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Antidiabetic and *in vivo* antioxidant activity of ethanolic extract of G*eniosporum prostratum* aerial parts on STZ induced diabetic rats

***Ashutosh Upadhayay[1], Kundlik Girhepunje[1], Ranju Pal[1] and Thirumoorthy N[2]**

[1]NIMS University, Jaipur, Rajasthan
[2]Cherraan's College of Pharmacy, Coimbatore, (T.N.)

ABSTRACT

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism that affects nearly 10% of the population every year. The treatment of diabetes mellitus has been confined to use of oral hypoglycemic agents and insulin, the former being reported to possess serious side effects. This leads to increasing demand for herbal products with antidiabetic factor with little side effects. Free radicals have been implicated in the pathogenesis of diabetes mellitus leading to various complications including atherosclerosis. The present study was designed to investigate the antidiabetic and antioxidant effects of ethanolic extract of Geniosporum Prostratum (GPEt). Type I diabetes was induced in rats by injection of streptozotocin (STZ) in a dose of 60 mg/kg bwt, i.p. for 3 consecutive days. GPEt was administered orally at a doses of 100, and 200 mg/kg of body weight for 21 day, after which liver tissue was assayed for the degree of lipid peroxidation by means of markers, lipid peroxidation, reduced glutathione content and activities of catalase, and superoxide dismutase. Treatment of diabetic rats with GPEt increased the antioxidant levels with significant decrease in LPO. GPEt at a dose of 200 mg/kg of body weight exhibited a significant effect as compared with 100 mg/kg of body weight. These effects were compared with glibenclamide, a reference drug.

Key words: Blood glucose, *Geniosporum Prostratum*, enzymic antioxidants, lipid peroxidation, streptozotocin induced diabetes.

INTRODUCTION

Diabetes mellitus, characterized by hyperglycemia, is the most common serious metabolic disorder that is considered to be one of the five leading causes of death in the world [1]. Various studies have shown that diabetes mellitus is associated with oxidative stress, leading to an

increased production of reactive oxygen species (ROS), including superoxide radical (O2 •−), hydrogen peroxide (H2O2), and hydroxyl radical (OH•) or reduction of antioxidant defense system [2, 3]. Implication of oxidative stress in the pathogenesis of diabetes mellitus is suggested not only by oxygen free radical generation but also due to non-enzymatic protein glycosylation, auto-oxidation of glucose, impaired antioxidant enzyme, and formation of peroxides[4, 5]. The treatment of diabetes mellitus in clinical practice has been confined to use of oral hypoglycemic agents and insulin, the former being reported to be endowed with characteristic profiles of serious side effects [6, 7]. This leads to increasing demand for herbal products with antidiabetic factor with little side effects. A large number of plants have been recognized to be effective in the treatment of diabetes mellitus [8]. *Geniosporum Prostratum* Linn (Lamiaceae**)** is a creeping, glabrous, succulent herb, rooting at nodes, distributed throughout India in all plain districts, ascending to an altitude of 1,320 m. The plant is reported to show sedative, antiepileptic, vasoconstrictor and anti-inflammatory activity [9,10]. It has been reported that the plant contains triterpenoid saponins, β-sitosterols, glycosides, alkaloids, phenols and flavonoids [11]. The purpose of this investigation was to evaluate the effects of *Geniosporum Prostratum* ethanolic extract (GPE**t**) on streptozotocin (STZ)-induced diabetes by measuring fasting blood glucose, glycosylated haemoglobin, antioxidant activity like SOD, CAT, and GSH.

MATERIALS AND METHODS

Plant material

The plant *Geniosporum Prostratum (L) Benth.* belonging to family “Lamiacea” are widely available in Tamil nadu. For present work the plant *Geniosporum prostratum (L) Benth.* was collected in the month of Jan. 2009, from Orakadam forest near Chennai. The plant was identified by Prof. P. Jayaraman Director, Plant Anatomy Research (PARC). Who authenticated the plant from available literature.

Preperation of plant extract

The shade dried aerial part of plant was broken into small pieces and powdered coarsely. 250 gm of powdered seeds were extracted in soxhlet apparatus with ethanol (99.9% v/v) for 72 hrs, yield of 3.43 gm of extract was collected under vaccum drying and the extract was preserved in vaccume descicator. The freshly prepared extract was phytochemically tested for the presence of various phytocontituents including steroids, flavonoids, tannins, phenols, glycosides, carbohydrate, protein and amino acids [12, 13].

Experimental Models

Adult male albino wistar rats (8 weeks), weighing 180 to 250 gm were used in this study. The animals were housed in clean polypropylene cages and maintained in a well-ventilated temperature controlled animal house with a constant 12 h light/dark schedule. The animals were fed with standard rat pelleted diet (Hindustan Lever Ltd., Mumbai, India) and clean drinking water was made available *ad libitum*. All animal procedures were performed after approval from the ethical committee and in accordance with the recommendations for the proper care and use of laboratory animals (CPCSEA/Reg No. 1283/c/09).

Effect on oral glucose tolerance in rats

After overnight fasting, a 0-min blood sample was taken from the tip of the tail of each rat of different groups under mild ether anesthesia. Without delay a glucose solution (2 g/kg) was administered by a gavage. Four more samples were taken at 30, 60, 90 and 120 min after glucose administration. All blood samples were taken for the estimation of the blood glucose. Estimation of blood glucose was carried out with the haemoglucostrips [14].

Induction of diabetes

Rats were injected intraperitoneally with a freshly prepared solution of STZ in 10 mM citrate buffer, pH 4.0 at a dose of 60 mg/kg of body weight to 12 hr fasted rats. Animals were kept fasted 3 hr after injection of STZ. After 72 hr blood glucose of all the animals were measured. The animals with blood glucose level between 250-350 were included in experiment [15, 16,17].

Experimental design

In the experiment, a total of 30 rats (24 diabetic surviving rats, 6 normal rats) were used. The rats were divided into five groups of six each, after STZ induced diabetes and the experiment was carried out for the period of 21 days

Group 1 : Normal control.
Group 2 : Diabetic control.
Group 3 : Diabetic rats treated with 100 mg/kg of plant extract.
Group 4 : Diabetic rats treated with 200 mg/kg of plant extract.
Group 5 : Diabetic rats treated with Glibenclamide 600 µg/kg.

Diabetic rats were given ethanolic extract of *Geniosporum Prostratum* daily using an intragastric tube for 21 days.

Sample collection

At the end of 21 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in tubes containing EDTA for the estimation of blood glucose and glycosylated heamoglobin. liver and pancrease was immediately dissected out, washed in ice-cold saline to remove the blood.

Estimation of Blood Glucose and Glycosylated Heamoglobin

Blood sample were collected from tip of rat tail vein and glucose levels were estimated using a glucose oxidase-peroxidase reactive strips using glucometer (Accu-chek, Roche Diagnostics, USA). Glycosylated heamoglobin were estimated by the method of Sudhakar Nayak and Pattabiraman (1981) [18].

Estimation of and plasma insulin

Plasma insulin was assayed by enzyme-linked immunosorbent assay by using a Boehringer-Mannheim kit with a Boehringer analyzer ES300. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate. Add 100 µl anti- C-Peptide antibody to each well and incubate for 1.5 hours. Discard the solution and wash wells 5 times with 1x Wash Solution (200 µl each). Add 100 µl of each standard, positive control and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C. Discard the solution and wash 4 times with 1x Wash Solution (200 µl each). Add 100 µl of prepared HRP-Streptavidin solution to each

well. Incubate for 45 minutes at room temperature. Discard the solution and wash 5 times with 1x Wash Solution (200 µl each). Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark. Add 50 µl of Stop Solution to each well. Read at 450 nm immediately [19].

Preparation of Tissue Homogenate

The tissues were weighed and 10% tissue homogenate was prepared with 0.025 M Tris-HCl buffer, pH 7.5. After centrifugation at 10,000 ×g for 10 min, the clear supernatant was used to measure thiobarbituric acid reactive substances (TBARS).

For the estimation of enzymic antioxidants, tissue was minced and homogenized (10% w/v) in 0.1 M phosphate buffer (pH 7.0) and centrifuged for 10 min and the resulting supernatant was used for enzyme assays.

Estimation of lipid peroxidation

Lipid peroxidation in tissues was estimated colorimetrically by thiobarbituric acid reactive substances and hydroperoxides according to the methods of Fraga et al. (1988). In brief, 0.1 mL of tissue homogenate (supernatant; Tris-HCl buffer, pH 7.5) was treated with 2 mL of (1:1:1 ratio) TBA-TCA-HCl reagent (0.37% thiobarbituric acid, 0.25 N HCl, and 15% TCA), placed in water bath for 15 min, cooled, and centrifuged at room temperature for 10 min. The absorbance of clear supernatant was measured against reference blank at 535 nm and expressed as millimoles per 100 g of tissue [20].

Determination of CAT and SOD

CAT was assayed colorimetrically at 620 nm and expressed as moles of H_2O_2 consumed per minute per milligram of protein, as described by Sinha.24 The reaction mixture (1.5 mL, vol) contained 1.0 mL of 0.01 M (pH 7.0) phosphate buffer, 0.1 mL of tissue homogenate (supernatant), and 0.4 mL of 2 M H2O2. The reaction was stopped by the addition of 2.0 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in a 1:3 ratio).

SOD was assayed according to the technique of Kakkar et al.25 based on inhibition of the formation of nicotinamide adenine dinucleotide, phenazine methosulfate, and amino blue tetrazolium formazan. A single unit of enzyme was expressed as 50% inhibition of nitroblue tetrazolium reduction per minute per milligram of protein [21, 22].

Determination of Reduced GSH

Reduced GSH was determined by the method of Ellman.27 Briefly, 1.0 mL of supernatant was treated with 0.5 mL of Ellman's reagent and 3.0 mL of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm. GPx activity was expressed as grams of GSH consumed per minute per milligram of protein and reduced GSH as milligrams per 100 g of tissue [23].

Statistical Analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the groups means were compared by Turkey multiple comparison test. Values were considered statistically significant if $p<0.001$.

Statistical Comparison

a : Group I - Group II *b : Group II – Group III* *c : Group II – Group IV*
d : Group II – Group V *e : Group III – Group IV*

Symbols : ** p < 0.001, ** p < 0.01 ns - non significant*

RESULTS AND DISCUSSION

Table 1. Effect of extracts of aerial part of *Geniosporum Prostratum* Linn on oral glucose tolerance test (OGTT) in normal male albino rats

Group	pretreatment	Plasma glucose concentration (mg/dl)			
		30 min	60 min	90 min	120 min
Vehicle treated	95.7±1.93	140.2±4.12	137.2±1.91	128.0±1.89	121.3±1.56
GPEt extract (100 mg/kg)	96.13±3.24 ns	128.4±2.52***	122.4±1.61***	118.0±1.81***	116.5±0.90***
GPEt extract (200 mg/kg)	95.42±1.88 ns	123.5±2.34***	119.0±1.86***	110.0±1.70***	103.5±1.20***

Data are expressed as mean SD; n=5 animals in each group. Values are statistically significant at P<0.001.

Table 2. Effect of extracts of arial part of *Geniosporum Prostratum Linn* on blood glucose (mg/dl) **level of the STZ induced male albino rats.**

Group	Blood glucose concentration (mg/dl)			
	Day 1	Day 8	Day15	Day 21
Normal control	85.51±1.43	86.18±1.72	85.35±1.70	85.17±1.41
Diabetic+ Vehicle treated	242.6±2.60	243.8±2.58	246.6±1.67	249.8±2.75
Diabetic + GPEt extract (100 mg/kg)	241.0±3.24 ns	234.6±8.44 ns	204.2±17.92***	178.0±28.52***
Diabetic + GPEt extract (200 mg/kg)	240.7±5.62 ns	199.3±4.02***	171.0±5.94***	151.4±3.69***
Diabetic + Glibenclamide (600 µg/kg)	241.1±3.83 ns	196.6±9.62***	187.1±6.15***	148.3±4.39***

Data are expressed as mean ±SD; n= 6 animals in each group. Values are stastically significant at P<0.001.

Table 3. Effect of extracts of aerial part of *Geniosporum Prostratum* Linn on plasma insulin and glycosylated haemoglobin level in normal male albino rats:

Group	Plasma insulin (µl/ ml)	Glycosylated hemoglobin %
Normal control	18.09±0.56	1.90±0.16
Diabetic+ Vehicle treated	7.80±0.46	8.90±1.31
Diabetic+ GPEt extract (100 mg/kg)	11.53±0.77***	6.38±1.26**
Diabetic+ GPEt extract (200 mg/kg)	12.97±0.41***	6.33±0.82**
Diabetic+ Glibenclamide (600 µg/kg)	16.31±0.63***	5.31±1.15***

Data are expressed as mean SD; n=5 animals in each group. Values are statistically significant at P<0.001.

Table 1 shows the blood glucose level of normal and experimental animals after oral administration of glucose (2 g/kg). Extract as well as standard drug treated animals showed more significant decrease in peak blood glucose level after 1 h. After 2 h, the extract treated animals tended to bring the values near normal. The results of Table 2 reveals that the extract produced significant decrease in the blood glucose level when compared with the controls in STZ induced hyperglycaemic rats in the single dose experiment at the tested dose level and is comparable with the standard drug glibenclamide. During diabetes the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin. The rate of glycosylation is directly proportional to concentration of blood glucose and with improvement of glycemic control glycosylated hemoglobin also decreases (Table 3). Hence the estimation of glycosylation of hemoglobin is a well established parameter useful in the management and prognosis of the

disease . Our study gave a clear view that the ethanolic extract prevented significant elevation of glycosylated hemoglobin.

Table 4. Effect of extracts of aerial part of *Geniosporum Prostratum* Linn on antioxidant level in liver of the STZ induced male albino rats

Enzyme Group	Catalase (Unit/mg protein)	GSH (Unit/mg protein)	LPO (Unit/mg protein)	SOD (Unit/mg protein)
Normal control	54.48±0.53	34.60±0.42	13.88±0.31	35.96±0.15
Diabetic+ Vehicle treated	26.70±3.00	15.66±1.62	32.54±1.98	19.60±0.93
Diabetic+ GPEt extract (100 mg/kg)	35.03±0.99***	23.35±0.73***	23.58±1.30***	24.41±2.20***
Diabetic+ GPEt extract (200 mg/kg)	37.73±1.34***	27.40±1.67***	21.67±2.60***	25.37±1.58***
Diabetic+ Glibenclamide (600 µg/kg)	40.58±1.77***	32.28±1.92***	16.94±1.26***	29.99±1.63***

*Data are expressed as mean ±SD; n= 6 animals in each group. * Values are stastically significant at P<0.001. Unit: mM of MDA liberated/100gm tissue/min, CAT : µM of H_2O_2 consumed per min, SOD : 1 unit of activity equals the enzyme reaction that gave 50% inhinition of nitroblue tetrazolium reduction in 1 minute.GPx : µg of GSH consumed/min, GSH : mg/100 mg tissue*

Lipid peroixidation is one of the characteristic features of chronic diabetes. STZ gives rise to dialuric acid, which undergoes oxidation and leads to generation of $O_2^{\cdot}$, H_2O_2 and $OH^{\cdot}$. In this context, a marked increase in the concentration of TBARS was observed in liver of diabetic rats. Increased lipid peroxide concentration in the liver of diabetic animals has already been reported. Administration of the exract and glibenclamide significantly decreased the levels of TBARS in diabetic rats (Table 4).

Glutathione (GSH), a tripeptide present in all the cells is an important antioxidant. Decreased glutathione levels in diabetes have been considered to be an indicator of increased free radical scavenger in the repair of radical caused biological damage. A decrease was observed in GSH in liver during diabetes. Administration of the extract and glibenclamide increased the content of GSH in liver of diabetic rats (Table 4). The cellular radical scavenging systems include the enzymes such as superoxide dismutase (SOD), which scavenges the superoxide ions by catalysing its dismutation and catalase (CAT), a haeme enzyme which removes hydrogen peroxide. Therefore, reduction in the activity of these enzymes (SOD, CAT) results in a number of deleterious effects due to the accumulation of superoxide anion radicals and hydrogen peroxide. Administration of ethanolic extract and glibenclamide increased the activity of SOD and catalase in diabetic rats (Table 4). STZ has been found to induce free radical generation and cause tissue injury. The ethanolic extract of *Geniosporum Prostratum* is reported to be rich in flavonoids. flavonoids are reported to possess antidiabetic and antioxidant activity. Presence of flavonoids in the ethanolic extract was confirmed through our preliminary phytochemical screening also. Thus, the flavonoids in the extract may be suspected to possess the activity that may be attributed to their protective action on lipid peroxidation and at the same time the enhancing effects on cellular antioxidant defense contributing to the protection against oxidative damage in STZ induced diabetes.

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