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The efficacy of aqueous extract of *Talinum triangulare* on carbohydrate metabolic, non enzymatic antioxidants and antioxidantive enzymes in alloxan induced diabetic rats

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ABSTRACT

The selected plant leaves, Talinum triangulare leaf extract (40 and 80 mg/kg bw) seems to be more efficient in the control of type II diabetes. Oral administration of aqueous extract of TT is able to control the diabetes induced alterations in enzymes related to carbohydrate metabolism. Both antioxidants like ascorbate, Vitamin C, Vitamin E and TTH and antioxidant defense enzymes like SOD, CAT and GPx levels also regulated by the supplementation of TT leaf extract to the rats during diabetes.

Keywords: Anti-oxidative enzymes, Antioxidant enzymes, Diabetes mellitus, *Talinum triagulare*

INTRODUCTION

Diabetes is a key health problem, touching approximately 150 million people worldwide and its frequency rate is likely to double during the next 20 years [1]. The global prevalence of DM for all age groups was estimated to be 4.2 % in 2000 and is projected to rise to 5.4% in 2025. The occurrence rates for type II diabetes in India are still increasing piercingly with the number of sufferers predicted to rise from 19.4 million in 1995 to 57.2 million in 2025 [2]. Diabetes is one of the most prevalent metabolic disorders and is characterized increased blood sugar levels and improper primary metabolism. Further- more, in diabetes mellitus, hyperglycemia can lead to the depression of the natural antioxidant system[3] several reports have demonstrated that elevated oxidative stress is common in chronic hyperglycemia as well as in both experimental models and human diabetes mellitus[4]. In diabetes, free radicals are formed harmfully through the following mechanisms: glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins [5,6] Increased oxidative stress resulting from abnormally high levels of free radicals and the depression of the antioxidant defense system may lead to the damage of enzymes within different cellular organelles,[7] increased lipid peroxidation, and the development of insulin resistance, all of which promote the development of complications associated with diabetes mellitus. Current experimental and clinical studies have uncovered new insights into the role of oxidative stress in diabetic complications, and these reports have demonstrated different and innovative approaches to employ natural, antioxidant therapies, e.g., flavonoids and saponin. Flavonoids possess several antioxidant mechanisms including scavenging or quenching free radicals, chelating metal ions, and inhibiting enzymatic systems responsible for free radical generation. The potent antioxidant activity of flavonoids may pro- vide the best protection against elevated oxidative stress [8-10] In the present study, we examined the effect of oral administration of *Talinum triangulare* aqueous extract (TT) on the levels of liver-specific enzymes as well as its effect on the activity levels of non enzymatic antioxidant and

antioxidative enzymes in the Alloxane-induced diabetic melitus (DM) in rats.

MATERIALS AND METHODS

2.1 Animals: Male albino rats (Wistar strain, weighing 150-200g) were purchased from Tamil Nadu Veterinary Animal Science University, Madhavaram, Chennai and housed under standard husbandry conditions (30ºC ± 2ºC, 60-70 % relative humidity and 12hr day night cycle) and allowed standard pelleted rat feed and water. Animal experiments were designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee (Sri Venkateswara University, Tirupati).

2.2 Plant material and extract preparation

The *Talinum triangulare* leaves were harvested and shade dried for 20 days. Then grinded mechanically and 100g of coarse powder was extracted by using water in soxhlet apparatus. Extract was concentrated to semi-solid water free material and final extract yield was 8.5%.

2.3 Induction of diabetes mellitus in rats

Diabetes was induced in male wistar albino rats by intraperitoneal injection with cold aqueous alloxan monohydrate (80 mg/kg body wt). Since alloxan is capable of producing hypoglycemic conditions the rats are fed with 15% glucose solutions. From 2nd day onwards blood sample were collected from the rats by cutting the tail and glucose estimation was made to know the induction of DM. After 12 days the rats were identified having hyperglycemic condition (blood glucose 250 mg/dl) and was selected as experimental material. All the animals were allowed to have free access to the tap water and pellet diet.

2.4 Experimental design

Animals were divided in to six groups of six animals each. Group I served as a control: group II had normal + TT(40 mg/ kg bw) rats; group III had normal + TT (80 mg/kg bw) and Group IV acts as diabetic control, V as diabetic + TT (40 mg/ kg bw) and VI comprised the diabetic + TT (80 mg/kg bw) rats treated with *talinum triangulare* aqueous leaves extract 40 and 80 mg/Kg bw/day respectively for 6 weeks, by oral incubation method. Rats were sacrificed at the end of 6 weeks and the blood samples were collected to analyze the effect of TT on biochemical parameters. Collection and processing of blood for estimation of glucose and other biochemical parameters.

2.5 Carbohydrate metabolic enzymes

Assay of hexokinase D (glucokinase): (ATP: D-hexose 6-13-phosphotransferase: (EC 2.7.1.1). Hexokinase D was assayed by the method of Brandstrup *et al.* (1957) [11].

Assay of glucose 6-phosphatase :(Glucose 6-phosphate phosphohydrolase: (EC 3.1.3.9) Glucose 6-phosphatase was assayed by the method of Koide and Oda, (1959)[12].

Assay of fructose 1, 6-bisphosphatase: (Fructose 1, 6-bisphosphate phosphohydrolase: EC 3.1.3.11). Fructose 1, 6-bisphosphatase was assayed by the method of Gancedo and Gancedo (1971) [13].

Assay of Hepatic Glycogen synthase and Glycogen phosphorylase: Hepatic Glycogen synthase and Glycogen phosphorylase were assayed by the method of Leloir and Goldemberg, (1962)[14].

Non enzymatic antioxidants

Estimation of reduced glutathione (TTH): Reduced glutathione in the plasma, erythrocytes and tissues was estimated by the method of Ellman (1959) [15].

Estimation of Ascorbic acid (vitamin C): Ascorbic acid in the plasma, erythrocytes and tissues was estimated by the method of Roe and Kuether (1943) [16].

Estimation of α--tocopherol (vitamin E): α-Tocopherol in the plasma, erythrocytes and tissues was estimated by the method of Baker *et al.* (1980)[17].

Estimation of plasma ceruloplasmin: Plasma ceruloplasmin was estimated by the method of Ravin (1961)[18].

Enzymatic antioxidants

Assay of superoxide dismutase (SOD, EC 1.15.1.1): Superoxide dismutase in the erythrocytes and tissues was assayed by the method of Kakkar *et al.,* (1984)[19].

Estimation of catalase (CAT, EC 1.11.1.6): The activity of catalase in the erythrocytes and tissues was determined by the method of Sinha (1972) [20].

Estimation of glutathione peroxidase (GPx, EC 1.11.1.19): The activity of GPx in the erythrocytes and tissues was measured by the method of Rotruck *et al.* (1973)[21].

2.6 Toxicity studies

The aqueous extract was administered orally to different groups of rats (n=6) in doses ranging from 100 mg-1g/kg of bw/day to 2-5g/kg of bw/day. The rats were observed for any lethal effects.

2.7 Statistical analysis

Statistical analysis was performed using the SPSS software package, version 9.05. The values were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMART). All the results were expressed as mean ± SD for six rats in each group and $p<0.05$ was considered as significant.

RESULTS AND DISCUSSION

The selected plant leaves, TT leaf extract is showing more antidiabetic properties; we concentrated by taking TT aqueous leaf extract. TT significantly improved the Hb content to 13.5 mg /dl when compare to that of control. Glyoxylated Hb increase is an indicator of diabetes and this increased is proportional to the fasting blood glucose level [22]. Therefore this measurement gives information about the glycemic index of the diabetic animal.

These blood glucose levels are regulated by either catalytic enzyme of anabolic enzymes. Therefore there is a need for the measurement of enzymes related to glycogen metabolism. They are glycogen phosphorylase or glycogen synthase. The measurements of two enzyme activities clearly indicated that there is a reciprocal relationship between the above enzymes (Table 1).

Table 1: Effect of TT - leaf extract on hepatic glycogen metabolizing enzyme levels in control and alloxan induced diabetic rats.

Groups	Glycogen synthase (µmoles of UDP formed/h/mg protein)	Glycogen phosphorylase (µmoles of Pi liberated/h/mg protein)
Normal	871.1±61.2	642.1±52.6
Normal + TT (40 mg/kg bw)	868.2±62.9 [b]	608.4±48.5 [b]
Normal + TT (80 mg/kg bw)	819.5±59.6 [b]	609.3±39.6 [b]
Diabetic control	561.7±42.5 [a]	843.7±68.7 [a]
Diabetic + TT (40 mg/kg bw)	801.2±59.7 [b]	615.1±42.8 [b]
Diabetic + TT (80 mg/kg bw)	799.1±53.6 [b]	660.3±47.3 [b]

Each value is mean ±SD for 6 rats in each group.
a: p<0.05 by comparison with normal rats.
b: p<0.05 by comparison with alloxan diabetic rats.
- : No significance

Glycogen phosphorylase activity increased in diabetic rats where as glycogen synthase activity decreased. The oral administration of TT leaf extract controlled the above two enzymes activity and maintains normal level of blood glucose. From the literature it is clear that the blood glucose levels are controlled by either utilization or generation, this process is mediated by glycolysis or gluconeogenesis are both. Therefore the activities of glycolytic enzymes and gluconeogenetic enzymes are measured (Table 2). The activity of hexokinase showed 60% decrease in its activity under diabetes and this activity brought to the normal levels due to the administration of TT treatment. Similarly the enzymes are related to the glucose synthesis like glucose 6 phosphotase and fructose 1, 6 bis phosphotase showed 2 fold increase in the activity under diabetic condition and activities are regulated to normal by the oral administration of TT leaf extract.

Table 2: Effect of TT - leaf extract on serum carbohydrate metabolizing enzymes levels in control and alloxan induced diabetic rats

Groups	Hexokinase mM of glucose (mg/dl)	Glucose-6-phosphatase (mg/dl)	Fructose-1,6-bis phosphatase (mg/dl)
Normal	126.75±9.10	17.12±1.22	7.54±0.51
Normal + TT (40 mg/kg bw)	118.31±9.21 [b]	14.13±1.11 [b]	7.18±0.48 [b]
Normal + TT (80 mg/kg bw)	120.12±9.31 [b]	13.91±1.01 [b]	7.49±0.56 [b]
Diabetic control	55.31±6.71 [a]	42.16±2.88 [a]	22.16±1.84 [a]
Diabetic + TT (40 mg/kg bw)	121.54±9.12 [b]	16.42±1.14 [b]	7.61±0.68 [b]
Diabetic + TT (80 mg/kg bw)	120.12±8.14 [b]	17.16±1.18 [b]	7.91±0.57 [b]

Each value is mean ±SD for 6 rats in each group.
a: p<0.05 by comparison with normal rats.
b: p<0.05 by comparison with alloxan diabetic rats.
- : No significance.

Table 3: Effect of TT - leaf extract on non-enzymatic antioxidant levels in control and alloxan –diabetic rats.

Groups	TTH (mg/dl)	Ascorbic Acid (mg/dl)	Vitamin E (mg/dl)	Ceruloplasmin (mg/dl)
Normal	25.68±1.71	1.92±0.16	2.45±0.12	21.12±1.61
Normal + TT (40 mg/kg bw)	27.12±1.88 [b]	2.08±0.17 [b]	2.66±0.17 [b]	22.68±1.57 [b]
Normal + TT (80 mg/kg bw)	26.92±1.76 [b]	1.99±0.18 [b]	2.17±0.16 [b]	21.61±1.65 [b]
Diabetic control	12.18±0.86 [a]	0.86±0.08 [a]	3.88±0.29 [a]	36.18±2.61 [a]
Diabetic +TT (40 mg/kg bw)	25.41±1.41 [b]	1.87±0.18 [b]	2.06±0.17 [b]	20.81±1.45 [b]
Diabetic + TT (80 mg/kg bw)	26.12±1.66 [b]	1.85±0.17 [b]	2.11±0.15 [b]	22.17±1.61 [b]

Each value is mean ±SD for 6 rats in each group.
a: p<0.05 by comparison with normal rats.
b: p<0.05 by comparison with alloxan diabetic rats.
- : No significance.

Table 4: Effect of TT - leaf extract on tissue superoxide dismutase (SOD) levels in control and alloxan – diabetic rats.

Groups	SOD (units/min / mg protein)
	Liver
Normal	9.17±0.77
Normal + TT (40 mg/kg bw)	10.46±0.91 [b]
Normal + TT (80 mg/kg bw)	10.68±0.89 [b]
Diabetic control	4.12±0.29 [a]
Diabetic + TT (40 mg/kg bw)	9.51±0.68 [b]
Diabetic + TT (80 mg/kg bw)	9.76±0.71 [b]

Each value is mean ±SD for 6 rats in each group.
a: p<0.05 by comparison with normal rats.
b: p<0.05 by comparison with alloxan diabetic rats.
- : No significance.

Table 5: Effect of TT - leaf extract on tissue catalase (CAT) levels in control and alloxan – diabetic rats.

Groups	CAT (n moles/ 100 g tissue)
	Liver
Normal	83.41±6.72
Normal + TT (40 mg/kg bw)	85.12±6.91 [b]
Normal + TT (80 mg/kg bw)	83.77±5.66 [b]
Diabetic control	42.66±3.91 [a]
Diabetic + TT(40 mg/kg bw)	79.44±5.12 [b]
Diabetic + TT (80 mg/kg bw)	78.58±5.45 [b]

Each value is mean ±SD for 6 rats in each group.
a: p<0.05 by comparison with normal rats.
b: p<0.05 by comparison with alloxan diabetic rats.
- : No significance.

To minimize the oxidative stress certain biomolecules like antioxidants namely glutathione, vitamin C, vitamin E and ceroloplasmin (plasma protein) are reported in literature .Therefore in this investigation an attempt has been made to measure the antioxidant levels in control diabetic and diabetic treated rats (Table 3). The levels of above antioxidants decreased by 50% in diabetic rats and supplementation of TT leaf extract have improved the levels to the normal due to its anti diabetic action. Erythrocytes membrane has a high ratio of poly unsaturated fatty acids to total lipids inductions susceptibility to lipid peroxidation more over erythrocytes are highly vulnerable to lipid

peroxidation due constant exposure of high oxygen tension and the presence of high Fe^{2+} ion concentrations [23] (Table 3). The antioxidant enzymes like SOD and CAT are considered primary enzymes, since they are involved in the direct elimination of ROS [24]. Pancreatic islet posses very low levels of free radical scavenging enzymes inducing SOD, CAT, GPx and are, therefore vulnerable to free radical toxicity [25]. Hence attempt has been to study the activities of above enzymes (Table 4-6). Due to induction of diabetes the enzyme levels have gone down by 50% and the supplementation of TT leaf extract improved the enzyme activities almost to the normal levels in diabetic treated albino rats.

Table 6: Effect of TT - leaf extract on tissue glutathione peroxidase (GPx) levels in control and alloxan – diabetic rats.

Groups	GPx (µg of TTH consumed/ min/mg protein)
	Liver
Normal	9.52±0.69
Normal + TT (40 mg/kg bw)	11.21±0.81[b]
Normal + TT (80 mg/kg bw)	10.27±0.83[b]
Diabetic control	4.12±0.50[a]
Diabetic + TT(40 mg/kg bw)	9.17±0.73[b]
Diabetic + TT (80 mg/kg bw)	9.24±0.76[b]

Each value is mean ±SD for 6 rats in each group.
a: p<0.05 by comparison with normal rats.
b: p<0.05 by comparison with alloxan diabetic rats.
- : No significance.

CONCLUSION

In this present study an attempt has been made to identify the potential plant in the control and alloxan induced type II diabetes. For this study the leaves of *Talinum triangulare* collected from the Tirumala hills. After preparation of the aqueous extracts of above four selected plants they were fed to the alloxan induced diabetic rats and the analysis has been made regarding the control of diabetes induced changes in carbohydrate, lipid metabolism and histopathology of pancreas in albino rats. After conducting the biochemical studies the following conclusions were made.

Alloxan is able to induce type II diabetes in experimental albino rats. Diabetes induction caused alterations in both carbohydrates as well as lipid metabolism. The activities of enzymes related to the catabolism of carbohydrates were gone down and the activities associated with anabolism have gone up. The selected plant leaves, *Talinum triangulare* leaf extract (40 and 80 mg/kg bw) seems to be more efficient in the control of type II diabetes. Oral administration of aqueous extract of TT is able to control the diabetes induced alterations in enzymes related to carbohydrate metabolism. Both antioxidants like ascorbate, Vitamin C, Vitamin E and TTH and antioxidant defense enzymes like SOD, CAT and GPx levels also regulated by the supplementation of TT leaf extract to the rats during diabetes.

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