinociceptive activity:**

Antinociceptive activity of the methanol extract of *Amaranthus viridis* whole plant was examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005). Briefly, mice were divided into six groups of six 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 400 mg per kg body weight, respectively. Groups 3-6 were administered methanolic whole plant extract of *Amaranthus viridis* 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 5 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 conducted for evaluation of antihyperglycemic activity of the plant extract and conducted in glucose-loaded mice, the extract was found to demonstrate dose-dependent significant antihyperglycemic activity. At extract doses of 50, 100, 200 and 400 mg per kg body weight of mice, serum glucose level was lowered, respectively, by 23.7, 27.1, 42.7 and 49.2%. The results were statistically significant at the three higher doses of 100, 200 and 400 mg extract administered per kg body weight. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, decreased serum glucose level by 46.1%, suggesting that whole plants of *Amaranthus viridis* possess considerable antihyperglycemic potential and may be used in cases of diabetic patients to lower high blood glucose levels. In antinociceptive activity tests, the extract when administered at doses of 50, 100, 200 and 400 mg per kg body weight in mice, reduced the number of acetic acid-induced writhings by 24.2, 30.4, 51.5 and 60.5%, when compared to control animals. The results were significant at the two higher doses of extract at 200 and 400 mg per kg body weight. A standard antinociceptive drug, aspirin, when administered at a dose of 400 mg per kg body weight reduced the number of writhings by 60.5%. The antinociceptive activity test results also suggest that the extract contain phytochemical constituent(s) with significant pain-killing ability.

The observed glucose lowering effect by the crude extract of whole plants may occur through several possible mechanisms. The extracts may potentiate the pancreatic secretion of insulin or increase the glucose uptake (Farjou et al., 1987; Nyunai et al., 2009). Alternately, the extracts may inhibit glucose absorption in gut (Bhowmik et al., 2009). Any of the above three mechanisms or a combination of mechanisms can contribute to the observed lowering of blood sugar. However, the exact mechanism of antihyperglycemic action remains to be elucidated. However, rutin and quercetin has been reported to be present in *Amaranthus viridis* (Ashok Kumar et al., 2009b). Rutin has been reported to have glucose-lowering effect in streptozotocin-induced diabetic rats (Mondal et al., 2012). Quercetin is also known to potentiate insulin secretion and protect INS-1 pancreatic beta-cells against oxidative damage (Youl et al., 2010).

Analgesia can be central or peripheral, and both central and peripheral analgesia can be detected with the acetic acid-induced writhing test (Shanmugasundaram and Venkataraman, 2005). Prostaglandins are considered as promulgators of pain. Production 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 extracts of the two mushroom species may be due to these extract’s ability to block synthesis of prostaglandins. This, in turn, 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* aqueous extract in acetic acid-induced gastric pain model (Sulaiman et al., 2008).

The bio-active components responsible for the antihyperglycemic and antinociceptive activities in *Amaranthus viridis* need to be isolated and identified in view of their possible uses as anti-diabetic and pain-killing agents. Such studies are now being undertaken in our laboratory. It is expected that novel compounds may be detected, which can prove to be more efficacious in the treatment of diabetes and pain.

**Table 1:** Effect of methanol extract of *Amaranthus viridis* whole plants on serum 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>Serum glucose level (mmol/l)</th>
<th>% lowering of serum glucose level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group 1)</td>
<td>10 ml</td>
<td>8.85 ± 0.66</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide (Group 2)</td>
<td>10 mg</td>
<td>4.77 ± 0.51</td>
<td>46.1*</td>
</tr>
<tr>
<td><em>Amaranthus viridis</em> (Group 3)</td>
<td>50 mg</td>
<td>6.75 ± 0.95</td>
<td>23.7</td>
</tr>
<tr>
<td><em>Amaranthus viridis</em> (Group 4)</td>
<td>100 mg</td>
<td>6.45 ± 0.54</td>
<td>27.1*</td>
</tr>
<tr>
<td><em>Amaranthus viridis</em> (Group 5)</td>
<td>200 mg</td>
<td>5.07 ± 0.55</td>
<td>42.7*</td>
</tr>
<tr>
<td><em>Amaranthus viridis</em> (Group 6)</td>
<td>400 mg</td>
<td>4.50 ± 0.52</td>
<td>49.2*</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 *Amaranthus viridis* whole plants 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>5.50 ± 0.85</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin (Group 2)</td>
<td>400 mg</td>
<td>2.17 ± 0.98</td>
<td>60.5*</td>
</tr>
<tr>
<td><em>Amaranthus viridis</em> (Group 3)</td>
<td>50 mg</td>
<td>4.17 ± 0.75</td>
<td>60.5*</td>
</tr>
<tr>
<td><em>Amaranthus viridis</em> (Group 4)</td>
<td>100 mg</td>
<td>3.83 ± 0.83</td>
<td>24.2</td>
</tr>
<tr>
<td><em>Amaranthus viridis</em> (Group 5)</td>
<td>200 mg</td>
<td>2.67 ± 0.61</td>
<td>30.4</td>
</tr>
<tr>
<td><em>Amaranthus viridis</em> (Group 6)</td>
<td>400 mg</td>
<td>3.17 ± 0.79</td>
<td>51.5*</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


