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PHYTOCHEMICAL ANALYSIS AND ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF *GENIOSPORUM PROSTRATUM* AERIAL PARTS ON STZ INDUCED DIABETIC RATS

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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 phytochemicals responsible for 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, i.p. for 3 consecutive days. GPEt was administered orally at a dose 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*, phytochemical analysis, enzymic antioxidants, lipid peroxidation, streptozotocin induced diabetes.

INTRODUCTION

Diabetes mellitus, characterized by hyperglycaemia, 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 defence 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]. Natural antioxidants present in the plants scavenge harmful free radicals from our body. In recent years there has been a tremendous increase in demand for herbal drugs due to its safety, efficacy and better therapeutic results. Due to its economic pricing as compared to synthetic or allopathic drugs, which have several therapeutic complications. Looking to the scope of herbal drug and increasing demand especially in disease of liver, hypertension, diabetes, cancer, renal diseases, inflammation, infectious diseases, arthritis and skin disease etc., hence, it is planned here to study the plant like *Geniosporum prostratum (L) Benth.* There is therefore the need to look inwards to search for herbal medicinal plants with the aim of validating the ethno-medicinal use and subsequently the isolation and characterization of compounds. The plant is reported to show sedative, antiepileptic, vasoconstrictor and anti-inflammatory activity [9,10]. It has been reported that the *Geniosporum Prostratum (L) Benth* plant contains triterpenoid saponins, β-sitosterols, glycosides, alkaloids, phenols and flavonoids [11]. The purpose of this investigation was to find out the phyconstituent and 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 Tamilnadu. 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.

PHARMACOGNOSTICAL STUDY

1. MICROMETRY

Micrometry studies of plant were done by evaluating the.

a) Determination of length and width of phloem fiber
b) Determination of dimension of calcium oxalate crystals
c) Determination of dimensions of starch grains
d) Determination of length and width of trichome [12.13]

2. Powder microscopy

The powder of shade dried aerial part of *Geniosporum prostratum (L) Benth* were studied for the identification of various plant components. The powder was passed through sieve number 60. First the macroscopic characters of the powder were observed. Then microscopically the powder was stained with phloroglucinol and concentrated hydrochloric acid in the ratio of 1:1 to study the lignified cells like xylem and sclerenchymatous tissues. A small portion of powder was mounted in chloral hydrate to identify calcium oxalate crystals [14].

The powdered drug exhibits the following characters:

(a) Phelloid cells:

(b) Crystals:

Calcium oxalate crystals of two types are seen in the powder

(i) Sphaerocrystals or druses:

(ii) Prismatic crystals:

(c) Sclereids:

3. **Ash values:**

   Various types of ash values were determined for powder of aerial part of *Geniosporum Prostratum* (L) Benth such as total ash, acid insoluble ash, water soluble ash and sulphated ash [15].

4. **Extractive values:** These values indicate the nature of the constituents present in a crude drug. Alcohol soluble extractive value and Water soluble extractive value were find out.

5. **Moisture content: (Loss on drying)**

   The percentage of active chemical constituents in crude drugs is mentioned on air dried basis [16].

6. **Fluorescence Analysis:**

   The fluorescence studies of powder of aerial part of *Geniosporum prostratum (L) Benth* were also performed under day light and UV- light [17].

PHYTOCHEMICAL STUDIES

Preparation 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 [18].

Qualitative phytochemical analysis

Various phytochemical test were performed to find out the chemical constituents like [15].

PHARMACOLOGICAL STUDIES:

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 [19].

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 [20, 21, 22].

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 haemoglobin. liver and pancrease was immediately dissected out, washed in ice-cold saline to remove the blood.

Estimation of Blood Glucose and Glycosylated Haemoglobin

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 haemoglobin were estimated by the method of Sudhakar Nayak and Pattabiraman (1981) [23].

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 [24].

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 [25].

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 [26, 27].

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 [28].

Stastical 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

PHARMACOGNOSTIC EVALUATION

Microscopical Study

Table 1 Data representing the values of microscopical study:

S. No	Microscopical parameter	Value
1.	**Phloem fiber**	**Length:**8.52-82.36 **Width :**1.09-1.42
2.	**Calcium oxalate crystals:**	**Length:**1.6-3.2 **Width :**1.4-1.6
3.	**Starch grains**	1.42-7.1
4.	**Trichomes**	**Length:**15.62-52.54 **Width** :1.42-2.84

Fluroscence Analysis

Table 2. Data for fluroscence analysis of powder of arial part of *Geniosporum Prostratum* (L) Benth

S. No.	Treatment	Day light	UV Light (254 nm)
1	**Powder as such**	Light Brown	Light green
2	**Powder + 1 N HCl**	Yellowish brown	Light green
3	**Powder + aqueous 1 N NaOH**	Yellow	Dark green
4	**Powder + alcoholic 1 N NaOH**	Pale Yellow	Yellowish green
5	**Powder + 5% I_2 solution**	Bluish black	Dark brown
6	**Powder + 50% HNO_3**	Yellowish orange	Light green

7	**Powder + 50% H_2SO_4**	Yellowish orange	Light green
8	**Powder + Methanol**	Pale Yellow	Emerald green
9	**Powder + 5% $FeCl_3$ solution**	Violet colour	Fluorescent green

Powder Microscopy

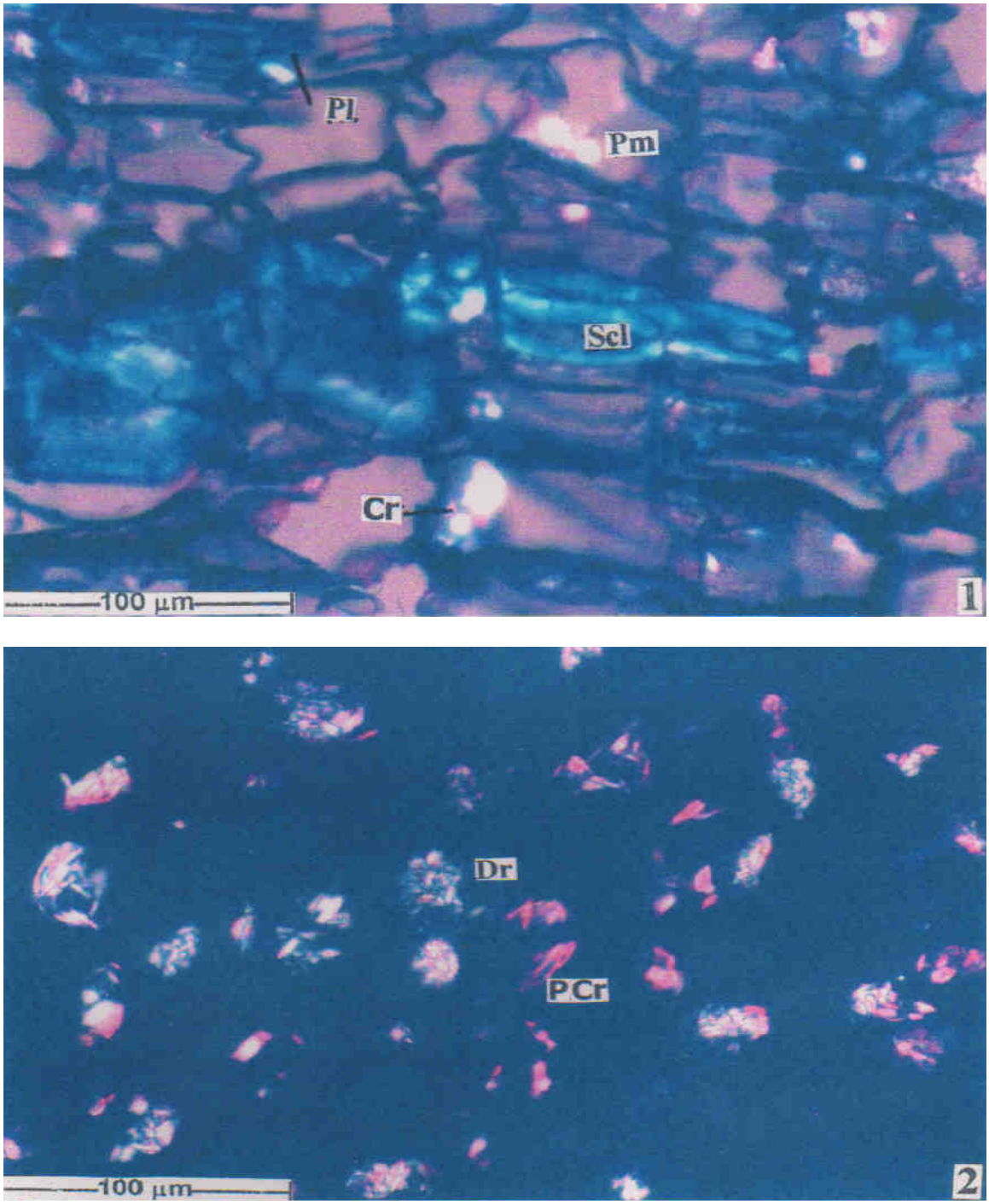

Fig. 1. Powder microscopy of aerial part of *Geniosporum prostratum* (L) Benth. (I) Pl- Phelloid cells, Pm- Phellem cells, Scl- Sclereids, Cr- Crystal, Dr- Druses, PCr- Prismatic crystal

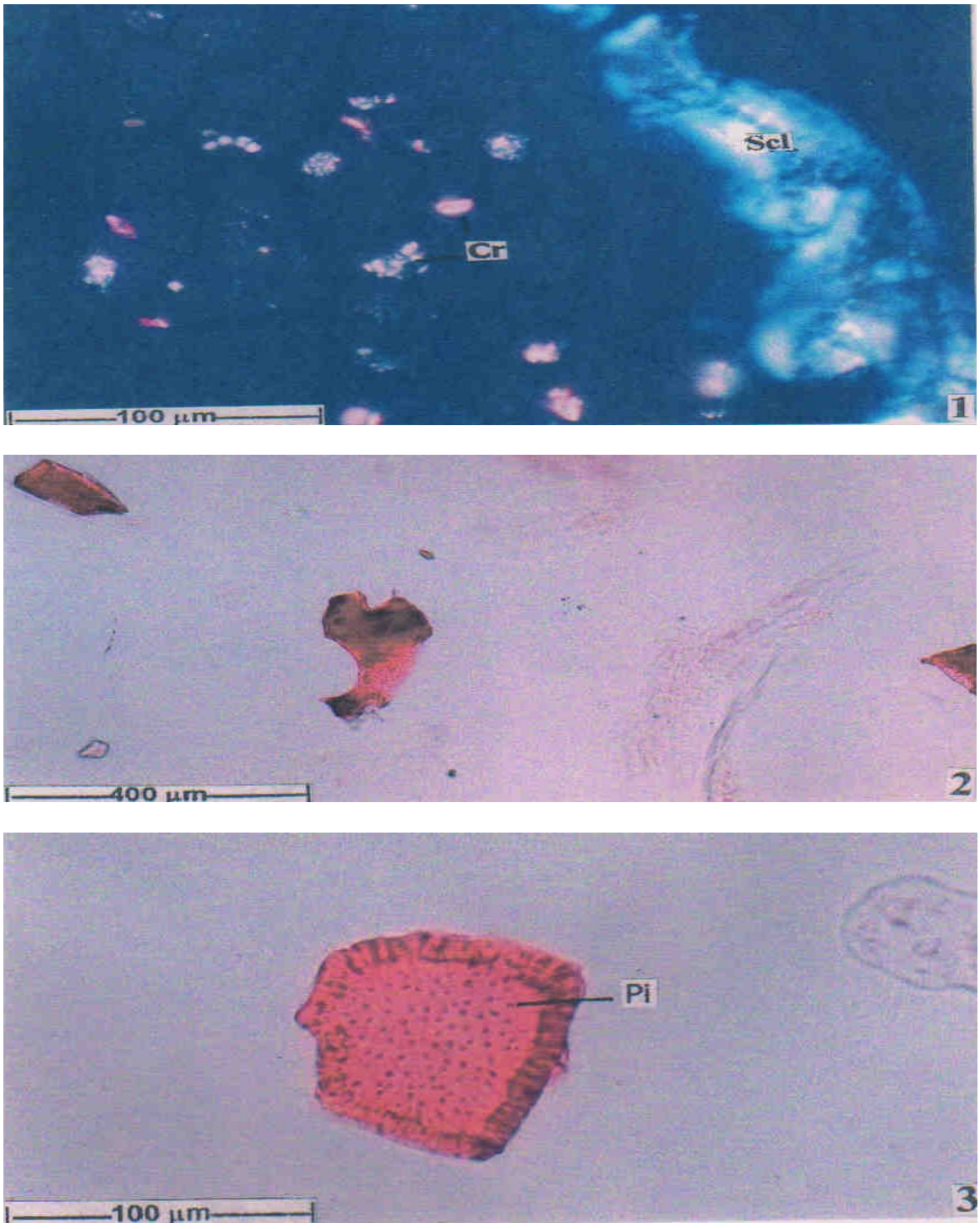

Fig. 2. Powder microscopy of aerial part of *Geniosporum prostratum* (L) Benth. (II) Scl- Sclereids, Cr- Crystal, Pi- Pits

Ash Values:

Table 3. Ash values of *Geniosporum Prostratum* (L) Benth powder

S. No.	Ash Value	(%w/w)
1.	Total ash	7.00
2.	Acid insoluble ash	0.45
3.	Water soluble ash	0.40
4.	Sulphated ash	2.70

Moisture Content:

Moisture content of the powder of aerial part of *Geniosporum prostratum* (L) Benth = 10% w/w.

Extractive Values:

Table4. Data for extractive values of powder of *Geniosporum prostratum* (L) Benth:

S. No.	Extractive value	% W/W
1.	Alcohol soluble extractive	6.25
2.	Water soluble extractive	4.70

PRELIMINARY PHYTOCHEMICAL EVALUATION:

Percentage Yield:

Table- 5. Percentage yields of ethanol and aqueous extract of *Geniosporum prostratum* (L) Benth:

S. No.	Extracts	% Yield
1.	Ethanolic extract	3.43
2.	Aqueous extract	8.1

Fluorescence Study:

Table 6. Fluorescence study of various extracts of powder of aerial part of *Geniosporum prostratum* (L) Benth:

S. No.	Extracts	Day Light	UV Light (254 nm)
1.	Ethanolic extract	Yellowish Brown	Dark Green
2.	Aqueous extract	Yellowish orange	Light Green

Qualitative Phytochemical Analysis:

Table 7. Qualitative phytochemical analysis of powder of aerial part of *Geniosporum Prostratum* (L) Benth:-

S. No,	Plant constituents	Observation
1.	Alkaloids	Present
2.	Saponins	Absent
3.	Glycosides	Absent
4.	Carbohydrate	Absent
5.	Tanins & Phenolic compounds	Present
6.	Flavonoids	Present
7.	Phytosterols	Present
8.	Proteins & amino acids	Absent
9.	Triterpenoids	Absent
10.	Fixed oil & fats	Absent
11.	Gums & Mucilage	Present

PHARMACOLOGICAL STUDIES

Table 8. Effect of extracts of aerial part of *Geniosporum prostratum* (L) Benth 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 9. Effect of extracts of arial part of *Geniosporum prostratum* (L) Benth 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 statically significant at P<0.001.

Table 10. Effect of extracts of aerial part of *Geniosporum prostratum* (L) Benth on plasma insulin and glycosylated haemoglobin level in normal male albino rats:

Parameter 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 11. 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 statically significant at P<0.001.

Units are as follows:

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

CAT : µM of H_2O_2 consumed per minute.

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

GSH : mg/100 mg tissue.

DISCUSSION

Macroscopical character of the aerial part of *Geniosporum prostratum* (L) Benth can serve as diagnostic parameters. Every plant posses a characteristic tissue structure, which can demonstrated through study of tissue arrangement. The characteristic feature of phloem fiber, calcium oxalate crystal, starch grains and trichomes estimated by micrometry. These distinguishing features can be used as anatomical markers. (Table no.- 1) In the powder microscopy of the powder of aerial parts shows superficial periderm, and this plant has two type phloems a) Collapsed phloem b) Non- collapsed phloem. Bark exhibits abundant number of calcium oxalate crystals, brachy sclereids with different shapes with thin lignified walls and numerous simple pits. Prismatic crystals, druses, phellem cell and pheloid cells are also seen in powder, which is characteristic of family (Fig.-1&2). The fluorescence studies were done for the powder of aerial part with different solvent extracts under day light and UV light. The characteristic colours were shown. (Table no. -2 & 6).

The physiochemical parameters like ash value give an idea about the quality and purity of drug during evaluation (Table no.-3). Extractive values of crude drug are useful for their evaluation especially when the constituent of drug can not be readily estimated by any other means. Present study shows the alcohol and water soluble extractive value. The above studies enable the identification of the plant material for future investigation and forms an important aspect of drug studies.

The aerial part of *Geniosporum prostratum* was powdered and extracted with ethanol and distilled water, and the yields were 3.43%w/w and 8.1%w/w respectively. (Table no.-4). All the extracts were subjected to preliminary phytochemical tests to find out the active constituents. In ethanolic extract alkaloids, flavonoids, phytosterols, gum & mucilage were present and in aqueous extract alkaloids, glycosides, carbohydrates, tannins, flavonoids, gums were present. These active moieties may be responsible for the present pharmacological activity. (Table no.-7).

Table 8 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 9 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 haemoglobin to form glycosylated haemoglobin. The rate of glycosylation is directly proportional to concentration of blood glucose and with improvement of glycemic control glycosylated haemoglobin also decreases (Table 10). Hence the estimation of glycosylation of haemoglobin 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.

Lipid peroxidation is one of the characteristic features of chronic diabetes. STZ gives rise to dialuric acid, which undergoes oxidation and leads to generation of $O2^{\cdot}$, 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 extract and glibenclamide significantly decreased the levels of TBARS in diabetic rats (Table 11).

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 11). 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 haem 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 11). 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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