

ANTIHYPERGLYCAEMIC AND ANTINOCICEPTIVE ACTIVITY EVALUATION OF
METHANOLIC EXTRACT OF WHOLE PLANT OF *AMARANTHUS TRICOLOUR* L.
(AMARANTHACEAE)

Mohammed Rahmatullah*, Mobasser Hosain, Shahnaz Rahman, Shiblur Rahman, Mahfuza Akter,
Farhana Rahman, Fatema Rehana, Mahmuda Munmun, Marjina Akter Kalpana

Department of Biotechnology & Genetic Engineering, mUniversity of Development Alternative,
Dhanmondi, Dhaka-1205, Bangladesh.

*Email: rahamatm@hotmail.com

Abstract

Amaranthus tricolor whole plants are used by folk medicinal practitioners of Bangladesh for treatment of pain, anaemia, dysentery, skin diseases, diabetes, and as a blood purifier. Thus far, no scientific studies have evaluated the antihyperglycaemic and antinociceptive effects of the plant. The present study was carried out to evaluate the possible glucose tolerance efficacy of methanolic extracts of *A. tricolor* whole plants using glucose-induced hyperglycaemic mice, and antinociceptive effects with acetic acid-induced gastric pain models in mice. In antihyperglycaemic activity tests, the extract at different doses was administered one hour prior to glucose administration and blood glucose level was measured after two hours of glucose administration (p.o.) using glucose oxidase method. The statistical data indicated the significant oral hypoglycaemic activity on glucose-loaded mice at all doses of the extracts tested. Maximum antihyperglycaemic activity was shown at 400 mg extract per kg body weight, which was comparable to that of a standard drug, glibenclamide (10 mg/kg body weight). In antinociceptive activity tests, the extract also demonstrated a dose-dependent significant reduction in the number of writhings induced in mice through intraperitoneal administration of acetic acid. Maximum antinociceptive activity was observed at a dose of 400 mg extract per kg body weight, which compared favourably with that of a standard antinociceptive drug, aspirin, when administered at a dose of 200 mg per kg body weight. The results validate the folk medicinal use of the plant for reduction of blood sugar in diabetic patients as well as the folk medicinal use for alleviation of pain. The results suggest that this plant may possess further potential for scientific studies leading to possible discovery of efficacious antihyperglycaemic and antinociceptive components.

Key words: *Amaranthus tricolor*, antihyperglycaemic, antinociceptive, Amaranthaceae

Introduction

Amaranthus tricolor L. (Amaranthaceae) is an ornamental plant known as Joseph's coat in English and 'laal shak' in the local (Bengali) language. The plant is both cultivated in Bangladesh, and can be found growing on fallow lands. It is well-liked for its taste and enjoys high consumer demand as a vegetable. The plant is also known by its synonyms, namely *Amaranthus gangeticus* L., *Amaranthus mangostanus* L., and *Amaranthus tristis* L. In Indian traditional medicine, the plant is used for treatment of a variety of ailments like coughs, throat infections, toothache, eczema, piles, diarrhoea, gonorrhoea, leucorrhoea, and impotence (Aneja *et al.*, 2011). Whole plants are used by folk medicinal practitioners of Bangladesh for treatment of pain, anaemia, dysentery, skin diseases, diabetes, and as a blood purifier.

The plant has been reported to possess hepatoprotective activity (Al-Dosari, 2010), antiviral activity (Roy *et al.*, 2009), cyclooxygenase enzyme inhibitory, and antiproliferative activity (Jayaprakasam *et al.*, 2004). Leaf extracts of the plant have been reported to possess very good antiulcer property in experimental animal models of gastric ulcers (Devaraj and Krishna, 2011). Phytochemical screening studies conducted with the plant have revealed the presence of carbohydrates (Behari and Sharma, 1984), flavonoids like betacyanins A and B, amaranthin, isoamaranthin, and quercetin (Piatteli *et al.*, 1964; Zakharova *et al.*, 1995), and various sterol compounds like spinasterol, cholesterol, campesterol, 24-methylene cholesterol, stigmasterol, β -sitosterol, fucosterol, and isofucosterol (Behari and Sharma, 1984; Fernando and Bean, 1984).

Since the plant is used for treatment of diabetes and pain by the folk medicinal practitioners of Bangladesh, the objective of the present study was to evaluate the antihyperglycaemic activity of methanolic extract of the plant using glucose-loaded mice in oral glucose tolerance tests (OGTT), and antinociceptive activity in acetic acid-induced gastric writhing pain model in mice.

Materials and Methods

Collection of plant material

Whole plants of *A. tricolour* were collected in November of 2011 from Savar in Dhaka district, Bangladesh. The leaves were identified by the Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. 35,577) and sample specimens have been kept over there.

Preparation of the test samples

Whole plants were cut into small pieces, air-dried in the shade, pulverised into a fine powder, and were mixed with methanol at a ratio of 1:6 (w/v). After 24 hours, the mixtures were filtered and the filtrate was collected. Filtrate was evaporated to dryness (approximate yield 5.35%) using rotary evaporator. Extracts were suspended in 1% Tween 80 in water prior to administration.

Animals

Swiss albino mice (male), weighing 15-20g bred in the animal house of ICDDR,B (International Centre for Diarrhoeal Disease and Research, Bangladesh) were used for the present experiments. All the animals were acclimatised one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature $25.0 \pm 2^\circ\text{C}$, and 12 hrs light-dark cycles). The animals were fed with standard diet from ICDDR,B and had free access to water. The study was approved by the Institutional Animal Ethical Committee of the University of Development Alternative, Dhaka, Bangladesh.

Antihyperglycaemic activity test

Antihyperglycaemic activity of the extract was studied through the glucose tolerance test method. Glucose tolerance test was performed following the procedure as described by Joy and Kuttan (1999) with slight modifications (Rahman et al., 2011; Ahmed et al., 2011). In brief, fasted mice were divided into six groups of six mice each. Each group received a particular treatment: Group 1 served as control and received vehicle (1% Tween 80 in water, 10 ml per kg body weight), while Group 2 received standard drug (glibenclamide, 10 mg per kg body weight). Groups 3-6 received extract of whole plants of *A. tricolour* at four different doses of 50, 100, 200 and 400 mg extract per kg body weight, respectively. Each mouse was weighed properly and the doses of the test samples, standard drug, and control materials were adjusted accordingly. Test samples, control, and glibenclamide were given orally. After one hour, all mice were orally treated with 2 g per kg body weight of glucose. Blood samples were collected two hours after glucose administration. Serum was separated and blood glucose levels were measured immediately by glucose oxidase method (Venkatesh et al., 2004).

Antinociceptive activity test

Antinociceptive activity of the methanol extract was examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005). Briefly, for methanolic extract of *A. tricolour* whole plants, 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 200 mg per kg body weight. Groups 3-6 were administered methanolic whole plant extract of *A. tricolour* at doses of 50, 100, 200 and 400 mg per kg body weight, respectively.

Statistical analysis

Experimental values are expressed as mean $\pm$ 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

The methanolic whole plant extract of *A. tricolour* exhibited significant and dose-dependent lowering of serum glucose levels in glucose-loaded mice in oral glucose tolerance (antihyperglycaemic) tests. The highest lowering of blood sugar (44.0%) was observed with the highest dose of extract tested, at 400 mg per kg body weight. Even with the dose of 200 mg extract per kg body weight, the lowering of serum glucose was by 43.1%, which compares favourably with that of the standard antihyperglycaemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight (44.7%). The results validate the folk medicinal use of the plant for treatment of diabetic patients.

In antinociceptive activity tests, the extract also demonstrated dose-dependent and significant reductions in the number of writhings induced by intraperitoneal administration of acetic acid in mice. At doses of 50, 100, 200 and 400 mg extract per kg body weight, the percent reductions in the number of writhings were, respectively, 32.7, 36.7, 40.9, and 47.0%. The percent reduction in the number of writhings at the highest dose of the extract tested compares favourably with that of a standard

antinociceptive drug, aspirin, when administered at a dose of 200 mg per kg body weight, which reduced the number of writhings by 51.0%. Thus, the folk medicinal use of the extract for treatment of pain was also found to be validated in the present study.

The observed glucose lowering effect by the crude extract may occur through several possible mechanisms. The extract may potentiate the pancreatic secretion of insulin or increase the glucose uptake (Farjou *et al.*, 1987; Nyunai *et al.*, 2009). Alternately, the extract may inhibit glucose absorption in gut (Bhowmik *et al.*, 2009). Although the present study did not identify the chemical component(s) responsible for the observed antihyperglycaemic effect, it may be noted that quercetin is one of the components observed to be present in *A. tricolour* (see Introduction, above). Methanol extract of *Moringa oleifera* pods containing quercetin and kaempferol has been reported to have antidiabetic and antioxidant effects when tested in streptozotocin-induced diabetic albino rats (Gupta *et al.*, 2012). Quercetin was also one of the components found in cell cultures of *Morus nigra* exhibiting hypoglycaemic efficacy (Abd El-Mawla *et al.*, 2011). Quercetin was also one of the major compounds found in rice bean (*Vigna umbellata*), when tested for antioxidant capacity and antidiabetic potential (Yao *et al.*, 2012). β -Sitosterol, another compound reported to be present in the plant, has also reportedly demonstrated antidiabetic and antioxidant potential in streptozotocin-induced experimental hyperglycaemia (Gupta *et al.*, 2011).

Both central and peripheral analgesia can be detected with the acetic acid-induced writhing test (Shanmugasundaram and Venkataraman, 2005). Production of prostaglandins [mainly prostacyclins (PGI₂) and prostaglandin- (PG-E)] has been shown to be responsible for excitation of A δ -nerve fibres, leading to the sensation of pain (Reynolds, 1982; Rang and Dale, 1993). As such, the antinociceptive activity exhibited by crude methanolic extract of *A. tricolour* may be due to the extract's ability to block synthesis of prostaglandins, which may be effected through inhibition of cyclooxygenase and/or lipoxygenase 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). It is noteworthy in this regard that cyclooxygenase enzyme inhibitory activity has been reported for *A. tricolour* (Jayaprakasam *et al.*, 2004), although the bioactive components responsible for this activity are yet to be elucidated.

Table 1: Effect of methanol extract of *Amaranthus tricolour* whole plant on serum glucose level in hyperglycaemic mice following 120 minutes of glucose loading

Treatment	Dose (mg/kg body weight)	Serum glucose level (mmol/litre)	% lowering of serum glucose level
Control	10 ml	7.38 $\pm$ 0.55	-
Glibenclamide	10 mg	4.08 $\pm$ 0.42*	44.7
<i>Amaranthus tricolour</i>	50 mg	5.32 $\pm$ 0.29*	27.9
<i>Amaranthus tricolour</i>	100 mg	5.00 $\pm$ 0.68*	32.2
<i>Amaranthus tricolour</i>	200 mg	4.20 $\pm$ 0.31*	43.1
<i>Amaranthus tricolour</i>	400 mg	4.13 $\pm$ 0.29*	44.0

All administrations were done orally. Values are represented as mean $\pm$ SEM, (n=6); **P* < 0.05; significant compared to hyperglycaemic control animals.

Table 2: Antinociceptive effect of crude methanol extract of *Amaranthus tricolour* whole plant in acetic acid-induced gastric pain model mice

Treatment	Dose (mg/kg body weight)	Mean number of writhings	% inhibition
Control	10 ml	8.17 $\pm$ 0.79	-
Aspirin	200 mg	4.00 $\pm$ 0.58*	51.0
<i>Amaranthus tricolour</i>	50 mg	5.50 $\pm$ 1.09*	32.7
<i>Amaranthus tricolour</i>	100 mg	5.17 $\pm$ 1.01*	36.7
<i>Amaranthus tricolour</i>	200 mg	4.83 $\pm$ 1.01*	40.9
<i>Amaranthus tricolour</i>	400 mg	4.33 $\pm$ 0.56*	47.0

All administrations (aspirin and extract) were done orally. Values are represented as mean $\pm$ SEM, (n=6); **P* < 0.05; significant compared to control.

Diabetes mellitus and pain are two afflictions which affect millions of people in the world on a daily basis. Allopathic medicine has no total cure for diabetes mellitus. Although pain can be treated by even 'over the counter' drugs like aspirin or paracetamol, continuous use of these drugs for chronic pain (as in rheumatoid arthritis) can lead to gastric ulcerations or hepatotoxicity. As such, *A. tricolour* is a promising plant for further scientific studies towards isolation of possible new compounds, which can prove their effectiveness against diabetes and pain without adverse side-effects. In our laboratory, work has begun on bioactivity guided fractionation for identification of possible antidiabetic and antinociceptive components present within this plant.

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