ANTIDIABETIC ACTIVITY OF LEAVES OF *TALINUM PORTULACIFOLIUM* (FORSSK) IN ALLOXAN – INDUCED DIABETIC RATS

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Summary

The present study was under taken to examine the effect of methanolic extract of *Talinum portulacifolium* (METP) leaves for its anti-hyperglycemic activity against alloxan induced diabetes. The anti-hyperglycemic effect of METP was evaluated in alloxan induced diabetic rats by monitoring its effect on blood glucose, serum lipid profiles (triglycerides and total cholesterol), liver glycogen, malondialdehyde and reduced glutathione (GSH) levels. The rats, pretreated with METP (400 mg/kg p.o) improved oral glucose tolerance compare to glucose fed rats. After oral administration of METP in diabetic rats for 15 days, the blood glucose, lipid profile and malondialdehyde has significantly decreased, while liver glycogen and reduced glutathione were increased significantly. Histopathological studies of the pancreas of these animals showed comparable regeneration by METP, which were earlier necrosed by alloxan. Anti-hyperglycemic activity of METP was compared with oral hypoglycemic agent, glibenclamide.

Key words: *Talinum portulacifolium,* Alloxan, antidiabetic activity, Glucose tolerance, oxidative stress**.**

Introduction

Herbal medicines for the treatment of diabetes mellitus have gained importance through out the world. As the number of people with diabetes multiplies worldwide, the disease takes an ever-increasing proportion of national and international health care budgets. It is projected to become one of the world's main disablers and killers within the next 25 years. Regions with greatest potential are Asia and Africa, where Diabetes Mellitus rate could rise to two to three-folds than the present rate. Diabetes mellitus is a complex disorder that characterized by hyperglycemia resulting from malfunction in insulin secretion and/or insulin action both causing by impaired metabolism of glucose, lipids and protein [1]. Currently available therapeutic options such as dietary modification, oral hypoglycemic and insulin have limitations of their own in treating non insulin dependent diabetes mellitus (NIDDM) [2, 3]. The development of new therapies that are able to improve glycemia management and even to cure diabetes is of great interest. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an area of active research. The World Health Organization has recommended the evaluation of the effectiveness of medicinal plants in condition where the conventional allopathic treatment of diabetes is not adequate [4, 5]. The available literature shows that there are more than 400 plant species showing hypoglycemic activity [6, 7, 8]. Though some of these plants have great reputation in the indigenous system of medicine for their antidiabetic activities, many remain to be scientifically established.

For a long time the tribal people of the Rayalaseema region in Andhra Pradesh, India have used the leaves of the plant *Talinum portulacifolium* (Forssk: Portulacaceae) to keep away from Diabetes [9]. The scientific basis of such a beneficial effect of plant leaves is not clear. It is possible that the compounds present in the extracts might contribute to it's over all protective effect. Therefore, this study was designed to investigate the antidiabetic activity of the methanolic extract of *T. portulacifolium* leaves to establish its potential therapeutic value. The genus Talinum consists of approximately 500 species across the world. The family is cosmopolitan and it has 19 genera and more centered in South Africa and America [10]. It is Perennial, suffrutescent, shrubby plant distributed from Rajasthan, India south wards in to the peninsular region; also found in Nepal. It is cultivated in Africa and, like spinach, is used as a vegetable. It is also said to be used as an aphrodisiac [11]. The leaf powder of this plant mixed with boiled milk is used to treat diabetes [12]. So far no work has been reported on the hypoglycemic and antihyperglycemic effects of this plant. Hence the present study was aimed to determine the antidiabetic property of methanolic extract of *Talinum portulacifolium* in alloxan – induced diabetic rats.

Methods

Plant material

The leaves of *T. portulacifolium* were collected from Tirupati (Andhra Pradesh, India) during October, identified and authenticated in Botanical Survey of India, Kolkata (No:CNH/I-I(72)/2006/Tech.II). The specimen was placed in the herbarium of Pharmacognosy and Phytotherapy Research Laboratory of Jadavpur University, Kolkata, India.

Preparation of extracts

The leaves were shade dried at room temperature. The dried leaves were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. The powder was packed in to soxhlet apparatus and extracted successively with petroleum ether (60 - 80˚C) and methanol. Further methanolic extract was air dried till solid to semi solid mass was obtained and stored in air tight container in refrigerator below 10˚ C. The suspension of methanolic extract was prepared by using 0.5 % Tween – 80.

Preliminary phytochemical analysis

The different extracts obtained were thoroughly analyzed for the presence of different chemical groups using standard methods [13].

Experimental animals

Wistar albino rats (200 – 250g) were obtained from the M/S B.M Gosh enterprises, Kolkata, India. Before and during the experiment, rats were fed with standard diet (Hindustan Pvt Ltd, Bangalore, India). After randomization into various groups and before initiation of the experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h *ad libitum*. The Study was approved by the institutional animal ethical committee and was performed by the guide lines of Care Prevention Control Supervision of Experimental Animals (CPCSEA), New Delhi, India.

Chemicals

Alloxan (S D Fine Chem, India), Glibenclamide (Aventis pharma, India), Thiobarbituric acid (SRL, India), Reduced glutathione (SRL, India) were used in this study. Other chemicals used were of analytical grade, obtained from Qualigens, India.

Acute oral toxicity

Acute oral toxicity study was performed as per OECD–423 guidelines [20], albino rats (n = 6) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level 5 mg/kg body weight by gastric intubation and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg body weight.

Experimental design

In the experiment a total of 30 rats (24 diabetic rats, 6 normal rats) were used. The rats were rendered diabetes by the intra peritoneal (i.p) injection of alloxan (150 mg/kg body weight). After two weeks when the condition of diabetes was stabilized, animals with blood glucose levels above 350 mg/dl were selected for the study. Further the rats were divided in to five groups after the induction of diabetes. In the experiment six rats were used in each group.

Group 1: Normal untreated rats
Group 2: Diabetic control
Group 3: Diabetic rats were given METP 200 mg/kg body weight in aqueous solution daily using an intragastric tube for 15 days
Group 4: Diabetic rats were given METP 400 mg/kg body weight in aqueous solution daily using an intragastric tube for 15 days
Group 5: Diabetic rats were given glibenclamide 600 µg/kg body weight in aqueous solution daily using an intra gastric tube for 15 days [16]

Effect on oral glucose tolerance

After overnight fasting, a 0–min blood sample was taken from the rats in different groups viz., normal, diabetic control, diabetic+METP (200mg/kg) and diabetic +glibenclamide (600µg/kg) by the orbital sinus puncture [14]. A glucose solution (2g/ kg) was administered by oral gavage. Three more samples were taken at 30, 60 and 120 min after glucose administration [15]. Blood glucose was estimated by commercially available glucose strips (Accu-Chek) using One Touch Glucometer (Johnson – Johnson, India).

Determination of blood glucose

Fasting blood glucose was estimated by commercially available glucose strips (Accu-Chek) using One Touch Glucometer (Johnson – Johnson, India).

Bio chemical assays

Blood samples were withdrawn from the retro orbital with a capillary for biochemical parameters determination serum cholesterol, triglycerides, total protein were measured by commercially availalable diagnostic kits (Span Diagnostics, Mumbai, India). Hepatic glycogen level was estimated by the colorimetric anthrone method [17]. On 16th day the liver was excised, washed with isotonic saline buffer, dried and stored at -20˚C. 10 % homogenate was used for the estimation of liver reduced glutathione [18] and lipid peroxidation levels [19].

Histopathology

The small portion of pancreas from each animal was removed after sacrificing the animal and was collected in 10% formalin solution, and immediately processed by the paraffin technique. Sections of 5 µ thickness were cut and stained by haematoxylin and eosin (H&E) for histological examination.

Statistical analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean ± standard error of mean (S.E.M.) and analyzed by one-way ANOVA followed by post hoc Dunnett's t-test. Differences between groups were considered significant at $P < 0.01$ levels.

Results

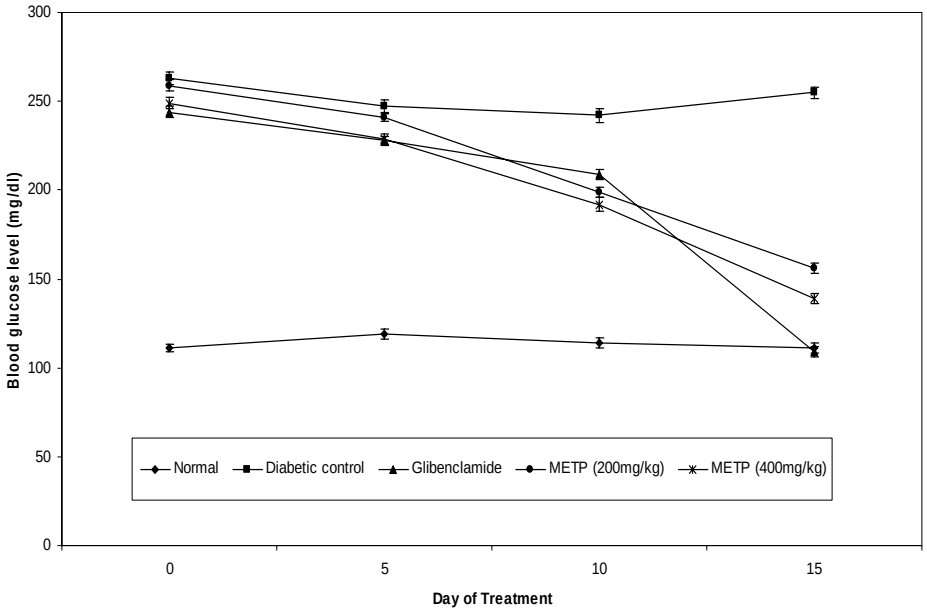
Preliminary phytochemical and toxicity studies

The preliminary phytochemical analysis showed the presence of steroids, triterpenoids and flavonoids in methanolic extract of *T. portulacifolium.* Acute toxicity studies were carried out according to the organization of economic co - operation and development (OECD) by acute toxic class method. The methanolic extract of *T. portulacifolium* did not cause any mortality up to 2000 mg/kg and were considered as safe [20]. No lethality or any toxic reactions were found up to the end of the study period.

Effect on blood glucose and glucose tolerance

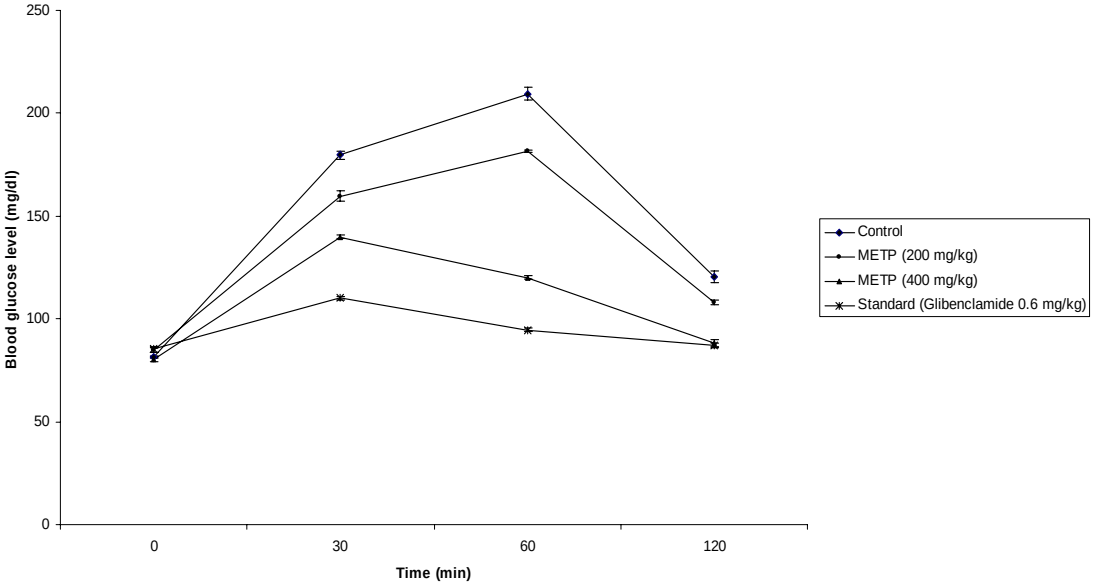
Oral administration of METP (200 and 400 mg/kg p.o.) for 15 days resulted in significant reduction in blood glucose. METP at the dose of 400 mg/kg body weight exhibited better sugar reduction than 200 mg/kg body weight and that produced by the standard drug, glibenclamide at the same period (figure 1). Results of the glucose tolerance test conducted in normal rats are shown in figure 2. METP (400mg/kg, p.o.) produced significant ($P<0.01$) increase in the glucose tolerance, 30 – min post – glucose loading in normal rats, while standard drug produced more glucose tolerance.

Figure 1. Effect of methanolic extract of *T. portulacifolium* leaves on blood glucose level in alloxan (150mg/kg) - induced diabetes in rats



N = 6 in each group, values are mean ± SEM
**P < 0.01 compared to Diabetic group (one-way ANOVA followed by Dunnett's test)

Figure 2. Effect of methanolic extract of *T.portulacifolium* on oral glucose tolerance test in normal rats



N = 6 in each group, values are mean ± SEM
**P < 0.01 compared to Diabetic group (one-way ANOVA followed by Dunnett's test)

Effect on Serum cholesterol, Triglycerides and Total protein

Control animals registered sharp rise in serum cholesterol and triglyceride levels. On the other hand there was a fall in serum total protein in the control diabetic rats. Lipid parameters viz., cholesterol and triglycerides showed improvement after 15 days of METP treatment compared to control values. Total protein levels showed significant improvement in METP treated diabetic rats (Table 1).

Effect on glycogen content

When compared with diabetic Control group, there was a significant elevation in hepatic glycogen content in both diabetic treated groups (METP 200 and METP 400 mg/kg). This effect was more pronounced in the METP 400 mg/kg treated group, and was statistically comparable to that observed in standard treated group (Table 2).

Effect on lipid peroxidation and reduced glutathione levels

Table 2 shows the effects of METP on biochemical variables suggestive of oxidative stress in alloxan treated rats. The liver glutathione levels significantly decreased, while Malondialdehyde level significantly increased in the alloxan treated diabetic rats. The normal control rats maintained optimal values of both parameters. METP treatment significantly decreased the elevated Malondialdehyde level, but also significantly increased the glutathione level towards their normal values in the liver.

Table 1: Effect of methanolic extract of *T. portulacifolium* on serum biochemical parameters of alloxan induced diabetic rats.

Biochemical Parameters	Normal control	Diabetic control	Methanolic extract of METP (200 mg/kg)	Methanolic extract of METP (400 mg/kg)	Standard Glibenclamide (600 µg/kg)
Cholesterol (mg/dl)	59.83±0.703**	105± 1.461	54±1.25**	53±1.065**	38.33±0.992**
Triglycerides (mg/dl)	60.5±0.991**	98±0.966	74.5±1.258**	69.5±0.991**	43.16±1.167**
Total protein (gm/dl)	7.23±0.108**	6.01±0.126	6.73±0.094**	6.81±0.113**	6084±0.095**
Body weight (g)					
Initial	220.83± 2.822	223.58± 3.50	212.5± 4.47	230.16± 5.11	232.33±3.499
Final	225.5± 2.89**	154.20± 1.872	184.83± 3.19**	219.5±2.93**	217.83±2.73**

N = 6 in each group, values are mean ± SEM
**p < 0.01 compared to Diabetic group (ANOVA followed by Dunnett's test)

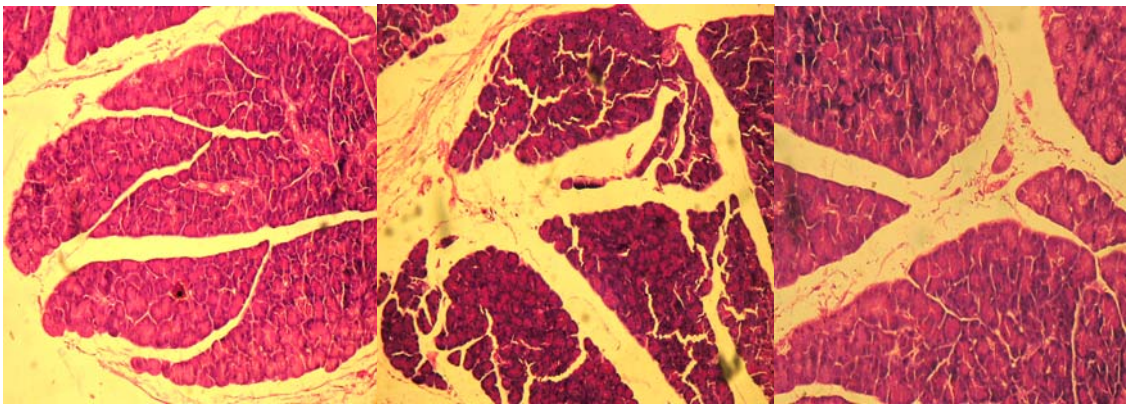
Table 2: Effect of methanolic extract of *T. portulacifolium* on liver lipid peroxidation and reduced glutathione (GSH) levels of alloxan induced diabetic rats

Groups	Lipid peroxidation (mM/100g tissue)	Reduced glutathione (mg/100 mg tissue)	Liver glycogen (mg/g)
Normal control	0.86 ± 0.007**	46.48 ± 0.511**	45.53 ± 0.341**
Diabetic control	2.05 ± 0.014	23.33 ± 0.455	6.91 ± 0.116
METP (200 mg/kg)	1.373 ± 0.008**	27.03 ± 0.306**	31.6 ± 0.453**
METP(400 mg/kg)	1.44 ± 0.006**	36.66 ± 0.392**	32.76 ± 0.364**
Glibenclamide (600 µg/kg)	1.59 ± 0.012**	40.40 ± 0.281**	39.28 ± 0.329**

N = 6 in each group, values are mean ± SEM
**P < 0.01 compared to Diabetic group (ANOVA followed by Dunnett's test)

Fig. 3. Photomicrographs of rat pancreas stained by haematoxylin and eosin of untreated (A) and alloxan – induced diabetic rats (B) and Effects of methanolic extract of *Talinum portulacifolium* (C) Microscope magnification (400x)

(A) **(B)** **(C)**



Discussion

The present study was conducted to study the antidiabetic activity of METP in rats as well as to provide an introductory approach for the evaluation of its traditional preparation in order to scientifically validate the therapeutic preparation of this plant in the control of diabetes. To the best of our knowledge, this is the first report that analyzes antidiabetic potential of *T. portulacifolium* in experimental diabetes.

The results reveal that the extract produced significant decrease in blood glucose level when compared with glucose loaded control rats and in the single dose treatment study at the tested dose levels for 15 days. This might suggest that the said effect may be due to extraintestinal action or probably by insulin releasing mechanism of the tested drug. [21,22]. A large amount of glycogen was also observed after treating diabetic rats with METP, suggesting the possibility of increased glycogen formation through enhanced glycogen synthase enzyme activity by METP as a probable mechanism of its hypoglycemic effect. The blood glucose data obtained clearly indicate that the METP produced significant and consistent anti–hyperglycemic effect in alloxan induced diabetic rats. Continuous treatment with METP for a period of 15 days produced a significant decrease in the blood glucose levels of diabetic rats. These results confirmed the use of *T. portulacifolium* leaves in folklore practice as an antidiabetic drug [9, 12]. Continuous administration of METP had decreased the blood glucose, total cholesterol and triglycerides significantly, while protein levels increased significantly. The results of serum glucose level, total cholesterol, triglycerides and protein are consistent with the finding of [23] and [24] in rats.

GSH is an important factor in detoxification and antioxidant systems [25]. GSH offers protection against oxygen derived free radicals and cellular lethality. A decreased glutathione level in diabetes has been considered to be an indicator of increased oxidative stress [26]. The decrease in GSH level in diabetic rats represents increased utilization due to oxidative stress [27]. An increase in GSH level in extract and glibenclamide treated diabetic rats might be due to less production of free radicals. Lack of rise in Lipid Peroxidation (LPO) level with progression of diabetes is probably associated with increased GSH level. In the literature, the intensification of lipid peroxidation in diabetic liver was stated [28, 29]. The present data may indicate a good correlation between lower LPO and higher GSH level in diabetic liver.

Histopathological section of vehicle treated rats showed normal cellular population of islets of langerhans in pancreas. Histologically diabetic rats with no treatment the most consistent findings in the sections of pancreatic tissues such as decreased cellular density, necrotic changes, shrunken in the islets of langerhans. In diabetic rats treated with METP there was a remarkable improvement in the islets of langerhans with distinct clarity, restoration of normal population of islets (figure 3).

Leaves of *T. portulacifolium* powder is claimed to be useful in diabetes. Results of hypoglycemic activity of *T. portulacifolium* leaf extract presented here may help to establish a scientific basis for the utility of this plant in the treatment of diabetes. Preliminary phytochemical and thin layer chromatographic examination of methanolic extract revealed the presence of flavonoids which may contribute to the hypoglycemic

activity. Flavonoids shown to possess potent antidiabetic activity in various experimental diabetes [30, 31].

The preliminary acute toxicity studies have revealed no signs of toxicity of the METP in normal rats. Therefore the present study has further encouraged the folklore practice of *T. portulacifolium* leaves for routine treatment of diabetes mellitus. Further studies regarding isolation and exact mechanism of action of *T. portulacifolium* are in progress.

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