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ABSTRACT

Herbal extract of *Amaranthus* species had been considered as effective and safe ethnomedicines for various ailments in Indian traditional system of medicine. The present study was aimed to explore scientifically the antidiabetic potential of *Amaranthus paniculatus* aqueous leaf extract as Amaranthus other species are reported to have potential of lowering of blood glucose, cholesterol and related ailments. Effect of variable doses of aqueous extract of *Amaranthus paniculatus* leaves on blood glucose level (BGL) of normal-, sub, mild and severe diabetic models have been studied and the results were compared with the reference drug Glipizide.

The dose of 200 mg kg⁻¹ showed the maximum fall of 24.5% in BGL after 6 h of oral administration in normoglycemic rats. In GTT studies the dose of 200 mg/kg produced a maximum fall of 38.4% in sub and 35.2% in mild diabetic rats in BGL after 2 h of glucose administration while the dose of 2.0 mg/kg of Gilipizide produced maximum fall of 36% and 32.8% in sub and mild diabetic rats respectively, after 2 h of glucose administration. In case of severe diabetic rats the FBG got decreased by 41.74% and 62.61% after one and two weeks treatment respectively. A fall of 24.14% in TC and 49.12% in TG were observed in treated severe diabetic rats after 14 days of treatment. There was also increase of 22.61% HDL cholesterol in the treated diabetic rats. A fall of 75% in urine sugar was observed after 14 days of treatment. These results clearly indicate that aqueous leaf extract of *Amaranthus paniculatus* possess antidiabetic and hypolipidemic effects in diabetic rats.
INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder caused due to altered carbohydrate, lipid and protein metabolism. Type 2 diabetes is the commonest form of diabetes constituting 90% of the diabetic population.\(^1\) The countries with the largest number of diabetic patients in the year 2025 will be India, China and United States.\(^2\) There are different groups of synthetic oral hypoglycemic agents available along with insulin to control diabetes mellitus but they have characteristic profiles of side effects \(^3,4\) and sometimes they are also not even effective in lowering the blood sugar in chronic diabetic patients.\(^5\)

India is a country with a vast reserve of natural resources and a rich history of traditional medicine.\(^6\) Ethnopharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their alleged hypoglycemic activity.\(^7\) The hypoglycemic activity of a large number of these plants/plant products has been evaluated and confirmed in animal models\(^8,9\) as well as in human beings.\(^10,11\)

*Amaranthus paniculatus* Linn. (Amaranthaceae) is commonly called, as ‘Rajgira’ in India. It is a shrub found wild and is also cultivated throughout Indian subcontinent for its leaves and seeds. The plant has widespread uses in folk and traditional medicines for respiratory infections, vision defects, tuberculosis, liver complaints and inflammations. In Ayurveda the decoction of leaves was used for chest afflictions and gastroenteritis. The seeds of Rajgira are nutritious and its leaves are important source of protein and vitamins, especially provitamin A (\(\beta\) carotene) and minerals like calcium and iron.\(^12\) It is also reported that amarnathus leaves are an alternative natural source of squalene.\(^13\) It is good natural sources of carotenoids, Vitamin C, high level of nutritional critical lysine and methionine amino acid and protein. The aqueous extract of *Amaranthus paniculatus* leaves shows good radioprotective efficacy.\(^14\) The leaf extract showed good radiomodulatory influence in swiss albino mice \(^15\) and improves learning after radiation stress.\(^16\) *Amaranthus paniculatus* has also been found to improve lipid profile in STZ induced diabetic rats \(^17\) and has antioxidant properties.\(^18\)

The leaves of other species of *Amaranthus* viz *Amaranthus spinosus* 50% ethanolic extract caused significant reduction in blood glucose in STZ induced diabetes in albino mice.\(^19\) Methanolic extract of *A. caudatus*, *A. spinosus* and *A. viridis* leaves showed significant anti-diabetic and anti-cholesterolemic activity in STZ induced diabetic rats.\(^20\) The mentioned studies present evidence that *Amaranthus* spp, is a potential natural source for management of hyperglycemia and associated lipidemia of diabetic persons. It was considered therefore worthwhile to investigate the aqueous extract of *A. paniculatus* leaves for its glycemic potential by evaluating its effect on blood glucose and lipid profile in normal and streptozotocin (STZ) induced diabetic rats. Phytochemical nature of the leaves extract has also been carried out based on qualitative chemical test.

MATERIAL AND METHODS

Chemicals:

Streptozotocin was purchased from Sigma-Aldrich Co. USA. Glucose, total cholesterol, HDL-cholesterol and triglyceride (TG) were assayed using Kits from Bayer Diagnostics India Limited, New Delhi, India. All other chemicals used were of analytical grade.

Preparation of the water extract

Fresh leaves of *Amaranthus paniculatus* (5 Kg) were collected from Botanical survey of India (Allahabad Branch) and got identified by by Prof. Satya Narayan, taxonomist, Department of Botany, University of Allahabad, Allahabad, India. A voucher specimen has been kept at the herbarium of the University. The leaves were shade dried and
were crushed to moderately coarse powder. The powder was extracted with distilled water using soxhlet at boiling temperature (100°C) up to 10 hours. This water extract was cooled and filtered to remove the residue. The extract was concentrated on rotavapour under reduced pressure and then lyophilized to get a powder (yield 8.8% w/w).

**Animals**

Healthy male Wistar albino rats (weighing 180-230gm) were used for the investigation. The animals were housed in standard conditions of temperature (21±2°C), humidity (55±10%) and a 12 hours light-dark cycle. The rats were fed with commercial diet (Pashu aahar, Varanasi) and water *ad libitum*. For experimental purpose the animals were kept fasting overnight but were allowed free access to water. The study was approved by the animal ethical committee of the institute.

**Induction of diabetes in rats**

A freshly prepared solution of streptozotocin (45 mg/kg bw) in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally to overnight fasted rats. FBG level was estimated at the time of induction of diabetes and PPG was checked regularly up to stable hyperglycemia, usually one week after streptozotocin injection. Depending on their fasting blood glucose level the animals were divided arbitrarily in to three groups:

(i) Sub diabetic animals, with nearly normal FBG of 80-120 mg/dl but showing abnormal glucose tolerance.
(ii) Mild diabetic animals with FBG of 120-250 mg/dl.
(iii) Severely diabetic animals showing FBG above 250 mg/dl.

**Estimations**

Blood glucose was estimated by using one touch glucometer for regular checkup and kit was used for weekly estimations. Blood glucose, total cholesterol (TC), High density lipoprotein cholesterol (HDL) and triglyceride (TG) levels in serum were measured spectrophotometrically by prescribed methods. Urine sugar was detected by reagent-based uristrix from Bayer. Low density lipoprotein cholesterol (LDL) was calculated from the above measurement by using Friedwald formula.

**Experimental Design**

Initial screening of the extract for the hypoglycemic activity was done in normal healthy rats. The antidiabetic effect was studied in diabetic animals by two methods:

(i) By studying the effect of different doses of the aqueous extract on blood glucose levels of sub and mild diabetic rats during glucose tolerance test (GTT).
(ii) By giving the most effective dose of extract (200 mg/kg) daily once for 14 days to STZ induced severely diabetic rats and observing the change in FBG, body weight, urine sugar and lipid profile.

**Assessment of hypoglycemic activity in normal healthy rats**

Initial testing was carried with the different doses of the leaves extract in 24 normal healthy male rats fasted overnight. The animals were divided into four equal groups. Control rats (group I) were given vehicle (distilled water) only while other groups II, III and IV received aqueous leaves extract suspended in distilled water orally at doses 100, 200 and 300 mg/kg, respectively. Blood glucose levels (BGL) were estimated before and after 2, 4, 6 and 8 hours of leaves extract administration.

**Assessment of activity of extract on glucose tolerance in sub and mild diabetic rats**

The hypoglycemic effect of aqueous extract of *A.paniculatus* in sub and mild diabetic rats was assessed by improvement of glucose tolerance. FBG was checked in overnight fasted rats and were divided into five groups of each. Control groups of sub and mild diabetic animals received vehicle (distilled water) only, whereas variable doses of 100, 200 and 300 mg/kg of aqueous leaf extract and a dose of 2.0 mg/kg of reference drug Gilipizide were administered orally to rest of the four
groups of each, sub and mild diabetic animals. The rats of all the groups were given glucose (3 g/kg) after 90 min of the extract and drug administration. Blood samples were collected just prior to glucose administration (0 h) and 1, 2 and 3 h after glucose loading.

Assessment of activity of extract in severely diabetic rats

Study was carried on three groups (V, VI & VII) of six rats each. Group V served as normal healthy control, group VI as diabetic control and group VII was orally treated with a single dose of 200 mg/kg b.w. of leaf extract suspended in distilled water daily for two weeks. Control rats (group V & VI) were given vehicle (distilled water) only. Fasting blood glucose, total cholesterol, HDL cholesterol and triglyceride levels were estimated and LDL cholesterol was calculated at the beginning and after 7 and 14 days of experiment. Changes in body weight and urine sugar were also assessed.

Preliminary phytochemical Investigation

The leaves crude extract was subjected to phytochemical analysis according to Kokate and Harborne to identify the presence of various phytoconstituents present in the extract. The plant extract powder (200 mg) was dissolved in 100 ml ethanol and filtered. 2 ml volume of conc. HCl was added followed by addition of Mg ribbon, tomato red color appeared indicating the presence of flavonoids in the extract. The amount of phenolic compounds in the extract were estimated using Folin-Ciocalteau reagent.

LD₅₀ experiment

Four groups of rats of both sex (6 animals per group, 3 females and 3 males) and weighing about 180-220 gm were administered orally a single dose of either 2.5, 5, 10 or 15 times of effective dose of water extract of leaves of A. paniculatus. Then rats were observed for gross behavioural, neurologic, autonomic and toxic effects at short intervals of time for 24 hours. Food consumption, faeces and urine were also examined at 2 h and then at 6 h intervals for 24 hr.

Statistical Analysis

The results are expressed as mean ± S.D. and all statistical analysis was performed by means of one-way analysis of variance (ANOVA), followed by Newman-Keuls Multiple Comparison Test. The data were analysed with Graph Pad Prism 4.0v for Windows (Graph Pad Software, USA). The significance of difference between and within various groups was determined. Differences were considered to be significant when P < 0.05.

Effect in normoglycemic rats

Results of the effect, of graded doses of aqueous extract of A. paniculatus leaves on blood glucose level of normal healthy rats are presented in Table 1. The leaves extract in all the three doses (100, 200 and 300 mg/kg) produced significant hypoglycemic effect after 6 h of administration. However, it was more marked in animals receiving aqueous leaves extract equivalent to 200 mg/kg body weight. (group III). This dose produces a significant fall of 24.5% in BGL after 6 h of oral administration. A fall of 16.1% and 19.4% was observed in BGL at dose 100 and 300 mg/kg respectively, after 6 h of oral administration. However, slight rise in BGL was observed after 8 h of extract administration.

Effect of A. paniculatus extract on glucose tolerance in sub and mild diabetic rats

In order to validate the most effective dose for the severe diabetic animals, different doses of aqueous extract (100, 200 and 300 mg/kg) were evaluated on glucose tolerance in sub and mild diabetic rats along with the synthetic drug Gilipizide (2.0 mg/kg). The dose of 200 mg/kg produced a maximum fall of 38.4% in sub and 35.2% in mild diabetic rats in BGL after 2 h of glucose administration (Fig. 1 & 2). The dose of 2.0 mg/kg of Gilipizide produced maximum fall of 36% and 32.8% in sub and mild diabetic rats respectively, after 2 h of glucose administration.
administration. The dose of 100 mg/kg produced a fall in BGL of sub diabetic rats by 10.8% in 1 h and 24.8% in 2 h whereas it produces a fall of 15.2% in 1 h and 20.8% in 2 h in BGL of mild diabetic rats. The higher dose of 300 mg/kg had more or less the same effect as that of 200 mg/kg. It, therefore, appears that 200 mg/kg of the aqueous extract of \textit{A.paniculatus} is the most effective dose as it produces significant hypoglycemic effect hence this dose was evaluated in hyperglycemic rats.

**Effect on FBG, lipid profile and urine sugar of severely diabetic rats**

It was intended to assess the effect of long term treatment on BGL, urine sugar and associated abnormal lipid profile in STZ induced severely diabetic rats. The effect of repeated oral administration of \textit{A.paniculatus} leaf extract on FBG and abnormal lipid profile in STZ rats is shown in table 2. The administration of extract produced marked antihyperglycemic effect in treated diabetic rats. The FBG got decreased by 41.74% and 62.61% after one and two weeks treatment respectively. The various parameters of blood lipid profile of severely diabetic rats were estimated before & after 7 and 14 days of treatment. The enhanced levels of TC, LDL cholesterol and TG were brought down significantly (p<0.001) after 14 days treatment period. A fall of 24.14% in TC and 49.12% in TG were observed in treated diabetic rats. There was also increase of 22.61% HDL cholesterol in the treated diabetic rats. A fall of 75 % in urine sugar was observed after 14 days of treatment (Table 3). The body weight in the treated diabetic group increased significantly (P<0.001) after 14 days as compared with vehicle treated (control) group (Table 3).

**Phytochemical Screening**

The extract was positive for alkaloids, phenolics, flavonoids and tannins. In the present study the total phenolic compounds of the extracts were expressed as gallic acid equivalent in mg/g and the flavonoids were expressed as quercetin equivalent in mg/g of plant material. The amount of total phenolic and flavonoid content was found to be 26.2mg/g and 12.3 mg/g respectively.

**LD\textsubscript{50} experiment**

The behaviour of the treated rats appeared normal. No toxic effect was reported up to 10 and 15 times of effective dose of the water extract and there were no death in any of these groups. Only the consumption of food was increased by 20 % in 10 and 15 time doses during 4 h but remaining normal afterwards.
Table 1: Hypoglycemic effect of graded doses of aqueous extract of A. paniculatus leaves in normal rats. (mean ± S.D.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg bw)</th>
<th>Pretreatment hour</th>
<th>Post treatment (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>90.2± 2.4</td>
<td>88.6± 3.8</td>
</tr>
<tr>
<td>Extract</td>
<td>100</td>
<td>85.6 ± 5.2</td>
<td>76.2 ± 4.6*</td>
</tr>
<tr>
<td>Extract</td>
<td>200</td>
<td>88.4 ± 3.6</td>
<td>75.8 ± 4.2*</td>
</tr>
<tr>
<td>Extract</td>
<td>300</td>
<td>86.2 ± 3.8</td>
<td>76.2 ± 3.6*</td>
</tr>
</tbody>
</table>

* P < 0.01 as compared to pretreatment hour.
Table 2: Effect of oral administration of the aqueous extract of A. paniculatus leaves on FBG and serum lipid profile in severe diabetic rats (mean ± S.D.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Pretreatment level</th>
<th>Post-treatment levels (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td><strong>FBG (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Control</td>
<td>85.2 ± 3.8</td>
<td>83.3 ± 3.4*</td>
<td>87.2 ± 4.8*</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>334.3 ± 6.2</td>
<td>339.2 ± 5.6*</td>
<td>331.2 ± 6.8*</td>
</tr>
<tr>
<td>Extract</td>
<td>200</td>
<td>316.2 ± 6.2</td>
<td>184.2 ± 6.8*</td>
</tr>
<tr>
<td><strong>Total cholesterol (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Control</td>
<td>54.3 ± 3.9</td>
<td>56.4 ± 4.2*</td>
<td>60.2 ± 5.2*</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>103.2 ± 4.8</td>
<td>116.2 ± 5.2*</td>
<td>125.2 ± 5.6*</td>
</tr>
<tr>
<td>Extract</td>
<td>200</td>
<td>105.2 ± 3.4</td>
<td>88.1 ± 2.6*</td>
</tr>
<tr>
<td><strong>HDL cholesterol (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Control</td>
<td>22.4 ± 3.6</td>
<td>23.6 ± 3.2**</td>
<td>25.2 ± 2.8**</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>35.4 ± 3.2</td>
<td>28.6 ± 3.8**</td>
<td>24.6 ± 3.6**</td>
</tr>
<tr>
<td>Extract</td>
<td>200</td>
<td>33.6 ± 3.6</td>
<td>37.8 ± 4.4**</td>
</tr>
<tr>
<td><strong>Triglyceride (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Control</td>
<td>84.3 ± 2.6</td>
<td>89.5 ± 3.6*</td>
<td>92.2 ± 3.2*</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>144.5 ± 3.4</td>
<td>149.2 ± 3.6*</td>
<td>157.6 ± 4.4*</td>
</tr>
<tr>
<td>Extract</td>
<td>200</td>
<td>147.8 ± 3.7</td>
<td>106.2 ± 3.6*</td>
</tr>
<tr>
<td><strong>LDL cholesterol (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Control</td>
<td>16.2 ± 4.0</td>
<td>16.6 ± 3.8*</td>
<td>16.3 ± 4.4*</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>45.2 ± 4.8</td>
<td>59.2 ± 4.5*</td>
<td>66.2 ± 5.2*</td>
</tr>
<tr>
<td>Extract</td>
<td>200</td>
<td>46.7 ± 3.2</td>
<td>28.9 ± 4.4*</td>
</tr>
</tbody>
</table>

TC: Total cholesterol; HDL: high density lipoproteins; LDL: low density lipoproteins; TG: triglycerides. N=6 in each group

* P < 0.001 as compared to pretreatment level.

** P < 0.05 as compared to pretreatment level.
Table 3: Effect of oral administration of the aqueous extract of *A. paniculatus* leaves on Body weight and Urine sugar in severe diabetic rats (mean ± S.D).

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Urine sugar (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 day 7 day 14 day</td>
<td>0 day 7 day 14 day</td>
</tr>
<tr>
<td>V</td>
<td>Normal Control</td>
<td>204.8±4.2 210.2±5.8 218.6±6.2</td>
<td>0 0 0</td>
</tr>
<tr>
<td>VI</td>
<td>Diabetic Control</td>
<td>196.6±7.8 184.2±8.2 163.2±7.6</td>
<td>+4 +4 +4</td>
</tr>
<tr>
<td>VII</td>
<td>Extract (200mg/kg)</td>
<td>198.8±6.4 202.4±8.2* 207.8±9.4*</td>
<td>+4 +2 +1</td>
</tr>
</tbody>
</table>

* *P* < 0.001 as compared to Diabetic control.
Fig. 1: Effect of oral administration of the graded dose of the aqueous extract on the glucose tolerance in sub diabetic rats (mean ± S.D.).

* P < 0.01 as compared to Diabetic control.
Fig. 2: Effect of oral administration of the graded dose of the aqueous extract on the glucose tolerance in Mild diabetic rats (mean ± S.D.)

**P < 0.001 as compared to Diabetic control.
DISCUSSION

The results of the present study indicates that *A.paniculatus* aqueous leaves extract reduces the glucose level in normal as well as in severe diabetic rats and improves glucose tolerance in sub and mild diabetic animals. The FBG decreases by 14.2% and 24.5% after 4 and 6 hours respectively in normal rats treated with a single dose of 200 mg/kg of leaves extract whereas the dose of 300 mg/kg produces a fall of 11.6% and 19.4% in FBG of normal rats after 4 and 6 hours of extract administration. Such a phenomenon of less hypoglycemic response at higher dose is not uncommon with indigenous plants and has already been observed in *Aegle marmelos* [28] and *Cinnamomum tamala*. [29] The dose of 200 mg/kg also showed a marked improvement in glucose tolerance of sub and mild diabetic rats in GTT after 2 h. This dose has almost same effect as of synthetic drug Gilipizide (2.0 mg/kg).

The daily treatment with 200 mg/kg of extract for 14 days brought FBG to near normal range in severe diabetic animals and prevented body weight loss. The levels of serum lipids is usually elevated in diabetes mellitus and such an elevation represents the risk factor for coronary heart diseases. [30] The marked hyperlipemia that characterizes the diabetic states may be regarded as consequence of the uninhibited actions of lipolytic hormones on the fat depots. [31] Lowering of serum lipids concentration through dietary or drug therapy seems to be associated with a decrease in the risk of vascular diseases. [32]

The results of this study reveals that a regular administration of *A.paniculatus* aqueous leaves extract for 14 days nearly normalized lipid profile in diabetic animals. The dose of 200 mg/kg not only lowered TC, TG and LDL but also enhanced the cardioprotective lipid HDL. The LD₅₀ of the extract is high (no death even with 15 times of effective dose) indicating high margin of safety. The fall of 50% and 75% in urine sugar of severely diabetic group after 7 and 14 days of treatment of most effective dose further confirms our findings.

CONCLUSION

The phytochemical studies present ingredients for the management of hyperglycemia [33], prevention of diabetic complications and for the overall health of diabetic person. From this study we can conclusively state that *A.paniculatus* aqueous leaves extract has beneficial effects on blood glucose level as well as improving hyperlipidemia due to diabetes. Further pharmacological and biochemical investigations are underway to elucidate the mechanism of the antidiabetic and hypolipidemic effect of *A.paniculatus* leaves.

REFERENCES


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