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Antidiabetic, antihyperlipidemic and antioxidant activities of methanolic extract of *Amaranthus viridis* Linn in alloxan induced diabetic rats

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ABSTRACT

The aim of this study was to investigate the antidiabetic, antihyperlipidemic and antioxidant activities of methanolic extract of whole plant of *Amaranthus viridis* (MEAV) in alloxan (ALX) induced diabetic rats. Diabetes was confirmed after 5 days of single intraperitoneal injection of ALX (140 mg/kg) in albino Wister rats. MEAV (200 and 400 mg/kg) and glibenclamide (10 mg/kg, p.o.) orally administered daily for 15 days, blood was withdrawn for glucose determination on 0, 1, 10 and 15 days respectively. On the 15th day, overnight fasted rats were sacrificed and blood was collected for the determination of high density lipoproteins cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), total cholesterol (TC), total glycerides (TG) and total proteins (TP). For *in vivo* antioxidant activity of MEAV, liver tissues were homogenized and the assay of lipid peroxidation and was measured as Malondialdehyde (MDA), glutathione (GSH), catalase (CAT) and total thiols (TT) were performed in control, ALX and MEAV treated rats. MEAV at doses of 200 and 400 mg/kg showed significant reduction is blood glucose, lipid profiles and significant improvement in MDA, GSH, CAT and TT when compared to diabetic control group. *In vitro* α-amylase inhibition activity of MEAV was also studied. We concluded that MEAV possess antidiabetic, antihyperlipidemic and antioxidant activities.

1. Introduction

The worldwide epidemic of type 2 diabetes (NIDDM) has been stimulating the search for new concepts and targets for the treatment of this incurable disease. Globally diabetes has shadowed the spread of modern lifestyle and it can be linked to an increase overweight and sedentary population (Vats et al., 2003). Hyperglycemia and hyperlipidemia are two important characters of diabetes mellitus, an endocrine based disease. Diabetic patients experience various vascular complications, such as atherosclerosis, diabetic nephropathy and neuropathy (Sheetz, 2002). It is now well established that the hyperlipidemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications (Goldstein et al., 1973; Kaur et al., 2002).

Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyruacil) has been commonly utilized as an animal model of diabetes. Alloxan exerts its diabetogenic actions when administered intravenously, intraperitoneal or subcutaneously. The action of alloxan in the pancreas is preceded by its rapid uptake by the insulin-secreting cells (β-cells) (Heikkila et al., 1976), and also due to autoimmune destruction of the β-cells of the pancreas (Atkinson and Maclaren, 1994).

Over the years, various medicinal plants and their extracts have been reported to be effective in the treatment of diabetes (Marles and Fransworth, 1995). Plants are rich sources of antidiabetic, antihyperlipedemic and antioxidant agents such as flavonoids, gallotannins, amino acids and other related polyphenols (Muruganandan et al., 2005; Miyake et al., 2006).

Amaranthus viridis L. (Amaranthaceae) commonly called as 'Chilaka Thota-Kura' in Telugu. *A. viridis* has been used in Indian traditional system and in Nepal to lesson labour pain and as antipyretic (Kirtikar and Basu, 1987; Mark and Turin, 2003). The Negritos of the Philippines apply the bruised leaves directly to eczema, psoriasis and rashes (Quisumbing, 1951). Other traditional uses are anti-inflammatory of the urinary tract, in venereal diseases, vermifuge, diuretic, antirheumatic, antiulcer, analgesic, antiemetic,

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laxative, improves appetite, antileprotic, respiratory problems, eye treatment and for asthma (Anonymous, 1988; Agra et al., 2007; De Fatima Agra et al., 2008; Kirtikar and Basu, 1987; Sher and Khan, 2006; Quershi et al., 2008; Dar, 2003; Arshad and Khan, 2000; Muhammad and Amusa, 2005). A novel antiproliferative, antifungal lectin, ribosome inactivating protein, β-carotene were isolated from *A. viridis* (Kaur et al., 2006; Kwon et al., 1997; Sena et al., 1998) and it possess antiviral activity (Obi et al., 2006). In the present study, we have evaluated the antidiabetic, antihyperlipedemic and antioxidant activities of methanol extract of whole plant of *A. viridis* linn.

2. Materials and methods

2.1. Collection of plant material and extraction

The fresh plant of *A. viridis* was collected from Chickballapur and was authenticated by Prof. B.K. Venkatesh, Department of Botany, Government First grade College, Chickballapur (Karnataka). A voucher specimen (SKVCP 11) was deposited in college herbarium. The whole plant was shade dried and coarsely powdered. The coarse powder was subjected to extraction with methanol by soxhlet apparatus and extract was concentrated to dryness in vacuum. The greenish brown extract was obtained and is dissolved in Tween 80 of pharmacological studies.

2.2. Preliminary phytochemical screening

The methanol extract of *A. viridis* was screened for the presence of various phytoconstituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, amino acids, proteins and phenolic compounds (Kokate, 1986).

2.3. Animals

Male Swiss Albino Wistar rats weighing 150–250 g were acclimatized to the experimental room at temperature 23 ± 2 °C, controlled humidity conditions (50–55%) and 12 h light and dark cycle. They were caged with a maximum of two animals in polypropylene cage and were fed with standard food pellets (Kamadenu Enterprises, Bangalore) and water *ad libitum*. All the studies conducted were approved by the institutional animal ethical committee of Sri K.V. College of Pharmacy (Reg. No. 117/2000), Chickballapur, Karnataka, according to prescribed guidelines of CPCSEA, Government of India.

2.4. Induction of diabetes

The animals were fasted for 12 h prior to the induction of diabetes as described by Joy and Kuttan (1999) with slight modification. ALX freshly prepared in 0.5% Tween 80 was administered intraperitoneally (i.p.) at single dose of 140 mg/kg. Development of diabetes was confirmed by measuring blood glucose concentration 5 days after the administration of ALX. Rats with blood glucose level of above 200 mg/dl were considered to be diabetic and used for the studies.

2.5. Experimental design

The rats were randomized into five groups comprising of six animals in each groups as given below. Solvent/MEAV (200 and 400 mg/kg)/glibenclamide (GLB) was administered orally using an intra-gastric tube once daily for 15 days.

- Group I: normal control rats, received 0.5% Tween 80.
- Group II: diabetic control received ALX in single dose (140 mg/kg. i.p.).
- Group III: diabetic rats received MEAV (200 mg/kg/day. p.o.), 5 days after ALX treatment.
- Group IV: diabetic rats received MEAV (400 mg/kg/day. p.o.), 5 days after ALX treatment.
- Group V: diabetic rats received with GLB (10 mg/kg/day, p.o.), 5 days after ALX treatment.

Blood samples were collected from retro-orbital plexus of each rat under mild anesthesia at 0, 1, 2 and 3 h after solvent/MEAV (200 and 400 mg/kg)/GLB administration and serum glucose was estimated by enzymatic glucose oxidase method. Percent reduction in serum glucose was calculated with respect to the initial level.

Five days before the termination of the experiment, the oral glucose tolerance test (OGTT) was performed to assess the glucose tolerance. For this purpose, overnight fasted rats were fed glucose (2 g/kg) orally and blood was collected at 0, 30, 60 and 120 min interval from orbital sinus for glucose estimation. On 15th day of the study, blood samples were collected for biochemical estimations. Later animals were sacrificed and liver was removed, cleaned and washed in ice-cold normal saline for biochemical study.

2.6. Biochemical analysis

Serum total cholesterol (Demacker et al., 1980), total glycerides (Foster and Dunn, 1973), LDL-c, VLDL-c (Friedwald et al., 1972) and HDL-c (Assmann et al., 1983) were estimated using standard enzymatic kits (ERBA diagnostic Mannheim GMBH, Germany) spectrometrically. Total protein was estimated by the method of Lowery et al. (1951) using bovine serum albumin as a standard. Lipid peroxidation was measured as malondialdehyde (MDA) (Gelvan and Saltman, 1990). GSH was determined according to the method of Moran et al. (1979), CAT activity was determined according the method of Claiborne (1985) and TT was determined according to the method of Moran et al. (1979).

2.7. In vitro α-amylase inhibition assay of MEAV

The α-amylase inhibitory activity for MEAV was determined based on the spectrophotometric assay using acarbose as the reference compound (Gella et al., 1997). The MEAV was dissolved in DMSO to give concentrations from 10, 50 and 100 μg/ml. The enzyme α-amylase solution (0.5 unit/ml) was prepared by mixing 3.246 mg of α-amylase (EC 3.2.1.1) in 100 ml of 40 mM phosphate buffer pH 6.9. Add 60 μl of 40 mM phosphate buffer (pH 6.9)/acarbose/MEAV and 30 μl of α-amylase enzyme are preincubated at 37 °C for 10 min and then 120 μl of 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNPG3) was added, mixed and incubated at 37 °C for 8 min. The absorbance was measured at 405 nm and control reaction was carried out without the extract. Percentage inhibition was calculated by expression:

$$\%\,\text{Inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

2.8. Statistical analysis

Results were expressed as the mean ± S.E.M. for statistical analysis of the data group means, were compared by one-way analysis of variance (ANOVA) followed by Tukey's post-test for multiple comparisons. $p < 0.001$ was considered to be statistically significant.