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HEPATOPROTECTIVE EFFECTS OF *AMARANTHUS TRICOLOR* LINN. EXTRACTS ON THE ALLOXAN DIABETIC RAT (*RATTUS NORWEGICUS*)

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ABSTRACT

The present study investigates the effect of *Amaranthus tricolor* on some hepatic enzyme activities in alloxan-induced diabetic rats. The serum levels of glucose, total protein, and bilirubin were significantly increased in alloxan diabetic rats as compared to control rats. A significant increase ($p \leq 0.05$) in the activities of transaminases, i.e. - aspartate aminotransferase (AST) and alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP), in the serum of alloxan diabetic rats was noted, whereas the activities of the same enzymes decreased in the hepatic tissue as compared to the control rats. Daily oral administration of *A. tricolor* aqueous and methanolic extracts (400 mg/kg body weight) for a seven day period significantly restored the disturbed enzyme activity to their normal levels. The serum total protein was also normalized and an improvement in body weight was seen in the extract fed rats when compared with the diabetic control group. The present study shows that *A. tricolor* besides its potent antioxidant and antidiabetic activities, upon regular consumption, may consequently alleviate or mitigate liver damage caused by diabetes.

Keywords: *Amaranthus tricolor*, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alloxan diabetes

INTRODUCTION

Diabetes mellitus arises from impaired glucose metabolism due to defective insulin secretion or action, and is initially characterized by hyperglycemia, glucosuria and increased thirst. In recent decades diabetes mellitus has assumed epidemic like proportions. Doubts about the efficacy and safety of the oral hypoglycemic agents have

prompted a search for safer and more effective drugs in the treatment of diabetes **[1]**. Even with the discovery of insulin, the search for novel and natural hypoglycemic agents as alternate sources for the treatment of diabetes has not ended. Many plants and natural therapies are still held in high regard as alternatives to conventional therapy, especially in poor areas where insulin and modern medicine is not readily available **[2]**.

Amaranths are well known cereal as well as leafy crops, known for their health benefits and easy availability. *Amaranthus tricolor* (Lal Saag, Tambdi Bhaji, Red Spinach), characterized by its purple red leaves is a highly nutritious source of protein with proven medicinal values. In Goan folk medicine this plant is consumed as a general tonic and is highly recommended for convalescing patients **[3]**. Based on these findings and claims, the present study was undertaken to evaluate the influence of oral administration of *Amaranthus tricolor* on the hepatic function of alloxan-induced diabetic rats.

MATERIALS AND METHODS

Plant Material and Extract Preparation

Fresh *Amaranthus tricolor* plants were collected from Fatorda, Goa, India. After identification of the plant, a sample specimen was deposited with accession number GUBH-PVAC-0515. The leaves were rinsed and blended with cold distilled water for 3 min with solid: liquid ratio of 1:1.5. The slurry thus formed was strained through a cheese cloth folded eight times to sieve out all fibers and yield the aqueous extract, which was immediately frozen until used. The residue was repeatedly milled and all filtered juice samples were combined to yield the aqueous extract. Fresh *A. tricolor* leaves after rinsing with distilled water were dried over night (50°C) in an oven and powdered. Twenty five grams of this dry leaf powder was macerated in 250 ml methanol at room temperature for 24 hours with intermittent shaking. The mixture was filtered and fresh methanol was added and kept for maceration again. This was repeated 3 times. All filtered methanol was combined and the filtrate was concentrated on a rotary evaporator to yield the methanolic extract.

Animals

Twenty four male albino rats (Wt. 110 -130 g) were housed in standard polypropylene cages, with a free access to water and standard pellet diet (Hindustan Lever, Bangalore, India), and maintained at room temperature (25°C) 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 **[4]**, which were followed throughout the study. After acclimatization, animals were divided into two groups, the control group and the experimental group.

Induction of Diabetes

Alloxan hydrate (150mg/kg body weight) was administered intraperitoneally to the animals as a single dose dissolved in chilled saline solution (0.9% NaCl, pH 7) prior to injection **[5]**. After 72 hours, rats with fasting blood glucose levels > 200mg/dl were included in the study. The extracts were administered orally by gavage. Control groups were given distilled water equivalent to volume of extract administered. Treatments with plant extracts started 72 hours after alloxan injection and establishment of hyperglycemia. Prior to administration of alloxan, the animals were fasted for 16h with free access to drinking water.

Experimental Design

The experimental group was used for the induction of diabetes. Rats with confirmed diabetes were divided into three subgroups (6 rats per group). The first subgroup was kept as diabetic control and received distilled water as vehicle along with the control group. The second subgroup received 400 mg/kg BW/day of aqueous extract and the third subgroup received 400 mg/kg BW/day of methanolic extract by gavage daily for duration of seven days.

Enzyme Assays

After the last dose, animals were fasted for 12 hours and sacrificed under anesthesia. Blood was collected in sterile glass centrifuge tubes and allowed to clot in the fridge. 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.

The liver was immediately excised, rinsed free of blood using chilled saline solution. The liver was homogenized (1% w/v) using ice cold saline (pH 7.4) in a pre-chilled mortar and pestle. The homogenate was centrifuged at 10,000g for 20min at 4°C, and the resultant supernatant was used for the enzyme assays.

Glucose, bilirubin, transaminases (AST and ALT), LDH and ALP were assayed from the serum and liver homogenate using commercially available kits (Crest Biosystems, Goa).

Serum and liver total protein concentration was assayed by the method of Lowry *et al*., 1951 **[6]** using bovine serum albumin as standard.

Statistical Analysis

The results obtained are presented as Mean ± SD. Student t-test was used to analyze data. Values were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Amaranthus tricolor is a commonly consumed, abundantly available leafy vegetable, which forms an integral part of the local staple diet. In a preliminary assessment Amaranths were reported to be important contributors to improvement of the nutritional content of rural & urban people, among other known leafy vegetables **[7]**. Normally consumed in the capacity of a traditional hepatoprotective and haematinic agent, the plant and its family are well known for their high antioxidant capacity. Recent studies have shown that *A. tricolor* possesses potent antidiabetic and anti-hyperlipidemic properties **[3]**. Such interesting claims warrant further study of the effect of the extracts on the major organs of the body, to check for any deleterious or beneficial side effects. As the liver plays a major role in the metabolism of drugs it is necessary to ascertain the effects of the aqueous and methanolic extracts on the liver function of the alloxan induced diabetic rats. Enzyme activities in tissues are used as 'markers' of early toxicity mediated by administered foreign compounds in experimental animals **[8]**. ALP is a membrane bound enzyme while ALT and AST are cytosolic and mitochondrial enzymes. These enzymes are highly concentrated in the liver and kidney, and found in minor quantities in other organs. They are only found in the serum in significant quantities when the cell membrane becomes leaky and even completely ruptured **[9]**. A rise in serum level or decrease in tissue level of these intracellular enzymes is an index of tissue damage especially of liver and kidney cells **[10]**. LDH activity in serum increases during tissue necrosis.

Treatment of alloxan-diabetic rats with the aqueous (reported earlier **[3]**) and methanolic extracts reduced the fasting blood glucose level as compared with the diabetic control group **(Figure 1)**.

In alloxan-diabetic rats the activities of serum AST, ALT, LDH and ALP **(Figure 2)** were significantly increased ($p \leq 0.05$) and serum total protein concentration was seen to be decreased **(Figure 5)** as compared to the non-diabetic control group.

In contrast, the activities of AST, ALT, LDH and ALP decreased **(Figure 3)** in the hepatic tissue of alloxan-diabetic rats. The increase in the activities of serum AST, ALT, LDH and ALP is generally seen in diabetes induced hepatic impairment, and may be mainly due

to the leakage of these enzymes from the necrotic liver into the blood stream **[11]**, indicating hepatotoxic effects of alloxan, vis-à-vis diabetes.

However oral treatment of the diabetic rats with the plant extracts over a period of 7 days, showed a significant decrease ($p \leq 0.05$) in the serum transaminases (AST, ALT) and an increase in the liver transaminases (AST, ALT) as compared to the diabetic control group. Treatment of alloxan diabetic groups with *A. tricolor* extracts for 7 days shows restoration of the activities of the enzymes to near normal levels, indicating that these treatments recover the liver damage induced by alloxan.

In diabetic animals, the variations in the levels of AST, ALT, ALP and LDH are directly related to changes in metabolism where in these enzymes are involved. The increased activities of transaminases in the blood of diabetics indicate increased gluconeogenesis and ketogenesis **[12]**. Diabetes and hyperlipidaemia also cause cell damage by altering the cell membrane architecture, which results in enhanced activities of ALP in diabetic rats **[12]**. Therefore rise in ALP activities in the present work suggests that the alloxan induced diabetes caused liver damage. Also the rise in serum ALP activities could be attributed to sloughing off of damaged cells and the release of ALP into the blood.

A decrease in serum AST, ALT, LDH and ALP activities in aqueous and methanolic extracts treated groups indicates the protective effect on liver function, particularly with reference to ketogenesis and gluconeogenesis.

Alloxan induced diabetic rats showed a significant decrease **(Figure 5)** in hepatic total proteins as compared to the control group, which could be attributed to hepatic injury. Besides serum total protein level were also elevated.

After treatment an increase in hepatic total protein concentration was seen **(Figure 5)**. Diabetes is well known to delay wound healing. Proteins are essential to wound healing because they build, maintain and repair body tissues. Increased protein intake speeds healing, where as inadequate protein intake is known to delay wound healing **[13]**.

Amaranthus extracts reduce protein breakdown and provide proteins. Weight loss is a very serious issue in the management of diabetes owing to the degeneration of the adipocytes and muscle tissues to make up for energy loss from the body due to frequent urination and over-conversion of glycogen to

glucose. Diabetic animals treated with *A. tricolor* extracts showed positive weight gain as compared to the diabetic controls **(Table 1)**.

Alloxan diabetes increased the level of serum bilirubin as compared to control rats **(Figure 4)**. This elevation indicates liver malfunction as confirmed by the changes in the activities of serum and liver enzymes. Administration of *A. tricolor* produced significant decrease ($p \leq 0.05$) in serum bilirubin of alloxan-diabetic rats as compared to the diabetic control rats **(Figure 4)**. This could be due to increase liver uptake of bilirubin and decrease production of bilirubin by liver. The increase in plasma bilirubin (hyper-bilirubenimia) may be a result of the decreased liver uptake, conjugation or increased bilirubin production from hemolysis **[14]**. Treatment of the diabetic rats with repeated doses of aqueous extract of *A. tricolor* shows restoration of the normal enzyme activities from the diabetic state. The methanolic extract also exhibits restorative behavior though to a lesser extent.

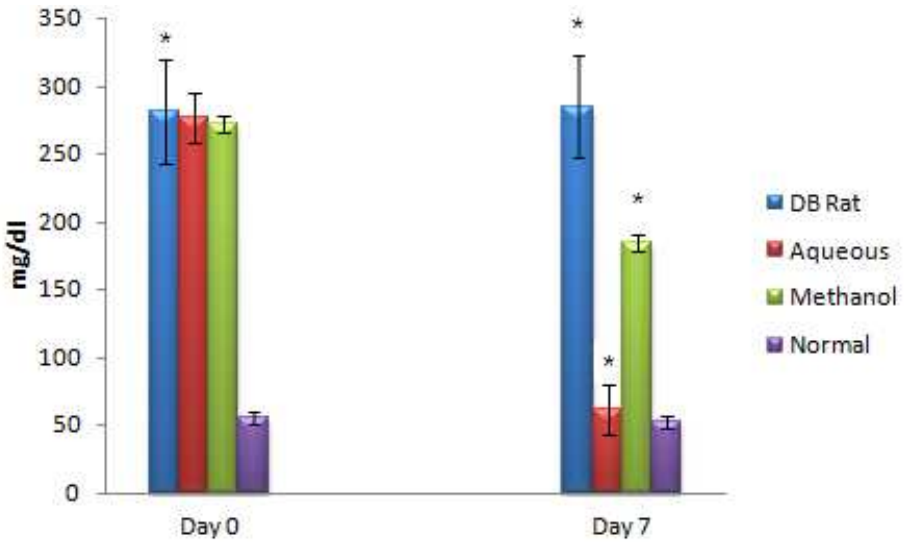

Figure 1: Effect of *A. tricolor* Leaf Extracts on Fasting Blood Glucose Level in Normal, Diabetic and Extract Fed Diabetic Rats [n=6 ± SD; *P < 0.05]

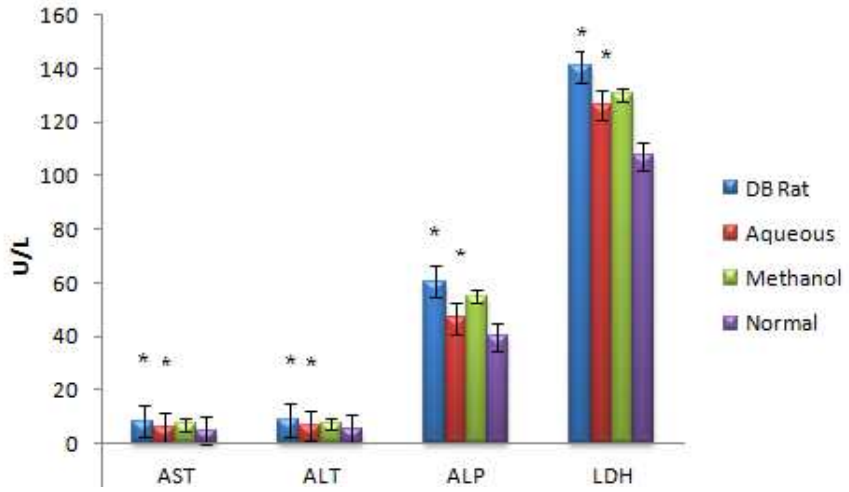

Figure 2: Effect of *A. tricolor* Leaf Extracts on Serum AST, ALT, LDH and ALP in Normal, Diabetic and Extract Fed Diabetic Rats [n=6 ± SD; *P < 0.05]

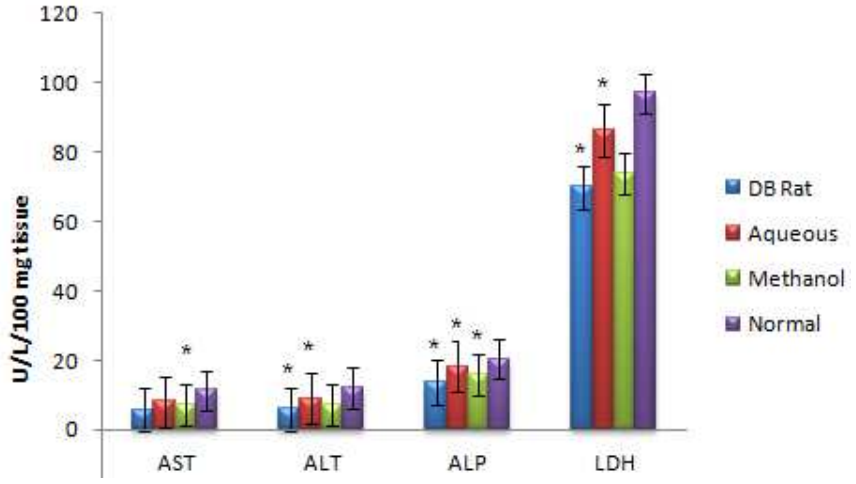

Figure 3: Effect of *A. tricolor* Leaf Extracts on Hepatic AST, ALT, LDH and ALP in Normal, Diabetic and Extract Fed Diabetic Rats [n=6 ± SD; *P < 0.05]

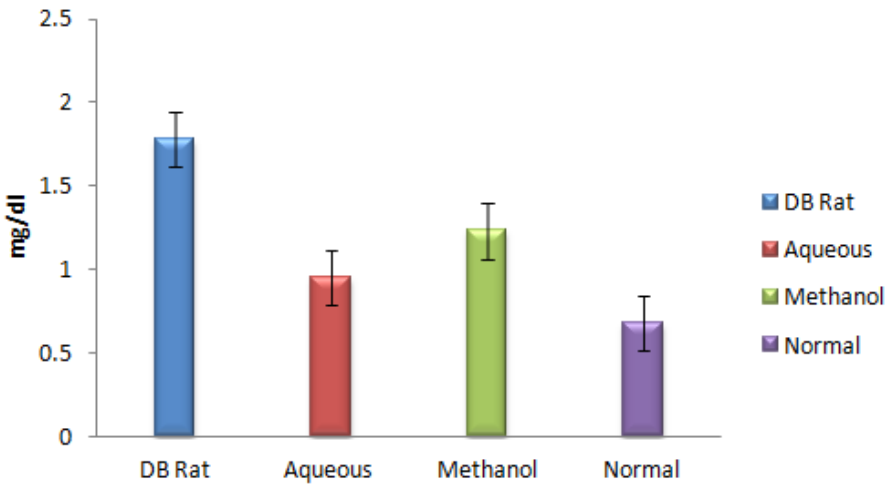

Figure 4: Effect of *A. tricolor* leaf extracts on Serum Bilirubin in Normal, Diabetic and Extract Fed Diabetic Rats [n=6 ± SD; *P < 0.05]

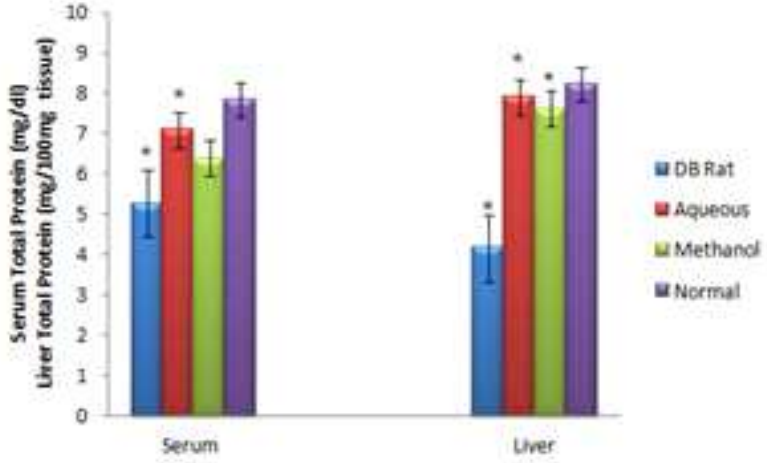

Figure 5: Effect of *A. tricolor* leaf extracts on total protein in serum and liver of normal, diabetic and extract fed diabetic rats [n=6; *P < 0.05]

Table 1: Effect of *A. tricolor* on the Body Weights of Normal, Diabetic and Extract Fed Diabetic Rats [n=6 ± SD; *P < 0.05]

	Normal	Diabetic	Aqueous treated	Methanol treated
Day 0	244.38 ± 4.196	210.11 ± 6.673	224.97 ± 7.257	219.67 ± 20.80
Day 7	240.98 ± 4.935	172.53 ± 8.352	197.36 ± 9.853	188.58 ± 16.817
% Change	1.39	17.886	12.27	14.153

CONCLUSION

The present study suggests that aqueous and methanolic extracts of *A. tricolor* have the potential to arrest cellular damage. These findings substantiate the folk medicinal and nutritional values attributed to *A. tricolor*, a popular leafy vegetable. Therefore increased intake of this vegetable could help diabetic patients in mitigating deleterious effects of diabetes. The present results suggest that *A. tricolor* exerts a protective effect on serum and liver of diabetic rats, and consequently may alleviate or reduce the liver damage caused by diabetes.

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