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Research Article

Evaluation of the Hematological, Hypoglycemic, Hypolipidemic and Antioxidant Properties of *Amaranthus Tricolor* Leaf Extract in Rat

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Abstract

Purpose: *To investigate the effect of Amaranthus tricolor leaf extract on some biochemical parameters in diabetic and normal rats*
Methods: *A. tricolor aqueous extract was assayed for antioxidant properties using ferric reducing ability of plasma (FRAP) assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and phosphomolybdenum assay. The effect of the leaf extract on serum glucose and triglyceride, total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL), elevated high density lipoprotein (HDL), body weight and hematological parameters were assessed in diabetic and normal rats. The extract doses used were 200 and 400 mg/kg body weight. Acute toxicity studies were also carried out*
Results: *In the extract doses, 200 and 400 mg/kg, reduced blood glucose levels in a dose-dependant manner, from 168.0 ± 18.5 mg/dl at 0 h to 43.0 ± 9.3 mg/dl at the 12th hour and from 146.50 ± 22.1 mg/dl at 0 h to 37.250 ± 6.3 mg/dl at the 12th hour, respectively. Oral administration of 400 mg/kg of the extract for 21 days significantly reduced ($p < 0.001$) serum glucose, serum triglyceride, total cholesterol, low density lipoprotein, and very low density lipoprotein, but elevated ($p < 0.05$) high density lipoprotein in diabetic experimental rats, compared to diabetic control. The extract prevented a decrease in body weight in treated diabetic rats and promoted an improvement in haemoglobin levels. Total antioxidant activity assay revealed that 1 g of dry leaf powder was equivalent to 0.035g/ml of ascorbic acid. The extract showed no toxicity up to 2 g/kg body weight.*
Conclusions: *This study shows that the aqueous extract of Amaranthus tricolor possesses some beneficial antidiabetic properties that warrant further research.*

Keywords: *Amaranthus tricolor, Anti-hyperglycemia, Anti-hyperlipidemia; Amaranthus, Antioxidant activity, Phosphomolybdenum*

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INTRODUCTION

Diabetes mellitus is a syndrome initially characterized by loss of glucose homeostasis [1]. One of the main challenges in managing diabetes is maintaining blood glucose at near normal level with almost no episode of major fluctuation.

The status and duration of hyperglycemia decide the degree of damage to vital organs while improvement in diabetic status defers the onset and progression of diabetic insult to vital organs [2]. Anemia is commonly found in hyperglycemic patients and is associated with an increased risk of diabetic complications leading to nephropathy, retinopathy and macro vascular diseases [3].

Treatment of diabetes starts with the introduction of oral hypoglycemic agents, dietary restrictions coupled with exercise regimen and eventually administration of insulin in more severe cases. Most hypoglycemic agents have various serious side effects. Insulin therapy results in sudden bouts of hypoglycemia that needs constant monitoring. This has led to increased demand for antihyperglycemic agents with fewer or no side effects. Recent decades has witnessed a resurgent of interest in traditional plant treatments for diabetes [2]. The World Health Organization, has recommended that indigenous plants be used as alternative medicine in the management of diabetes mellitus, particularly in developing countries where safe modern drugs, health centers and resources are limited or lacking [4].

A. tricolor (Tambdi Bhaji/Lal Saag) is native to a large part of India and forms an integral part of the Goan staple diet. Its mild spinach like flavor, high nutritive value, ability to grow in hot weather and lower cost, have made it a very popular vegetable. The plant is well known for its purple betalain pigments, such as amaranthine and isoamaranthine [5]. An antiviral protein that imparts high resistance to sunnhemp rosette virus has been purified from the dried leaves of *A. tricolor* [6]. Three galactosyl diacylglycerols (1-3) with potent cyclooxygenase and human tumor cell growth inhibitory activities have also been isolated from the leaves and stems of *A. tricolor* [7]. Linolenic, palmitic acid and spinasterol are also reported to be present in the leaves of the plant [8].

Goan/Indian folklore suggests that the plant is a good liver tonic and therefore recommended as a vegetable for diabetic and anemic patients. The present study was conducted to evaluate the antidiabetic, hypolipidemic, hematological and antioxidant effects of *Amaranthus tricolor* on alloxan-mediated diabetes in rats.

EXPERIMENTAL

Plant material

Fresh Amaranthus plants were collected in the month of February 2007 from a local source in Fatorda, Goa, India. The plant was identified by Dr. Janarthanam, Department of Botany, Goa University and a voucher specimen (GUBH-PVAC-0515) was deposited at the Botany Herbarium Unit of Goa University, Goa, India. The leaves were blended with cold distilled water for 3 min with solid: liquid ratios of 1:3 (AE I) and 1:1.5 (AE II). In each case, the slurry was strained through a cheese cloth folded eight times to sieve out all fibers and yield the aqueous extract. The residue was severally milled and the filtrates combined. One milliliter of the extract was dried in an oven at 40 °C to constant weight to determine its yield.

Qualitative and quantitative determination of phytoconstituents

Qualitative determination of phytoconstituents of the extract was carried out using standard analytical procedures. Lipids were estimated using a Soxhlet apparatus with petroleum ether (b.p. 60 – 80 ℃) [9] as solvent. Quantitative determination of phytosterols was by the Libermann-Buchard method [10] using β-sitosterol (1mg/ml) as standard.

Polyphenol assay was carried out by Folin-Denis method [11] using tannic acid (1 mg/ml) as standard. Assay of tannins was carried out by using a standard method [12].

Animals

Male Albino rats (Wt. 110 - 130 g) were housed in standard polypropylene cages, allowed free access to water and standard pellet diet (Hindustan Lever, Bangalore, India), and maintained at room temperature (25 ± 2 ℃) throughout the study. Ethical approval was obtained from the Institutional Animal Ethics Committee (ref no. 206/C - 2007), based on the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines [13], which were followed throughout the study.

Determination of LD_{50} and acute oral toxicity

LD_{50} determination was carried out as per OECD guidelines using AOT425 software [14]. Single oral doses of the extract (0.1 - 2 g/kg BW) were administrated to different groups of rats. The animals were observed continuously for the first 4 h and intermittently for the next 48 and 72 h, following administration of the plant extract. The rats were observed for grooming, hyperactivity, sedation, respiratory rate, convulsion and reflexes.

Experimental design

Alloxan (140mg/kg BW) was administered intraperitoneally to the animals [15]. After 72 hours, rats with fasting blood glucose levels > 110mg/dl were included in the study. The extracts were administered orally by gavage. Control groups were given distilled water equivalent to the volume of the extract administered. Treatments with plant extracts started 72 h after alloxan injection and establishment of hyperglycemia.

Acute effects

The control and alloxan-treated rats were divided into four groups of 6 rats each. Group I – normal, received distilled water; Group II – diabetic, received distilled water; Group III – diabetic, received AE I (200 mg/kg BW) orally; and Group IV – diabetic, given AE II (400 mg/kg BW) orally.

Blood glucose was estimated [15] using a glucometer (One Touch Horizon, Johnson & Johnson) at 0, 3, 6, 9 and 12 h after administration of a single dose of the vehicle/extract.

Chronic effects

Based on the results of the acute tests, the more effective dose (400 mg/kg BW) was chosen for chronic treatment and was administered once a day for 21 days [15]. The rats (N=18) were divided into three groups of 6 rats each. Group I – normal, given distilled water orally; Group II – diabetic rats, given distilled water orally; Group III – diabetic rats, given AE II (400 mg/kg BW). Body weight was taken on days 1, 7, 14, and 21 of the experiment. Blood glucose levels, lipid profile and hematological parameters were estimated on day 21 of the experiment. All determinations were made in the pre-prandial state.

Biochemical determinations

After the last dose, animals were fasted for 12 hours and sacrificed under anesthesia. Blood was collected by cardiac puncture into clean sterile vials containing EDTA, for the study of hematological parameters. The remaining blood was collected in glass centrifuge tubes and allowed to clot in the fridge to facilitate serum separation. The separated serum was aspirated out into sterile centrifuge tubes and centrifuged at 3500 rpm for 10 min. Each serum sample was stored in duplicate in clean sterile micro centrifuge tubes at -4° C until analysis. glucose, total cholesterol, triglycerides, HDL,

LDL, and VLDL were assayed using commercially available kits (Crest Biosystems, Goa).

Antioxidant activity

Antioxidant activity of the water extract was measured by ferric reducing ability of plasma (FRAP) assay [16] and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [17]. Total antioxidant capacity was measured by phosphomolybdenum assay [17]. DPPH radical scavenging activity was calculated as (% inhibition) according to Eq 1.

$$\%Inhibition = \frac{100(C-5)}{C} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where C and S are the absorbance of control and test sample, respectively, at 517 nm.

FRAP activity was calculated according to Eq 2.

$$FRAPvalue = \frac{Sa \times FS}{St} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where Sa and St are changes in the absorbance of the sample and standard, respectively, between 0 and 4th min, and FS is the FRAP value of the standard.

Statistical analysis

The results obtained were expressed as mean ± SD. Significant difference between groups were determined using Student *t*-test (Microsoft Office Excel 2003) and differences were considered significant at $p < 0.05$; $p < 0.001$ was considered highly significant.

RESULTS

The effects of acute and chronic treatments on the diabetic rats are presented in Figs 1 and 2, respectively. Alloxan treatment elevated blood glucose level beyond that found in controls indicating a diabetic state was achieved. The rats that received a single dose of either AE I (200 mg/kg) or AE II (400 mg/kg) exhibited significant reduction in blood glucose level by the 3rd hour. At the lower dose of the extract (200mg/kg), steady reduction in blood glucose level was maintained between the 9th and 12th hour. However, the higher dose of the extract (400mg/kg BW) caused a more sustained reduction of blood glucose levels beyond the 9th hour, further reduced by the 12th hour, a highly significant ($p < 0.001$) decrease in blood glucose level occurred at the end of 21 days. This suggests a prolonged antidiabetic effect by the extract (Fig. 2).

Serum cholesterol, triglyceride and LDL levels were significantly higher ($p < 0.001$) while HDL levels were significantly decreased ($p < 0.05$) in diabetic controls, compared to normal control. Following treatment of diabetic group with AE II, a significant reduction ($p < 0.001$) in serum cholesterol, triglyceride and LDL and a significant increase ($p < 0.05$) in HDL were observed.

As Fig 3 shows, diabetic controls exhibited a significant decrease ($p < 0.001$) in body weight compared with normal controls. The diabetic animals given 400 mg/kg extract showed a lower weight loss, compared with the diabetic controls (Fig. 3).

Toxicity study revealed that none of the extract doses up to 2g/kg caused any visible symptoms of distress or toxicity In the rats, The effect of the 400 mg/kg extract dose on Hb, PCV, and RBC and WBC counts is presented in Table 1. The diabetic controls showed a highly significant ($p < 0.001$) decrease in Hb level, PCV, and RBC and WBC counts, compared with normal control. On the other hand, the extract increased significantly ($p < 0.05$) Hb level in the diabetic group, compared to control. However, rise in WBC and RBC counts was negligible in the extract-treated group, compared to diabetic control, but PCV increased significantly ($p < 0.05$). The normal control group that received the extract showed a decrease ($p < 0.05$) in WBC count, compared to the normal control.

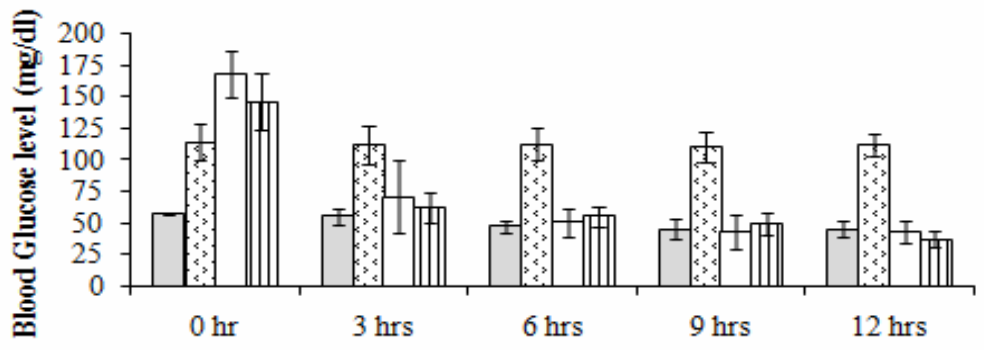

Fig 1: Dose dependant reduction of hyperglycemia by *A. tricolor* extract. ***Note*:** = normal; = diabetic; = diabetic + 200 mg dose extract dose; = diabetic + 400 mg dose extract dose

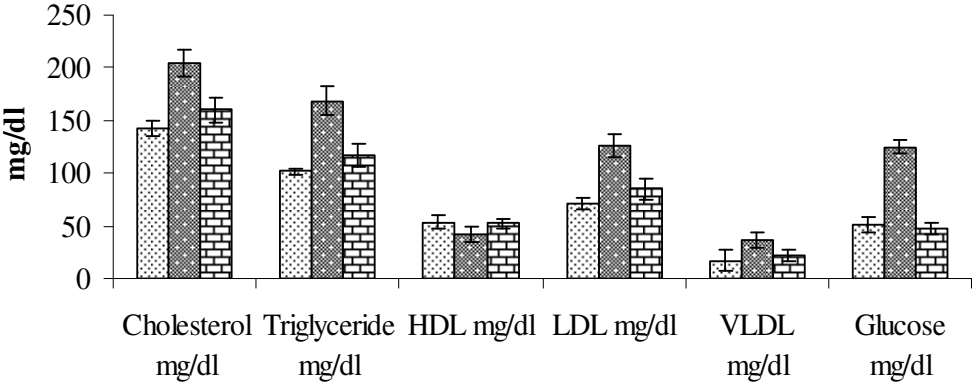

Fig 2: Effect of treatment of *A. tricolor* on glucose and lipid profile. ***Note*:** = normal; = diabetic; = diabetic + 400 mg dose extract dose

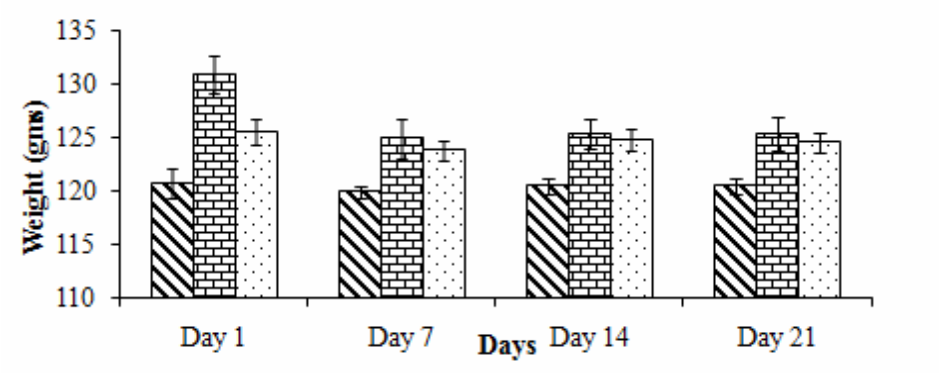

Fig 3: Effect of *A. tricolor* on body weights of normal and treated rats**. *Note*:** = normal; = diabetic; = diabetic + 400 mg dose extract dose

Table 1: Effect of *A. tricolor* on the hematological values in normal and diabetic rats

Parameter	Normal	Diabetic	Diabetic + Extract*
WBC (x $10^3/\mu L$)	5.38 ± 0.47	3.12 ± 0.16 [b]	3.36 ± 0.27
RBC (x $10^6/\mu L$)	5.6 ± 0.34	4.36 ± 0.49 [b]	4.78 ± 0.71
PCV (%)	38.40 ± 2.33	33.85 ± 2.85 [a]	37.58 ± 1.78 [y]
Hb (g/dl)	12 ± 0.71	9.54 ± 0.97 [b]	10.96 ± 0.90 [y]

a = p ≤ 0.05, b = ≤ 0.001 when compared to normal; y = p ≤ 0.05, z = ≤ 0.001 when compared to diabetic

The results of phytochemical analysis (Table 2) indicate the presence of tannins and phenols in the aqueous extract. The major phytoconstituents detected in 1 g of dry sample of *A. tricolor* extract were lipids (2.00 %), polyphenols (1.56 %), tannins (1.25 %) and phytosterols (0.18 %).

Table 2: Some phytoconstituents present in the leaf extract of *A. tricolor*

Phytoconstituent	Content (%w/w)
Lipid	2.0
Phytosterols	0.178
Polyphenols	1.561
Antioxidants	0.035g/ml*
Tannins	1.25

** Equivalent to ascorbic acid*

Data obtained on total antioxidant activity revealed that 1 g of dry leaf powder was equivalent to 0.035g/ml of ascorbic acid, while the free radical scavenging activity of the aqueous extract was almost comparable with standard butylated hydroxy toluene (BHT). FRAP data confirmed that the aqueous extract had very high free radical scavenging activity.

The free radical scavenging activity of the extract of obtained by DPPH assay is comparable to the standard butylated hydroxy toluene (BHT), with the aqueous extract exhibiting an inhibition of 65.0 % at 30 min, compared with the standard BHT inhibition value of 78.1 %. The antioxidant capacity of the aqueous extract, measured by ferric reducing ability (FRAP) assay, was much higher (14.26mM/L), compared with $FeSO_4$ standard (2mM/L).

DISCUSSION

Alloxan induces diabetes mellitus by disrupting the balance between cellular antioxidant defenses and free radical formation as well as by partially destroying β-cells in the pancreas [18]. Diabetes-induced hyperlipidemia is attributable to excess mobilization of fat from adipose tissue due to underutilization of glucose [19].

Amaranthus tricolor is traditionally used in folk medicine as a general tonic, to improve immunity vis a viz overall health improvement. The present study shows that *A. tricolor* is not only a hypoglycemic but also a weight-enhancing agent. Normally, diabetic animals and patients exhibit a decrease in body weight, due to loss of fluid from the body or loss of tissue mass. On treatment with the extract, the weight remained almost constant in the diabetic experimental group.

Administration of the aqueous extract of *A. tricolor*, to diabetic rats, significantly reduced cholesterol, triglyceride & LDL level, and increased HDL level. Tannic acid, a major component of tannins, has the capacity to decrease blood glucose level, by stimulating glucose transport, while inhibiting adipogenesis [20]. Various studies have shown that phytosterols and polyphenols have the potential to reduce hyperlipidemic conditions and blood glucose level [8]. Therefore, reduction in blood glucose and blood lipid levels in the diabetic rats can be attributed to the action of the phytosterols, polyphenols and tannins present in *A. tricolor*. The presence of antioxidants in *A. tricolor* can mitigate damage to the insulin-producing β cells of pancreas. The differential blood count showed decrease in WBC in the extract-fed normal rats. Tannin may be responsible for cell lyses of WBCs as tannin is known to cause cell lysis [21]; however,

there is need to investigate further whether tannin selectively promotes lysis of WBCs.

In diabetes mellitus, additional factors may contribute to anemia, such as increased hemolysis due to advanced glycosylation end-products (AGE) on the RBC membranes or iron and vitamin B12 deficiencies [22]. Recent studies have shown that anemia is a key indicator of early impairment of organ functions in diabetic patients and if left untreated, can cause significant renal and cardiac damage [23] resulting ultimately in death. AE II-treated rats showed significant increase in Hb concentration, which could be partly attributed to its high iron content [24], suggesting *A. tricolor*'s antianaemic properties.

The aqueous extract analysis showed the presence of tannins and phenols, which together constitute the polyphenolic group. Polyphenols are known to have antioxidant, antidiabetic, antihypertensive, hypolipidemic, anticancer and antimicrobial activities. Therefore, the antidiabetic activity may be due to the polyphenol group.

The test plant extract showed high free radical scavenging activity, indicating its potential in arresting cellular damage. In the last decade, interest in the antioxidant activity of plant extracts has grown considerably due to the knowledge that free radicals responsible for onset and aggravation of ailments can be quenched by antioxidants of plant origin. Antioxidants, due to their radical scavenging activity, are useful for the management of those ailments.

The oral safety dosage of the extract using Acute Oral Toxicity Program (AOT 425) guidelines was greater than 2000 mg/kg body weight. Any compound with an oral LD_{50} > 1000 mg/kg can be considered a low toxicity and safe agent [25]. Since the administration of the aqueous extract of *A. tricolor* resulted in a significant reduction in blood glucose level and an increase in haemoglobin, we conclude that this plant is a potential natural source of an agent for antidiabetic, hypolipidemic and blood tonic. Though, the plant has been used traditionally as a general tonic and good natural source of iron, it should be consumed with caution, as this study shows that it has a tendency to induce reduction in WBC count.

CONCLUSION

The current study shows that *A. tricolor* may be useful in the management of hyperglycemia and associated lipidemia. The mechanisms of action of this plant extract on the hyperglycemic state and its related complications need to be studied further. The plant may also find use as a prospective food supplement and for the management of the overall health status of diabetic patients. Furthermore, *A. tricolor* appear to be a potential natural source of ingredients for the management antidiabetic, antihyperlipidemic and antioxidants

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