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To evaluate aqueous extract of *Geniosporum prostratum* (L) Benth in normal and streptozotocin (STZ) induced diabetic rats. Diabetes was induced by intraperitoneally (i.p) streptozotocin (60mg/kg) adult male albino wister rats (8 weeks), weighing 200 to 250 g. Blood glucose levels were determined after oral administration of G. Prostratum (200 mg/kg) in diabetic groups. At the end of 21 days after daily oral administration of aqueous extract of G. Prostratum (100 & 200 mg/kg) and standard drug (600 µg/kg) in diabetic rats, rats were autopsied and liver was removed. The effect of extracts of G. Prostratum on antioxidant like catalase, superoxide dismutase, lipid peroxidase and glutathione were estimated in the diabetic and non-diabetic rats. There was significant increase in catalase, superoxide dismutase, liver peroxidase and glutathione in extracts treated diabetic rats. These results indicate that G. Prostratum reduce oxidative stress in diabetic rats.

Key words: Catalase, *Geniosporum prostratum*, Glutathione, Lipid peroxidase, Oxidative stress, Superoxide dismutase.

Introduction

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 [1,2]. 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 [3,4]. 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 [5,6].

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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 [7]. *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 [8,9]. It has been reported that the plant contains triterpenoids, saponins, β-sitosterols, glycosides, alkaloids, phenols and flavonoids [10]. The purpose of this investigation was to evaluate the effects of *Geniosporum Prostratum* extracts in oxidative stress on streptozotocin (STZ)-induced diabetes by measuring antioxidant activity like superoxide dismutase, catalase, lipid peroxidase and glutathione.

Materials and methods**Plant Material**

The plant *Geniosporum Prostratum* (L) Benth belonging to family "Lamiaceae" 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 & herbarium.

Preparation of Plant Extract

The shade dried aerial part of plant was broken into small pieces and powdered coarsely. Powdered drug packed well in Soxhlet apparatus and extracted with distilled water until the completion of the extraction. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely and dried in a desiccator. Weighed the extract and calculated its percentage in terms of air dried powdered crude material [11,12].

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).

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 [13].

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 aqueous extract of GP.

Group 4 : Diabetic rats treated with 200 mg/kg of aqueous extract of GP.

Group 5 : Diabetic rats treated with Glibenclamide 600 µg/kg.

Diabetic rats were given aqueous 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, liver was immediately dissected out, washed in ice-cold saline to remove the blood.

Preparation of Tissue Homogenate

Liver was removed, blotted free of mucous and blood and weighed. The tissue was homogenized quantitatively at 4° C in 0.15 M Tris-HCl Buffer/ 0.1 phosphate buffer, centrifuged at 15000 rpm for 15 minutes in refrigerated centrifuge and the supernatant was used for determination of catalase, SOD, LPO. For estimation of GSH tissue was homogenized in 0.1 M phosphate buffer 7.4 pH, 10% [14].

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 [15].

Determination of CAT and SOD

CAT was assayed colorimetrically at 620 nm and expressed as moles of H₂O₂ consumed per minute per milligram of protein, as described by Sinha et al. The reaction mixture (1.5 mL) 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 based on inhibition of the formation of nicotinamide adenine dinucleotide, phenazine methosulfate, and amino blue tetrazoliumformazan. A single unit of enzyme was expressed as 50% inhibition of nitrobluetetrazolium reduction per minute per milligram of protein [16,17].

Determination of Reduced GSH

Reduced GSH was determined by the method of Ellman et al 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. GSH activity was expressed as grams of GSH consumed per minute per milligram of protein

and reduced GSH as milligrams per 100 g of tissue[18].

Statistical Analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the group's 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 V

d : Group II– Group VII

Symbols: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, $p > 0.05$ -ns (non-significant).

Table 1: Effect of extract 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	54.48±0.53	34.60±0.42	13.88±0.31	35.96±0.15
Diabetic+ Vehicle treated	26.70±3.00 a***	15.66±1.62 a***	32.54±1.98 a***	19.60±0.93 a***
Diabetic+ GPAq extract (100 mg/kg)	29.58±1.69 b ^{ns}	18.09±1.47 b ^{ns}	28.69±1.87 b*	20.33±1.85 b ^{ns}
Diabetic+ GPAq extract (200 mg/kg)	31.72±2.02 c**	21.43±2.75 c*	27.38±2.39 c**	21.85±1.62 c ^{ns}
Diabetic+ Glibenclamide (600 µg/kg)	40.58±1.77 d***	32.28±1.92 d***	16.94±1.26 ***	29.99±1.63 d***

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

Units are as follows:

Unit: mM of MDA liberated/100gm tissue/min.

CAT : µM of H₂O₂ consumed per minute.

SOD : 1 unit of activity equals the enzyme reaction that gave 50% inhibition of nitrobluetetrazolium reduction in 1 minute. GPx : µg of GSH consumed/minute.

GSH : mg/100 mg tissue.

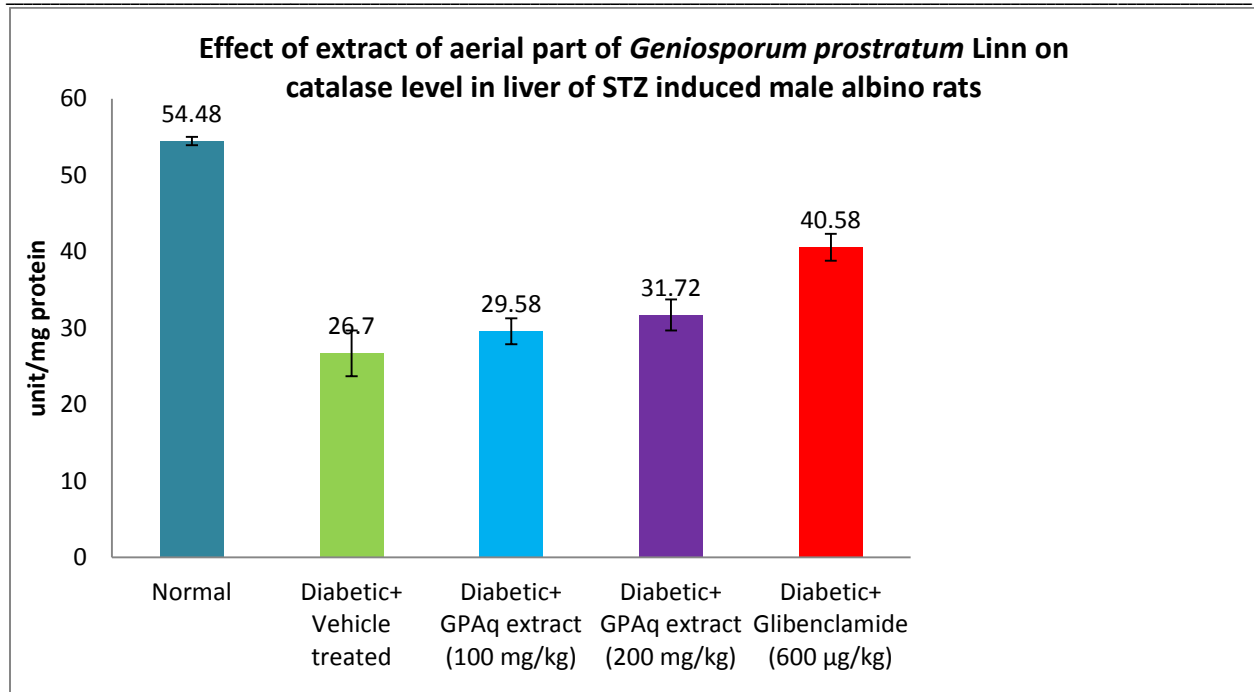


Fig 1:Effect of extract of aerial part of *Geniosporum prostratum* linn on catalase level in liver of STZ induced male albino rats

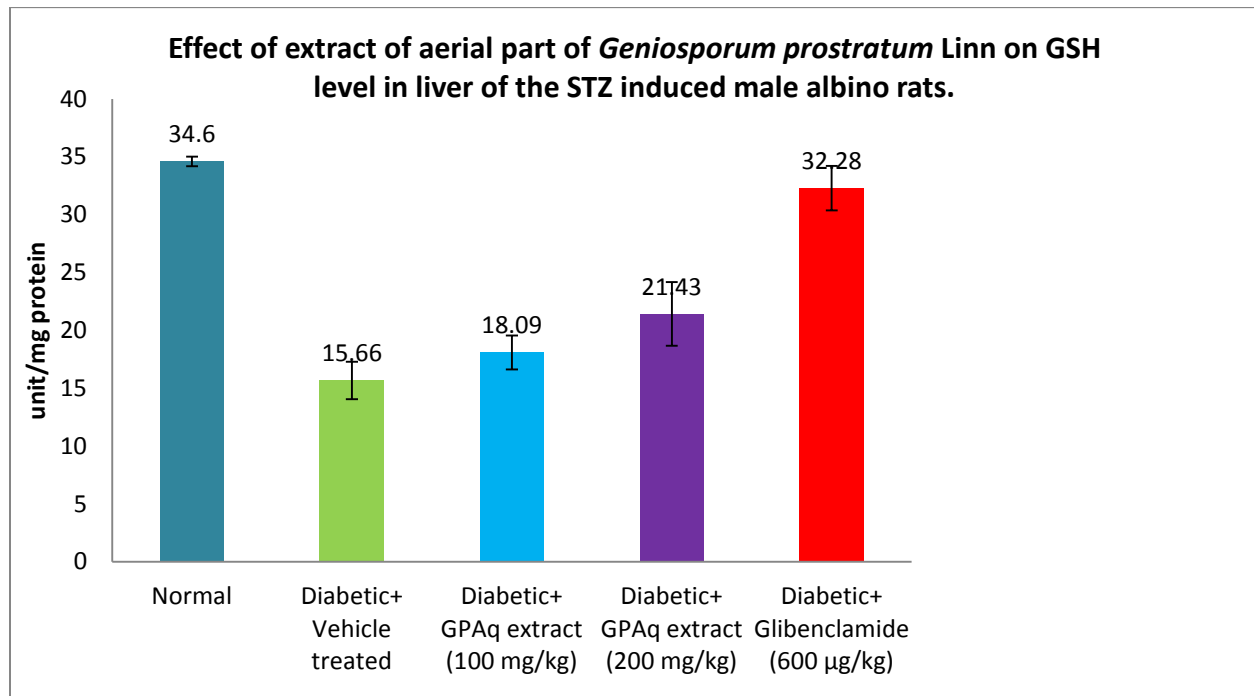


Fig 2: Effect of extract of aerial part of *Geniosporum prostratum* linn on GSH level in liver of STZ induced male albino rats

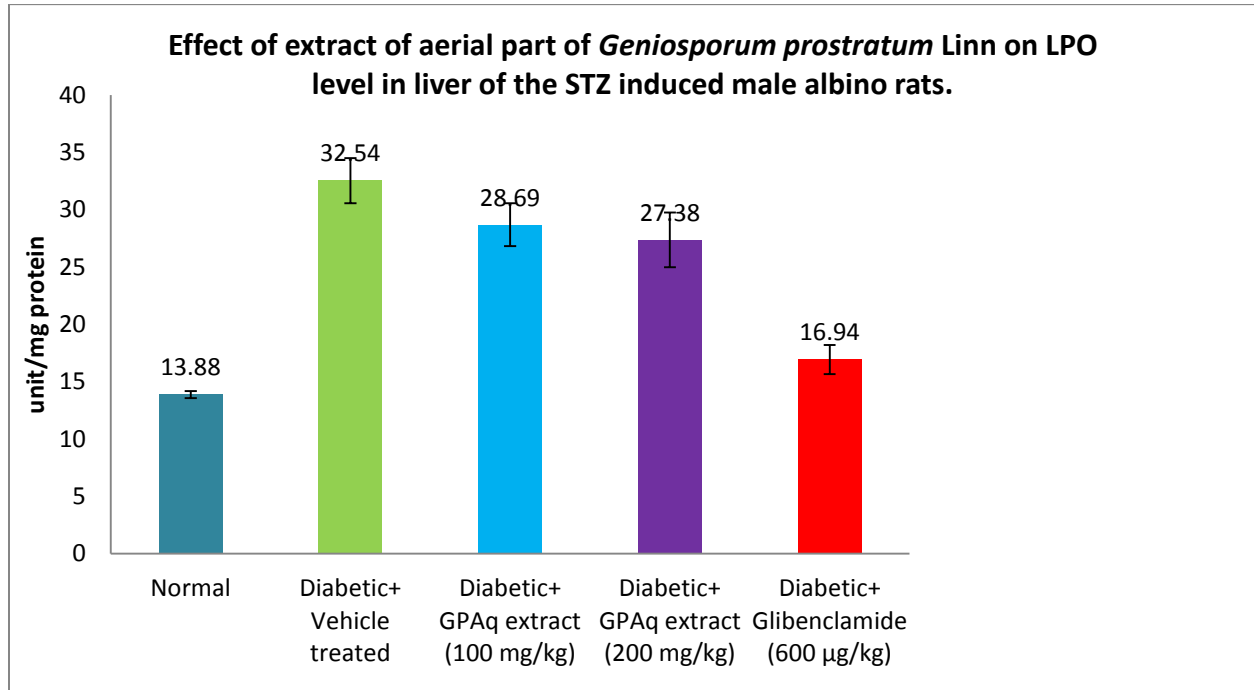


Fig 3: Effect of extract of aerial part of *Geniosporum prostratum* linn on LPO level in liver of STZ induced male albino rats

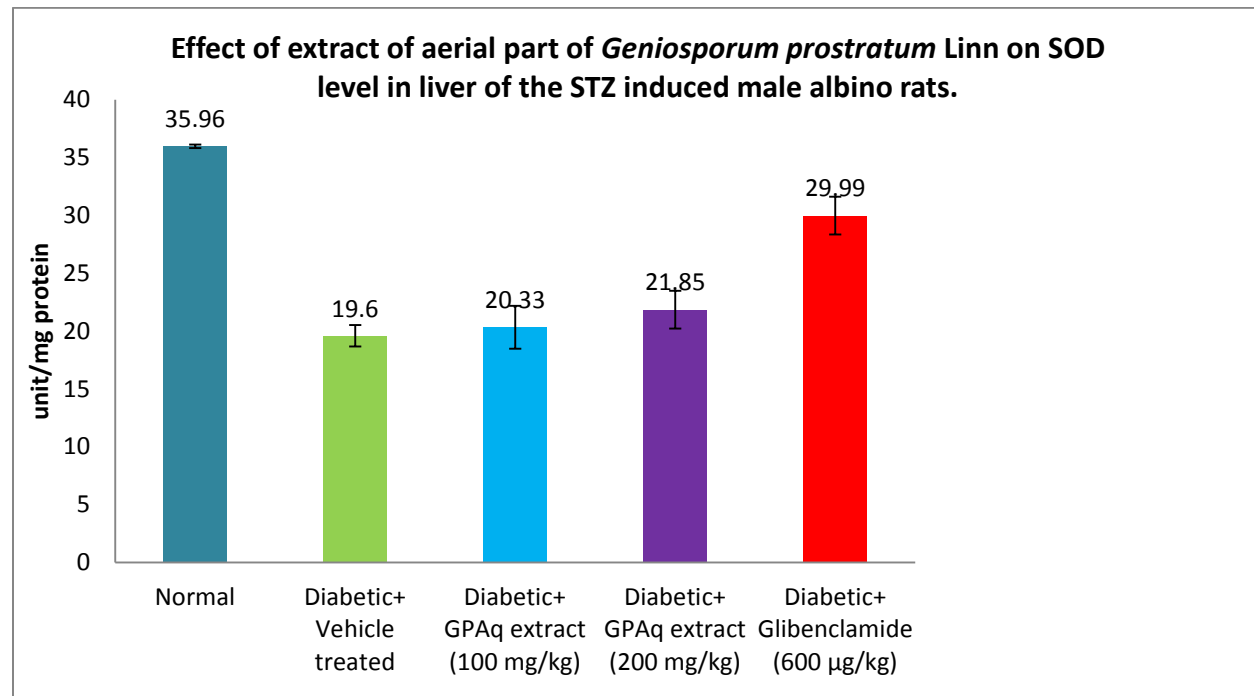


Fig 4: Effect of extract of aerial part of *Geniosporum prostratum* linn on SOD level in liver of STZ induced male albino rats

Discussion

Lipid peroxides is the secondary products of oxidative stress and are unleashed as a result of the toxic effect of reactive oxygen species produced during lipid peroxidation in diabetes. The elevated levels of lipid peroxides in the liver tissues reveal the degree of lipid peroxidation in hepatic tissues and are considered as the indicator of hepatocyte damage. There are several reports in the literature demonstrated the elevated levels of lipid peroxides in the hepatic tissues of experimental diabetic models. The results of the present study also in line with the previous studies, in which *G. prostratum* administration to diabetic group of rats notably declined the levels of liver lipid peroxides, This normalization may be accomplished by the antioxidant and free radical quenching nature of *G. prostratum*. GSH is a major non-protein thiol in living organisms which plays a central role of coordinating the body's antioxidants defense process. It is implicated in cellular defense against xenobiotics and naturally occurring deleterious compounds such as free radicals. The decrease in GSH level represents increased utilization for neutralizing free radicals. However, administration of *G. prostratum* to STZ induced rats attained near normal level. It indicates that the plant extract possessed free radical scavenging nature. Superoxide dismutase and Catalase are considered as primary enzymes since they are involved in the direct elimination of oxygen species. Superoxide dismutase is an important defense enzyme, which catalyzes the dismutation of superoxide radicals, and catalase is a hemoprotein, which catalyses the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals. The reduced activity of superoxide dismutase and catalase in the liver and kidney observed in diabetes may pose deleterious effects as the result of the accumulation of superoxide anion radicals and hydrogen peroxide. A reduced activity of SOD and catalase in liver has been observed during diabetes and this may result in number of deleterious effect due to the accumulation of superoxide radicals and hydrogen peroxide. Plants extract treated rats showed decreased lipid peroxidation which is associated with increased activity of SOD and CAT. This means that the extract can reduce reactive oxygen free radicals and improve the activities of the hepatic antioxidant enzymes. The results of present study suggest that there was significant increase in level of catalase, GSH and SOD. When we was comparing from the results of LPO there is significant reduction of LPO level in extract treated rats as compared to diabetic control. When we was comparing both aqueous extracts to each other observed that there

was more alteration in high dose of aqueous extract treated rats at dose 200 mg/kg then aqueous extract at dose 100 mg/kg.

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