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Original Research Article

Antidiabetic activity of *Amaranthus dubious*ethanolic leaf extract on alloxan induced diabetic mice

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A B S T R A C T

Diabetes mellitus is a metabolic disorder in which the body does not produce or properly use insulin. *Amaranthus dubious* is an indigenous plant, has a folk reputation in Asia, Europe and Africa. It is used for the conventional therapy of several diseases such as hypertension and cardiovascular disease; regular consumption reduces blood pressure and cholesterol levels. The present study was carried out to evaluate the antidiabetic effect *Amaranthus dubious* and to study the activities of liver glycolytic and gluconeogenic enzymes such as glucose 6-phosphatase and fructose1,6-disphosphatase in the liver of control and alloxan diabetic mice. Oral administration of ethanolic leaf extract of *Amaranthus dubious* (400 mg/kg body weight) to diabetic mice for 45 days resulted in reduction of blood glucose and applied doses did not cause any acute toxicity changes. The glucolytic enzyme activity of glucokinase in liver showed minimum effect in diabetic mice but diabetic mice treated with plant extract showed elevated effect and their values were near normal. The gluconeogenic enzyme activities of glucose-6-phosphatase and fructose 1,6-diphosphatase in liver showed higher level in diabetic mice but diabetic mice treated with the *Amaranthus dubious*ethanolic extract showed lower level and there value were near normal value. Glibenclamide was used as reference drug in this investigation.

Keywords

Diabetes; alloxan; antidabetic; glibenclamide; *Amaranthus dubious*

Introduction

Type-2 diabetes is a chronic disease caused by inherited and/ or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin production. Such a deficiency results in increased concentration of glucose in the blood, which in turn damage many of the body's systems, in particular, the blood vessels and nerves. The hyperglycemia due to decreased insulin production (Type 1) or insufficient insulin utilization (Type 2)(Marshal and Bangret, 2004)of this type 2 diabetes is the major problem of today and it account for nearly 95% of total diabetic population of about 246 million and is projected to

become one of world's disablers and killer within the next 25 years(Mycek and Harvey et al., 2000; Jayakumar, 2010). The commonly practiced treatment of diabetes includes oral anti-diabetic drugs, insulin injection and management through diet and physical exercise. Apart from currently available therapeutic for the treatment of diabetes, traditional plant medicines are used throughout the world for a range of diabetic presentations.

Amaranthus dubious is an herb is native to Asia, Europe and Africa. It can grow All parts of the plant are edible and employed for therapeutic Several studies have shown that like oats, amaranth seed or oil may be of benefit for those with hypertension and cardiovascular disease; regular consumption reduces blood pressure and cholesterol levels, while improving antioxidant status and some immune parameters(Czerwiński and Bartnikowska et al. 2004; Gonor KV, Pogozheva et al. 2006; MartirosyanandMiroshnichenko et al. 2006). This plant is used in various herbal preparations for different diseases. Therefore, the present investigation was undertaken to study the effect of *Amaranthus dubious* ethanolic leaf extract on alloxan induced diabetic mice.

Materials and Methods

Plant material and preparation of extract

The leaves of *Amaranthus dubious* were collected in July 2012 from Area around Gondar College of Medicine Health Sciences, University of Gondar, Ethiopia. The plant was identified and authenticated in the Department of studies in Botany. The fresh leaves of the plant were shade dried on a laboratory table for 6 days and reduced to powder by using dry grinder. This powder was packed into soxhlet apparatus and extracted using absolute ethanol (40-50 °C). The extraction was carried out for 38h till the total extraction was achieved. The extract obtained was dried at 45 °C in hot air oven till semisolid mass was obtained(Nagappa A.N. et al., 2003). The yield obtained was 5.5% and the extract was stored in a refrigerator at 4 °C until used.

Animals

Laboratory bred normal 3-4 months old adult virgin male Swiss albino mice weighing 25-30 g were used under standardized animal housing conditions (temperature 25 ± 2 °C facility with 12 h light/ dark cycle) with unlimited access to pellet diet and water *ad libitum* throughout study. Animals descried as fasted were deprived of food for 16 h, but had free access to water.

Induction of experimental diabetes

Diabetes was induced in male Swiss albino mice by intraperitoneal administration of alloxan monohydrate (200 mg/kg body weight) dissolved in normal saline, second intraperitoneal injection was given after 48 h of first injection, since alloxan is capable of producing fetal hypoglycemia as a result of massive pancreatic insulin release, mice were treated with 30 percent glucose solution orally at different time intervals after six hours of alloxan induction, and 5 percent glucose solution was kept in bottles in their cages for next 24 h to prevent hypoglycemial(Okokon J. E. et al., 2006). After 72 h of second injection mice with diabetes mellitus having glycosuria (indicated by Benedict's test) and hyperglycemia with blood glucose range of 200 to 350 mg/dl were used for this

experiment. Initial and final body weight was recorded in the experiment.

Experimental design

Animals were divided into four groups of five mice each. Standard pellet diet and water was provided *ad libitum*to the animals.

Group I. Normal untreated mice given only vehicle (1% percent gum acacia)

Group II. Diabetic control mice

Group III. Diabetic mice were given single dose of ethanolic extract of *Amaranthus dubious* leaves (400 mg/kg body weight) 1ml with vehicle by oral administration daily, for 45 days.

Group IV. Diabetic mice were given a single dose of glibenclamide (600 μg/kg body weight) 1 ml with vehicle by oral administration daily for 45 days.

Evaluation of anti-diabetic activity

The blood glucose level (BGL) was monitored after alloxanisation in blood samples collected by amputation of the tail tip under mild anesthesia. A drop of blood was placed on a blood glucose test strip (Glucocard™ 01 sensor) and was inserted into a glucometer Glucocard 01-mini Blood glucose testing system. After 72 h. Swiss albino mice having BGL beyond 200 mg/dl of blood were selected for the study. The 400 mg/kg body weight dosage of *Amaranthus dubious* ethanolic extract or 600 μg/kg body weight of glibenclamide was given to mice of respective groups. Blood glucose levels were tested before the treatments on fasting mice and then blood glucose levels were tested after 45 days of treatment. The mice of all four groups were fasted and sacrificed by cervical decapitation. The liver was dissected out and washed with ice-cold saline immediately. A portion of the tissue was homogenized using a homogenizer, and the extract was used for estimation of glucokinase(Ananthi J. et al., 2003), glucose 6-phosphatase(Sowmia C. et al., 2009), and fructose 1, 6-diphosphatase(King J., 1965).

Ethical consideration

Ethical clearance was obtained prior to start this study for Institutional Ethical Committee.

Results and Discussion

Changes in blood glucose and body weight in normal, diabetic and on treatment of diabetic mice with *Amaranthus dubious*extract, glibenclamide are presented in table1. An increase in blood glucose and reduction in body weight were observed in diabetic mice when compared with control mice. O ral administration of *Amaranthus dubious*extract (400 mg/kg body weight) for 45 days showed reduction in blood glucose and an improvement in body weight in diabetic mice compared with untreated diabetic mice.

This was almost similar to changes accrued in diabetic mice after treatment with reference drug glibenclamide. Effect on the hepatic hexokinase and glucose 6-phosphatase and Fructose 1-6, diphosphatase, due to administration of *Amaranthus dubious* extract and glibenclamide diabetic mice is given in table 2. The activity of hepatic hexokinase wasdecreased while glucose-6-phosphatase and Fructose 1- 6-

Table.1 Blood glucose and body weight changesin control and diabetic mice treated with *Amaranthus dubious*leaf extract and glibenclamide

Groups	Blood glucose Level (mg/dl)		Body weight (g)	
	Initial	Final	Initial	Final
I	102.20 ± 3.6	111± 3.4	30.20±.10	31.60 ± 0.18
II	309.22± 8.22	348± 5.16	30.29±.22	27.24± 0.14
III	311.22± 7.82	138± 2.4	30.30±.18	27.24± 0.14
IV	312.40 ± 7.62	130± 6.67	30.36±.20	26.28 ± 0.28

Values are mean ± S.E.M (n=5)

Table.2 Effect of *Amaranthus dubious* leaf extract on the activities of hepatic enzymes in control and experimental animal

Groups	**Glucokinase[a]**	**Glucose-6-phosphatase[b]**	**Fructose 1-6-diphosphatase[c]**
I	135.67±4.27	0.171±0.06	0.249±0.023
II	97.94±1.89	0.238±0.038	0.417±0.09
III	122.46±2.78	0.192±0.028	0.280±0.026
IV	140.76±3.21	0.168±0.02	0.274±0.027

a, moles of glucose phosphorylated/g/h.
b, moles of pi liberated/min/mg.
c, moles of pi liberated/min/mg.
Values are mean S.E.M (n=5)

diphosphatase were elevated in alloxan diabetic control mice. The administration of *Amaranthus dubious* for 45 days showed increased activity of hexokinase and decreased the activity of glucose 6-phosphatase and fructose 1, 6 diphosphatase in diabetic mice. The administration of glibenclamide to diabetic mice also showed similar results. The results showed that *Amaranthus dubious* extract lowered the blood glucose level in (Tomada. M., et al., 1985), terpenoids and tannins(Schimizu M., et al., 1984), steroid(Recher G., et al., 1991), diabetic mice by stimulating the activity of hepatic enzymes involved in glucose metabolism.

A number of plants have been used traditionally in treatment of diabetes and some have been proven scientifically and reported to have hypoglycemic activity. These plant extract contain compound like polysaccharides (Bhavapriya and Govindrajsamy, 2000), flavonoids polypeptides (Ivorra M. D., et al., 1989) and alkaloids(Karawya, M.S. and Wahab S.A., 1984), to be responsible for the

action. *Amaranthus dubious* extract is reported to contain compounds like lighans, alkaloids, flavonoids, galloatnoids and glycosides (Czerwiński and Bartnikowska et al. 2004)**.** The blood glucose level increased as expected in alloxan- injected mice, since alloxan causes a massive reduction in insulin release, by the destruction of the β cells of the islets of Langerhans and inducing hyperglycemia (Khanna P. and Jain S.C., 1981). Oral administration of *Amaranthus dubious* extract (400 mg/kg body weight) resulted in a reduction of blood glucose and improvement in body weight compared to untreated diabetic mice. The loss or degradation of structural proteins in diabetic mice resulted in reduction of body weight. The structural proteins are known to contribute the body weight (Recher G., et al., 1991). Protein synthesis is affected by insulin deficiency in alloxan – induced diabetic mice.

The ability of the *Amaranthus dubious*extract to protect from maximum body weight loss seems to be due to its ability to reduce hyperglycemia. The plant antihyperglycemia action may be by potentiation of pancreatic secretion of insulin, which was clearly evidenced by the increased level of insulin in diabetic mice treated with *Amaranthus dubious* extract. Other plants have also showed anti hyperglycemic and insulin release stimulatory effect (Khanna P. and Jain S.C., 1981), *P. amarus* extract is also known for its liver protective action (Ivorra M. D., et al., 1989). Liver is an insulin dependent tissue, which plays a vital role in glucose and lipid homeostasis and is severely affected during diabetes. Decreased glycolysis impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver (Ivorra M. D., et al., 1989; Bagur N. Z., 1998).

Insulin influences the intracellular utilization of glucose in a number of ways. Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase, phosphofructokinase, and pyruvate kinase. Hexokinase is universally present in cells of all types. Hepatocytes also contain a form of hexokinase called hexokinase D or glucokinase that is more specific for glucose and differ from other forms of hexokinase in kinetic and regulatory properties(Khanna P. and Jain S.C., 1981). Glucokinase catalyzes the conversion of glucose to glucose -6-phosphatase and play a central role in the maintenance of glucose homeostasis. In the liver enzyme is an important regulator of glucose storage and disposal (Doherty O., et al., 1989). In the present study, the hexokinase activity was decreased in alloxan- diabetic mice which may be due to insulin deficiency insulin stimulated and activates glucokinase in liver. *Amaranthus dubious*extract or glibenclaimide, elevates the activity of glucokinase in liver. *Amaranthus dubious*extract like glibenclamide, may stimulate insulin secretion which may activate glucokinase thereby increasing utilization of glucose and thus increased utilization leads to decreased blood sugar level. Insulin decreased gluconegenesis by decreasing the activity of key enzymes such as glucose -6-phosphatase, fructose 1,6, diphosphatase, phosphoenolpyruvate carboxykinase, and pyruvate carboxylase (Arati G. and Sachdanandam, 2003). In present study, increased activities of glucose-6-phosphatase and fructose-1,6, diphosphatase were observed in the liver of alloxan-diabetic mice. Glucose 6-phosphatase, one of the key enzymes in

the homeostatic regulation of blood glucose levels, catalyzes the terminal step in both gluconeogenesis and glycogenolysis (Arati and Sachdanandam, 2003) and fructose 1,6-diphosphataes, catalyzes one of the irreversible step in gluconeogeneis, and serves as a site for the regulation of the process (Bagur, 1998). Increased activities of these two gluconeogenic enzymes may be due to insulin insufficiency. In *Amaranthus dubious* extract in treated mice, the activities of these two enzymes were reduced in liver. This may be due to increased insulin secretion which is responsible for the suppression of the gluconeogenic key enzymes however, further investigation is essential to understand the content and mechanism of action of *Amaranthus dubious* leaf extract on alloxan induced diabetic mice.

This study was concluded that *Amaranthus dubious* leaf extract exhibited antidiabetic activity by improving the peripheral utilization of glucose by altering the impaired liver glycolysis and by limiting its gluconeogenic formation similar to insulin. This effect may be due to the presence of alkaloids, flavanoids, galloatnoids, and other constituents present in the leaf which could act by synergistically in improving the activity of glycolytic and gluconeogenic enzymes. Further research is needed to comprehend the content and mechanism of action of *Amaranthus dubious* leaf extract on alloxan induced diabetic mice.

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