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A BREIF INSIGHT TO THE PHARMACOGNOSTIC PROPERTIES AND SHOWING THE ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF *GOMPHRENA SERRATA* L. ON STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The present work was undertaken to investigate the Antidiabetic and Antioxidant Activity of Gomphrena serrata L. on Streptozotocin-induced Diabetic Rats. For its evaluation, pharmacognostical and pharmacological evaluation studies were performed. The pharmacological assessment of Water and Methanolic extract of G. serrata was performed using various models such as DPPH assay, Malon-di-aldehyde & Glutathione levels for evaluation of Antioxidant activity whereas for the evaluation of Antidiabetic activity Acute(1 day) and Sub-acute(15 days) studies were performed. Currently available therapy for diabetes and diabetic dyslipoproteinemia include insulin and various oral antidiabetic agents such as sulfonylurea, metformin, α-glucosidase inhibitors, troglitazone and antidyslipoproteinemic agents as gemfibrozil and flavastatin are used. But they cause a number of serious adverse effects in patients. On the other hand it is considered that natural products are free from side effects and safe. This study was therefore, planned to explore antidiabetic, antioxidant and lipid-lowering activity of *Gomphrena serrata* in streptozotocin induced diabetic rats.

INTRODUCTION

The incidence of diabetes mellitus is growing worldwide. India is no exception, and about 25 million Indians are estimated to be suffering from diabetes (Kochupilai N. 2000). Further predictions indicate that India will have the most number of diabetic patients by the year 2025 (King H, *et al.* 1998). The life of patients with diabetes has improved with newer insulins and oral hypoglycaemic agents. Unfortunately chronic complications of the disease show a rising trend among diabetics living longer. Diabetes, earlier thought to be a problem of glucose metabolism, actually produces large number of micro and macro vascular complications affecting mainly cardiovascular system (Grundy SM, *et al.*

1999). Patients with diabetes are characterised by an increased possibility for developing congestive heart failure through increased coronary artery disease (CAD), hypertension, specific cardiomyopathy and endothelial dysfunction. Over the last three decades, a number of epidemiological, clinical and autopsy studies have proposed the presence of diabetic heart disease, as a separate clinical entity (Zhi YF, *et al.* 2004). It is ranked seventh among the leading causes of death and third when it's fatal complications are taken in to account (Trivedi NA, *et al.* 2004). Diabetes is a syndrome, initially characterized by a loss of glucose homeostasis. The disease is progressive and is associated with oxidative stress with high risk of diabetic dyslipidemia which is responsible for micro and macro vascular complications of diabetes mellitus (Pavan P, *et al.* 2007). Currently available therapy for diabetes and diabetic dyslipoproteinemia include insulin and various oral antidiabetic agents such as sulfonylurea, metformin, α-glucosidase inhibitors, troglitazone (Rao BK, *et al.* 2003) and antidyslipoproteine- inemic agents as gemfibrozil and flavastatin are used (Schweitzer M, *et al.* 2002). The American Diabetes Association reported in 2009 that there are 23.6 million children and adults in the United States 7.8% of the population, who have diabetes. While an estimated 17.9 million in the US alone have been diagnosed with diabetes, nearly one in four (5.7 million) diabetics are unaware that they have the disease (Tierney LM, *et al.* 2002).

Herbal drugs which are mentioned in our ancient text books and are still used in the treatment of diabetes includes *Allium sativum*, *Eugenia jambolana*, *Momordica charantia* *Ocimum sanctum*, *Phyllanthus amarus*, *Pterocarpus marsupium*, *Tinospora cordifolia*, *Trigonella foenum graecum* and *Withania somnifera*. These are available as single drugs or are used in combination with other drugs so as to reduces the secondary complication associated with diabetes. The World Health Organization (WHO) has estimated that 80% of the populations of developing countries still rely on traditional medicines, mostly plant drugs, for their primary health care needs. Demand for medicinal plant is increasingly felt, in both developing and developed countries due to growing needs of natural products being non-toxic and bereft of side-effects, apart from availability at affordable prices. The medicinal plant sector has traditionally occupied a pivotal position in the socio cultural, spiritual and medicinal areas of rural and tribal families (World Health Organization, 2002-2005). A number of plants and their isolated constituents has been studied for their role in treatmet of diabetes this study was therefore, planned to explore antidiabetic, antioxidant and lipid-lowering activity of *Gomphrena serrata* in streptozotocin induced diabetic rats.

MATERIALS & METHODOLOGY

DRUGS, CHEMICALS & REAGENTS

Drug: Streptozotocin and Glibenclamide were purchased from Sigma Aldrich. Chemicals Petrolium ether, Ethyl acetate, Methanol, Ethanol,Chloroform, Dextrose, Sodium chloride, Potassium chloride, Potassium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium citrate, Citric acid were purchased from Central Drug House Laboratory (CDH).

All Reagents & Chemicals used in the research work were of analytical grade.

COLLECTION & AUTHENTIFICATION

The roots of *Gomphrena serrata* were collected in the month of August, 2011, from was collected from the side of Railway track, Meerut city station, U.P. India. The roots were dried under normal environmental conditions and were authenticated by Dr. Anjula Pandey, Principal Scientist, National Bureau of Plant Genetic Resources (Indian council of Agricultural Research, New Delhi). The voucher no. NHCP/NBPGR/2011-37/ has been

deposited at the Department of Pharmaceutical Technology, M.I.E.T, Meerut and NBPGR, New Delhi.

PREPARATION OF EXTRACTION OF GOMPHRENA SERRATA

Extraction of plant Was done by Successive solvent extraction. Soxhlet apparatus was used for the successive solvent extraction. The solvents for extraction selected on the basis of their polarity. The order of use of solvents for extraction was petroleum ether, chloroform, ethyl acetate, methanol, ethanol, and water. Petroleum ether is the most nonpolar solvent among these four solvents.

Gomphrena serrata was dried undershade, reduced to moderately coarse powder, loaded into soxhlet extractor and was subjected to successive extractionwith Petroleum Ether, Chloroform, ethyl acetate, Methanol, Ethanol andWater to get different extracts.

QUALITATIVE IDENTIFICATION TESTS

The detection of active principles in medicinal plants plays a strategic role in phytochemical investigation of crude plant extracts and is very important in regards to their potential pharmacological effects. The identification of the chemical groups in the various extracts was performed with the help of various qualitative chemical tests followed by their confirmations from specific tests.

Table 1.1: Chemical Tests

S.No.	Name of Test	Procedure
1	**Test for Carbohydrates:** **Molisch test** **Benedict Test**	Extracts+ few drops of α -naphthol solution in alcohol & add conc. H_2SO_4 Equal volume of Benedict's reagents and plant extracts and Heated in boiling water bath for 5 min.
2	**Test for Flavanoids:** **Shinoda test**	To the extracts+ 5ml of 95% alcohol, few drops of conc. HCl and 0.5 mg of magnesium turning were added. To a small quantity of extracts +$Pb(CH_3COO)$ solution was added
3	**Test for Alkaloids:** **Mayer's test** **Wagner's test** **Dragendorff's test** **Hager's test**	Extract + pot. HgI_2 solution Extract +I_2-KI solution Extract + Pot. bismuth iodide solution Extract + Picric acid solution.
4	**Test for Protein:** **Biuret test**	In 3 ml of aqueous solution of extract added 4% NaOH & few drops of 1% $CuSO_4$ solution.
5	**Test for Glycosides:** **Keller-killani test** **Froth formation test**	Extract the drug with chloroform then evaporate to dryness + glacial acetic acid+ $FeCl_3$ sol Drug solution In water in a test tube and shake well.
6	**Test for Steroids:** **Salkowski test** **Libermann-Burchard test**	Extract's residue + 2 ml of $CHCl_3$ and 2ml of conc.H_2SO_4 were added. Extract +$CH_3(CO)_2O$, boil then cool+ Conc. H_2SO_4 added
7	**Ferric chloride tannin test**	Extract +$FeCl_3$ solution

MICROSCOPICAL EXAMINATION OF LEAF

Microscopic study was performed by simple free hand sectioning of the fresh leaves of the plant for thin sections.

The thin sections were cleared by chloral hydrate solution and treated with the following stains:

- Phloroglucinol and Hydrochloric acid in the ratio of 1:1
- Iodine solution
- Aniline blue
- Toluidine blue
- FABIL (Basic Fuschin, Aniline blue & Iodine solution in lactophenol)

Thin stained and unstained sections were observed using OLYMPUS BX41 microscope and Photomicrography was done using OLYMPUS C7070 camera.

PHARMACOLOGICAL ASSESSMENT

ANIMALS

Adult Wistar albino female rats, weighed 180-200 Gms, in equal numbers per group (i.e.6) were used for the study. The animals were fed with standard pellet diet and drinking water was supplied ad libitum. At the commencement of the study the weight variations of animals used was kept minimal and not exceeded ± 20% of the mean weight of each animal. Normal weight of rats was 180-200 gm. The animals were housed in polycarbonate cages at a temperature of 22ºC (±3ºC) with 12hr light and 12 hr dark cycle and relative humidity of 50–60%. The study was performed as per the protocols and recommendation of the Institutional Animal Ethics Committee (IAEC). Animals were caged in small number of the same group. Healthy young adult female rats were randomly assigned to the control, standard and treatment groups. The animals were identified uniquely (i.e., via marking at the base of the tail) and acclimatized for not less than 5 days in their cages prior to the start of the study.

The animals were grouped into Control, Normal control, Standard and *Gomphrena serrata* extracts treated (100, 200, 400 mg/kg body weight) groups.

TOXICITY STUDY

Administered test dose (2000 mg/kg) to animal. If animal die, then conduct the main test to determine LD_{50}. If animal survive then give test dose to four additional animals. If three animals die, limit test is terminated and main test to be performed. In case three or more animals survive, its mean LD_{50} is greater than 2000 mg/kg. For main test administer dose to one animal in sequence usually at 48 hr interval. If the animal survives, the second animal receives a higher dose. If the first animal dies or appears moribund, the second animal receives a lower dose. Dosing continues depending on the fixed-time interval (e.g., 48-hour) outcomes of all the animals up to that time. The testing stops when one of the following stopping criteria first is met

(a) 3 consecutive animals survive at the upper bound;

(b) 5 reversals occur in any 6 consecutive animals tested;

(c) At least 4 animals have followed the first reversal and the specified likelihood-ratios exceed the critical value. At last, the 10% of maximum dose was considered safe to carry out the research work.

EXPERIMENTAL DESIGN

INDUCTION OF DIABETES

Animals were divided into seven groups and for each group six animals were

taken. Group I (Normal control) (0.9% NaCl; 5ml/kg.b.w.p.o) and Diabetes was induced to other rats by intraperitoneal injection of streptatozotocin (60mg/kg.b.w.i.p). After 24h, animals showing plasma sugar level more than 250mg/dl were considered diabetic. The diabetic animals were stabilized for five days and the next day (day 0) experiment was started. Group II served as diabetic control, Group III ,IV, V and VI received plant extract (100, 200 and 400 mg/kg.b.w.p.o: water and 200 mg/kg b.w.p.o: methanol) once a day for 14 days and Group V received (Glibenclamide 0.5mg/kg.b.w.) once a day for 14days and served as standard. Blood glucose was measured on 0^{th}, 5^{th}, 10^{th}, and 15^{th} day. At the 15^{th} day all the animals were sacrificed and evaluated for the biochemical and *in vivo* antioxidant status (AL-Awadi, FM *et al,* 1985).

DOSING OF DRUGS

The extract was dissolved in water and required doses were administered orally to different groups of male rats (n=6) once a day for 15 days.

ANTIDIABETIC ACTIVITY

(A) ORAL GLUCOSE TOLERANCE TEST

(i) Male wistar albino rats of 180-220gm body weight were selected and kept for a overnight fasting.

(ii) Then animals were divided in to six group of 6 rats each.

(i) **Group 1** served as control.

(ii) **Group 2** served as standard treated with Glibenclamide 0.5mg/kg.b.w.

(iii) **Group 3** served as test group treated with 100 mg/kg *Gomphrena serrata* methanolic extract orally.

(iv) **Group 4** served as test group treated with 200 mg/kg *Gomphrena serrata* water extract orally.

(v) **Group 5** served as test group treated with 400 mg/kg *Gomphrena serrata* water extract orally.

(vi) **Group 6** served as test group treated with 200 mg/kg *Gomphrena serrata* water extract orally.

(iii) After 30 minute of administration of respective treatment in each group, glucose (2 gm/kg b.w) was given.

(iv) Then blood glucose level was analyzed at 0, 30, 60, 90 and at 120 minutes of each rat with the help of glucometer.

(B) SUB-ACUTE METHOD FOR EVALUATION OF ANTIDIABETIC ACTIVITY

For Sub-acute method, the animals were grouped six animals in each group as following:

(i) **Group 1** served as control

(ii) **Group 2** served as standard treated with Glibenclamide 0.5mg/kg.b.w.

(iii) **Group 3** served as test group treated with 100 mg/kg *Gomphrena serrata* water extract orally.

(iv) **Group 4** served as test group treated with 200 mg/kg *Gomphrena serrata* water extract orally.

(v) **Group 5** served as test group treated with 400 mg/kg *Gomphrena serrata* water extract orally.

(vi) **Group 6** served as test group treated with 200 mg/kg *Gomphrena serrata* methanolic extract orally.

ANTIOXIDANT ACTIVITY

(A) DIPHENYL PICRYLHYDRAZYL ASSAY (DPPH ASSAY)

1, 1-Diphenyl-2-picrylhydrazyl free radical scavenging assay (DPPH) was carried out according to the following procedure. Each plant extract at various concentrations (20, 40, 60, 80 and 100 µg/ml) was added to DPPH in methanol and the reaction mixture was shaken vigorously. The amount of DPPH remaining was determined at 520 nm, and the radical scavenging activity was obtained from the following equation: Radical scavenging activity (%) = {(OD control –OD sample) / OD control} x 100. The antioxidant activity of plants extracts was partially expressed as IC50, which was defined as the concentration (in µg/ml) of extract required to inhibit the formation of DPPH radicals by 50%(Dolphin, D.,*et al.* 1989).

(B) MELON DI-ALDEHYDE (MDA) / THIOBARBITURIC ACID REACTIVE SPECIES (TBARS) ESTIMATION IN LIVER HOMOGENATE

The levels of melon di-aldehyde and Thio barbituric acid reactive species increases whenever there is tissue damage and therefore it is an important parameter in the study of antioxidant activity.

In this method, the animals were grouped six animals in each group as following:

(i) **Group 1** served as control

(ii) **Group 2** served as standard treated with Glibenclamide 0.5mg/kg.b.w.

(iii) **Group 3** served as test group treated with 100 mg/kg *Gomphrena serrata* water extract orally.

(iv) **Group 4** served as test group treated with 200 mg/kg *Gomphrena serrata*water extract orally.

(v) **Group 5** served as test group treated with 400 mg/kg *Gomphrena serrata* water extract orally.

(vi) **Group 6** served as test group treated with 200 mg/kg *Gomphrena serrata* methanolic extract orally.

1. 1 gm of rat liver is taken in a tube. In that 4.5 ml of phosphate buffer (pH-7.4) and 3 mM EDTA is taken.
2. Homogenized at 2000 rpm using 10 strokes.
3. Centrifuged at 7000 G for 10 min.
4. Supernatant liquid is to be removed from the tube.
5. Take supernatant and add 100 µl of 8.1% SDS + 750 µl of 20% acetic acid +750 µl of 0.8% Thiobarbituric acid (TBA) in a glass tube.
6. Volume is made up to 2 ml with Distilled Water (D.W.).
7. Heat it over water bath at 95ºC for 60 minutes.
8. In the above step wrap the mouth of test tube with an Aluminium Foil.
9. Then test tube is to be taken out and cool under tap water. In this step colour of the sample becomes pinkish.
10. Again centrifuge at 10,000 rpm for 10 min.
11. Take 300 mcl supernatant in a microtitre plate with blank as D. W. in a cuvette which is ready for absorbance and absorbance is taken at 532 nm.

(C) ESTIMATION OF GSH LEVEL IN LIVER HOMOGENATE

Glutathione is released as a part of protective mechanism whenever there is tissue damage, so its level indicate the health of the tissue *i.e* higher the level better will be the

condition of the tissue

1. 1 gm of liver will be taken in a test tube containing 5 ml of chilled phosphate buffer (pH-7.4) and 3 mM EDTA.
2. Homogenize it at 2000 rpm at 10 strokes. Centrifuge at 7000 rpm for 10 min.
3. Take 500 µl of supernatant and add 500 µl of 5% chilled sulfosalicylic acid.
4. Vortex it and keep in ice for 30 min. Centrifuge at 10,000 rpm for 10 min.
5. Supernatant will be separated from pellet and will be stored in freezer.
6. For test 450 µl of PB (pH 7.4) will be taken and add 50 µl of sample.
7. For blank 500 µl of PB (pH 7.4) will be taken in a test tube. **(Ohkawa, H., *et al.*1997).**

3. RESULT & DISCUSSION

PHARMACOGNOSTICAL STUDY

After evaluation of various parameters the following results were concluded:

PERCENTAGE YIELD

The petroleum ether, chloroform, ethyl acetate, methanol, ethanol and aqueous extracts were obtained by performing as per standard procedure with the help of analytical techniques. The percentage (%) yields were 1.37%, 0.95%, 0.63%, 12.6%, 0.96% and 25.5% w/w respectively.

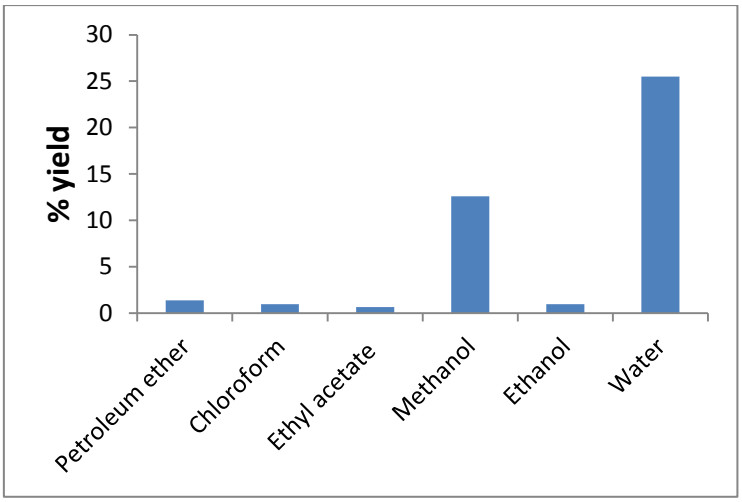

Figure 1.1: Figure depicting Percentage Yield of Different Extracts

PHYTOCHEMICAL SCREENING

The following chemical groups were found in the various extracts by using qualitative chemical identification tests, results shown in the table.

Extraction of plant material was performed by using soxhlet apparatus using the following solvents: Petroleum ether, Chloroform, Ethyl acetate, Methanol, Ethanol, and water successively. The extracts thus obtained were collected and stored at 4º C. Phytochemical investigation of various extracts of Gomphrena serrata revealed the nature of phytochemicals present which are summarized in the table along with the chemical test applied for the different phytoconstituents.

Table 1.2: Phytochemical Testing of G. serrata plant extracts

	Pet. ether	Chloroform	Ethyl acetate	Methanol	Ethanol	Water
Alkaloid						
Dragendorff's Test	-	-	-	+	+	-
Mayer's reagent	-	-	-	+	+	-
Wagner's reagent	-	-	-	+	+	-
Hager's reagent	-	-	-	+	+	-
Amino acids						
Ninhydrin test	-	-	-	-	-	-
Carbohydrates						
Molish's test	-	-	-	-	-	+
Barfoed's reagent	-	-	-	-	-	+
Flavonoids						
Shinoda test	-	-	-	+	+	+
Zn. HCl test	-	-	-	+	+	+
Alkaline reagent test	-	-	-	+	+	+
Steroids						
Salkowski test	-	+	+	+	+	+
Tannins						
Ferric chloride test	-	+	+	+	+	+
Glycoside						
Fehling test	+	-	-	-	+	+
Saponin test	+	-	-	-	+	+
Fats & Oils	+	-	-	-	-	-

Where + means Present and – means Absent

Upon examination, it was found that *G. serrata* plant extract contains abundant amount of alkaloids, flavonoids, triterpenes, carbohydrates saponins, steroids, and glycosides. Methanol and aqueous extracts were found to contain flavonoids. Methanolic extract was found to contain maximum no. of phytoconstituents. Therefore, methanolic extract and water extract were used for the evaluation of antidiabetic & antioxidant activity.

MICROSCOPICAL CHARACTERISTICS TRANSVERSE SECTION OF LEAVES OF *GOMPHRENA SERRATA*

The thin transverse sections of leaf were taken which were treated with appropriate reagents and mounted on a glass slide. Transverse section of a leaflet shows a

dorsiventral structure. Following are the important tissues in the lamina and the midrib region.

Lamina: It shows regular upper and lower epidermis with well-developed thin cuticle and stomatal pores. Stomata of anomocytic type were observed as shown in the Fig 5. (10).

Midrib: Upper and lower epidermis layers continuous over the midrib, followed by a patch of collenchymatous cells below the upper and above the lower epidermis. The epidermal cells show similar features as seen in the lamina region. The rest of the midrib is occupied by the parenchyma cells.

Transverse Section of Lamina

Palisade mesophyll was found in patches which are densely packed by the collenchymatous cells. Loosely arranged parenchymatous cells were found below the palisade cells. The leaves were dorsiventral as palisade cells were observed only on one side.

Transverse Section of Midrib

The zone of cortex consisting of collenchymatous cells was observed. Collenchymatous layer surrounds the parenchymatous cells. The outer wall consists of cellulose which is evaluated by the treatment with I_2 solution and dil. H_2SO_4. The spongy mesophyll consists of shaped parenchymatous cells which covers the central portion of midrib. The central portion of midrib consists of group of vascular bundles. The vascular bundles are arranged in circular pattern with one vascular bundle (in centre) surrounded by these vascular bundles. The vascular bundles are covered with single layered parenchymatous bundle sheath. Vascular bundle is ovoid in shape consist of sclerenchyma cell, phloem region and xylem vessels as depicted in the Figure 5.4 (11).

SURFACE MICROSCOPY OF LEAF

The surface of the leaf was cleared and cuticle layer was peeled out for surface microscopic study. Epidermis was found with 5 to 6 subsidiary cells which were attached to the stomata and evaluated as parasitic stomata as depicted in the Image 5.3 (10).

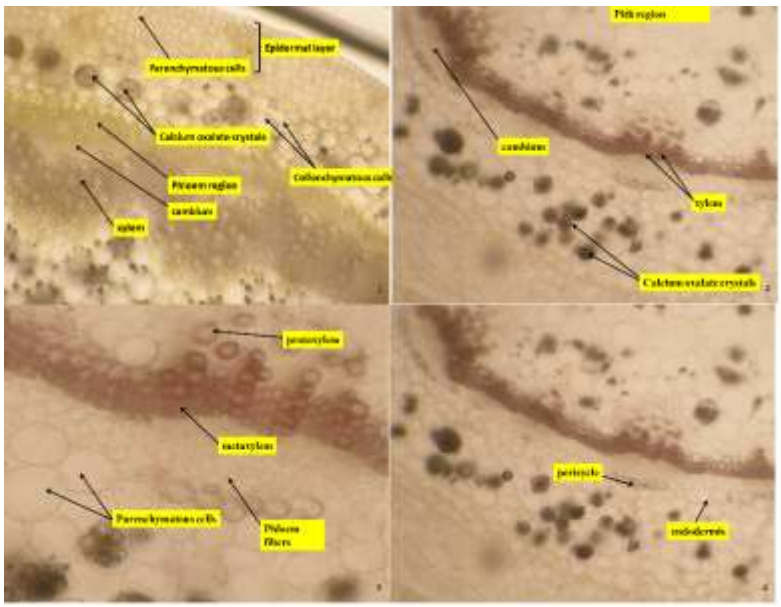

Figure 1.2: Figure depicting(1) Ca oxalate crystals, Collenchyma, Cambium & Vascular bundles (2) Pith region (3) Proto & Meta Xylem (4) Pericycle & Endodermis.

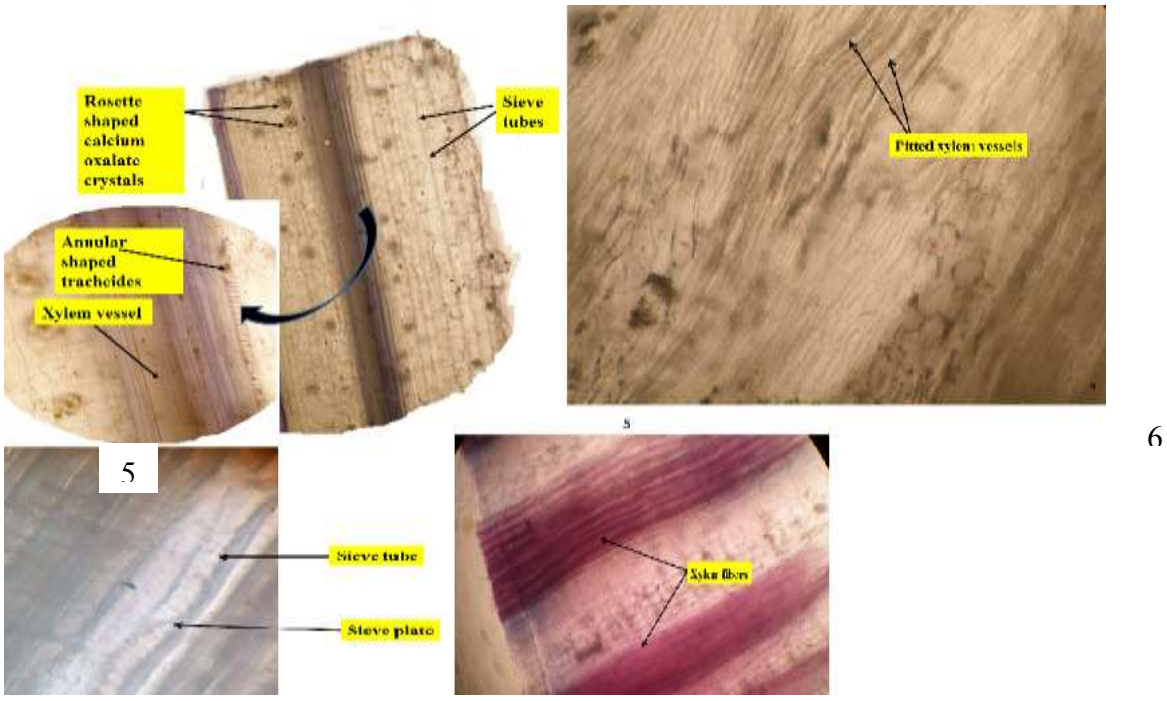

Figure 1.3: Figure depicting (5) Rosette shaped Ca oxalate crytals, annular shaped tracheids & sieve tubes (6) Pitted xylem vessels (7) Sieve tube & Sieve plates (8) Xylem fibres

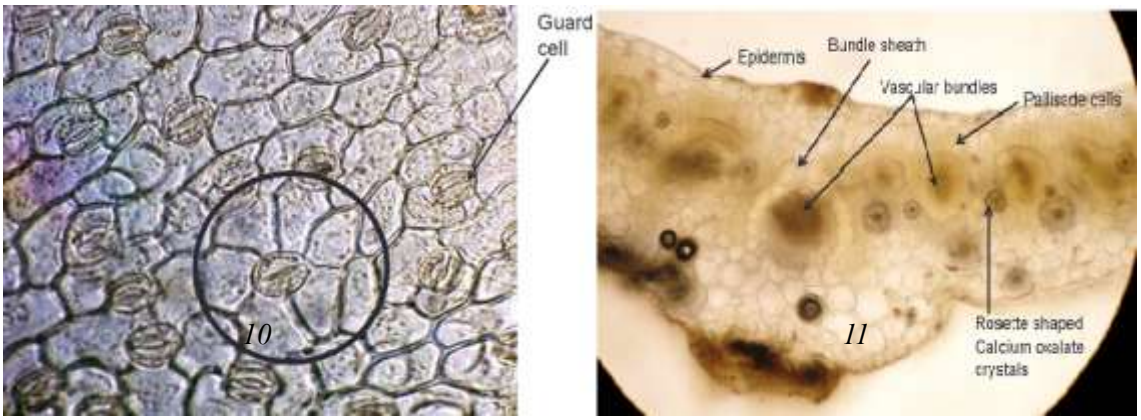

Figure 1.4: Figure depicting leaf of G. serrata (10) Guard cells and Anomocytic stomata (11) Rosette shaped Ca oxalate crystals, scattered vascular bundles.

ACUTE ORAL TOXICITY

G. serrata plant extract (ethanolic, and aqueous), at the doses of 2000 mg/kg (orally) had no effect on mice behavioural responses and no mortality was observed during 72 hour time period. These results indicate that *G. serrata* ethanolic extract have low/no toxicity profile. Therefore 200mg/kg was taken as the therapeutic dose and 100mg/kg and 400mg/kg were taken as lower and higher doses, respectively.

PHARMACOLOGICAL SCREENING

ANTIDIABETIC ACTIVITY OGTT

The normal rats were tested for the Oral Glucose Tolerance Test by administering glucose with the simultaneous administration of the test drug, the results are given below.

Table 1.3: Effect of Glibenclamide and G.Serrata extract on Streptozotocin Induced Diabetic Rats

Groups	Time Interval (Minutes)				
	Blood glucose level before drug administration (mg/dl)	Blood glucose level after glucose (2g/kg b.w) administration mg/dl (Mean±SEM)			
	0 min.	30 min.	60 min.	90 min.	120 min.
Control	82.16±1.62	121.8± 2.72	115.8± 2.54	108.66±2.56	102.33± 2.83
Glibenclamide (5mg/kg)	71.33±1.11	112± 1.78	91.83± 1.77	80.66± 0.95	74.66± 1.45
GSME (200 mg/kg)	81.661.14	114.33± 2.82	99.33± 2.06	92.33± 1.45	87.16± 0.94
GSWE (100 mg/kg)	74.661.11	122.66± 2.06	105.16± 1.44	97.16± 1.07	89.83± 1.16
GSWE (200 mg/kg)	75.57±1.24	116.56±1.81	99.25±2.10	91.58±1.32	84.79±0.87
GSWE (400 mg/kg)	79.351.13	113.61±1.84	93.04±1.82	89.24±1.06	84.15±1.36

GSME- Gomphrena serrata Methanolic extract, GSWE – G. serrata water extract
Values are expressed as Mean ± SEM
*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n = 6 animals in each group.

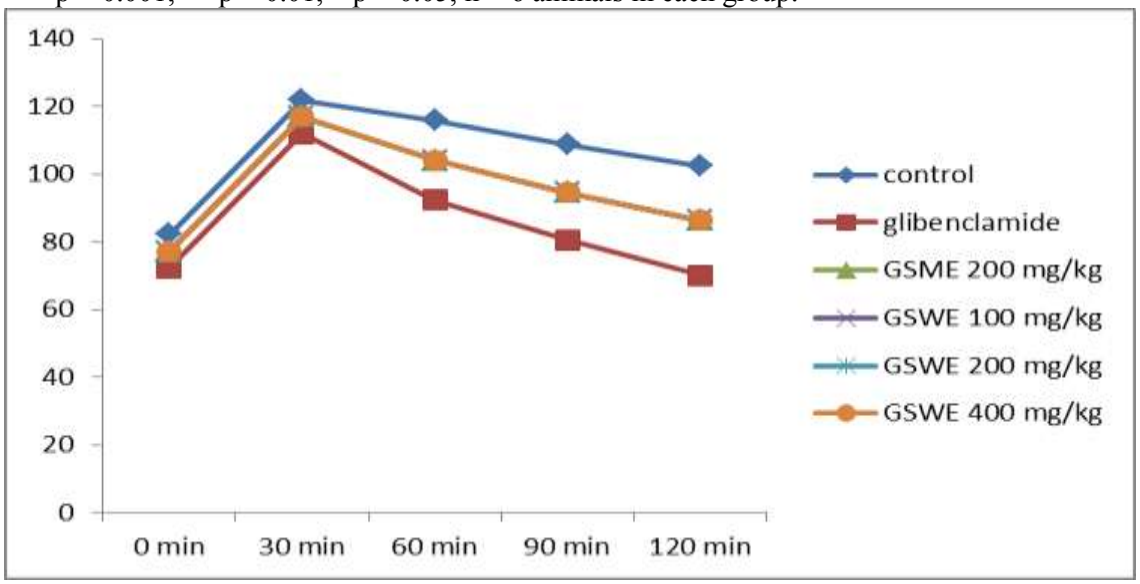

Figure 1.5: Graph showing plasma glucose levels of plant extracts of G. serrata at different time intervals.

Gomphrena serrata methanolic extract(200mg/kg) and aqueous extract (100, 200, 400 mg/kg) showed significant reduction in plasma glucose level as compared to control at 30 min, 60 min & 120 min duration. GSWE as depicted in fig 1.5.

HYPOGLYCEMIC ACTIVITY

SUBACUTE STUDY (15 DAYS STUDY)

The rats were induced with diabetes and stabilized for at least two days and then single dose of standard and test drugs were administered for 15 days consecutively and the results are discussed below.

Table 1.4: Plasma Glucose Levels (Subacute study)

GROUPS	TIME IN DAYS			
	1st day	5th day	10th day	15th day
Control	480.83±4.621	475.00±3.864	478.50±3.844	473.83±4.238
Standard	486.16±5.952	227.33±7.521 53.29%↓	163.66±8.256 *** 66.4%↓	96.33±4.793 *** 80.24%↓
GSME (200 mg/kg)	504.50±13.613	322.16±15.59 36.11%↓	190.16±8.960 ** 62.30%↓	135.00±3.577 ** 73.21%↓
GSWE (100 mg/kg)	544.16±13.08	322.16±5.504 40.8%↓	265.50±4.745 ** 51.28%↓	186.66±6.800 ** 65.8%↓
GSWE (200 mg/kg)	516.83±10.02	360.16±6.959 30.23%↓	215.16±8.010 ** 58.33%↓	122.16±2.120 ** 76.35%↓
GSWE (400 mg/kg)	541.00±14.233	256.33±6.897 52.7%↓	149.50±3.403 *** 72.4%↓	108.66±2.740 *** 80.03%↓

GSME- Gomphrena serrata Methanolic extract, GSWE – G. serrata water extract
Values are expressed as Mean ± SEM
**** p< 0.001, ** p< 0.01, * p < 0.05, n = 6 animals in each group.*

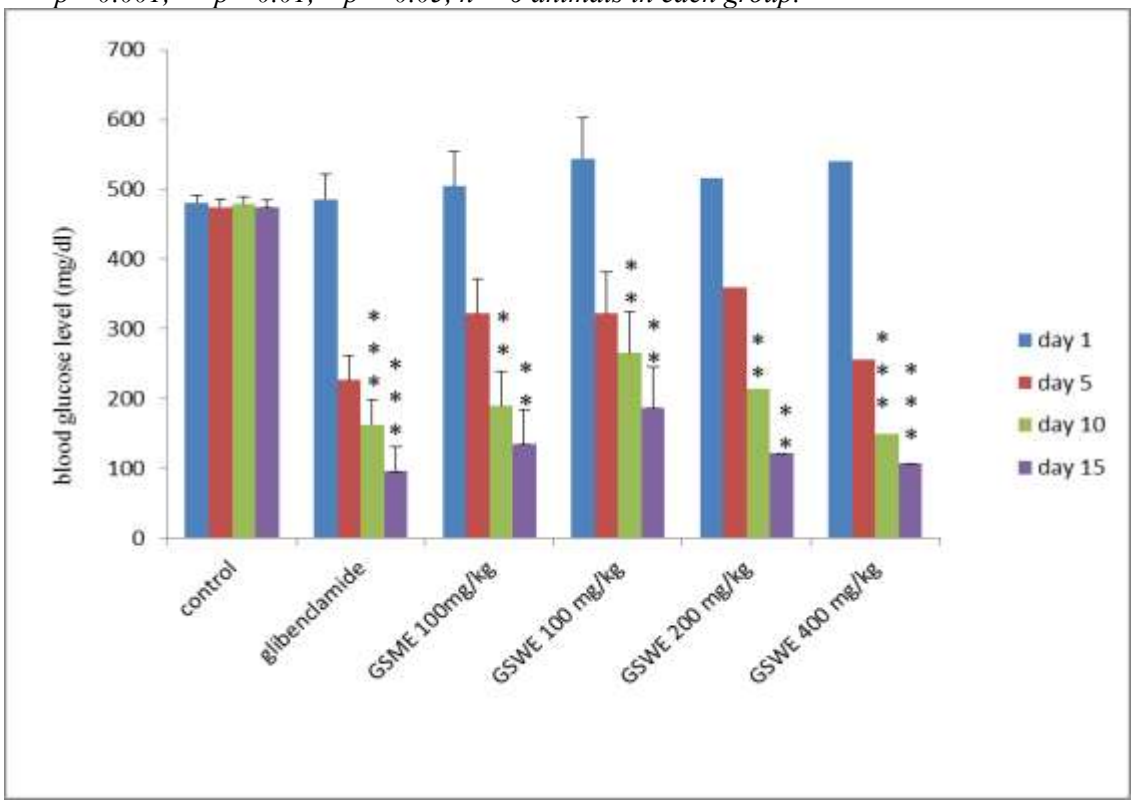

Figure 1.6: Graph depicting plasma glucose levels (Sub acute study)

From the different extracts of *Gomphrena serrata* plant GSME (200mg/kg) GSWE(200 & 400mg/kg) showed significant reduction in plasma glucose level as compared to Control on 5th, 10th & 15th day of the study as depicted in the Figure 5.6. The aqueous extract at 400 mg/kg showed a marked reduction in the blood glucose level *i.e.* 80.03 % which is comparable to the standard (80.24%). Hence, it indicates that *G. serrata* extracts possesses significant Antidiabetic potential which supports its traditional use as

mentioned in the literature.

ANTIOXIDANT ACTIVITY

DPPH ASSAY

The extracts were tested for their free radical scavenging activity against Di-Phenyl Picryl Hydrazyl and compared with ascorbic acid. The results are given below.

Table 1.5: % Inhibition values of Methanolic and Water extract as compared to Standard Ascorbic acid

Conc. (mcg/ml)	*% inhibition (Ascorbic acid)*	% inhibition (GSME)	% inhibition (GSWE)
0	*0*	0	0
20	*54.9*	24.49	22.58
40	*62.91*	31.34	28.8
60	*86.74*	42.88	46.12
80	*85.51*	52.87	69.34
100	*89.6*	76.6	84.82

GSME- *Gomphrena serrata* Methanolic extract, GSWE – *G. serrata* water extract

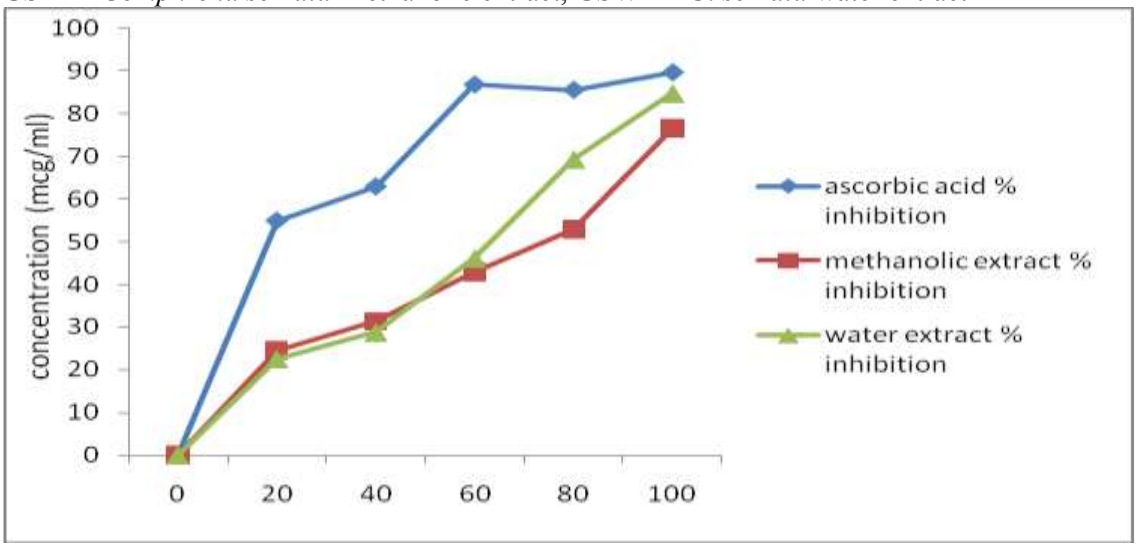

Figure 1.7: % Inhibition values of Water and Methanolic extract of *G. serrata* as compared to the standard Ascorbic Acid.

Water and methanolic extract exhibited free radical scavenging activity. At higher concentrations, the water extract shows significant anti-oxidant activity when compared to the standard i.e. ascorbic acid.

Table 1.6: MDA and GSH levels

Groups	MDA levels (µmol/mg of protein)	GSH levels (mmol/L)
Toxic Control	68.29 ± 12.350	3.67 ± 0.525
Control	156.65 ± 7.327	6.57 ± 0.024
Standard	27.38 ± 5.123***	5.22 ± 0.101***
GSWE(100mg/kg)	59.55 ± 3.899	2.02 ± 0.045
GSWE(200mg/kg)	50.37 ± 2.456	2.789 ± 0.105*
GSWE(400mg/kg)	42.86 ± 2.146**	3.47 ± 0.092*
GSME(200mg/kg)	62.46 ± 3.84	2.08 ± 0.035

GSME- *Gomphrena serrata* Methanolic extract, GSWE – *G. serrata* water extract
Values are expressed as Mean ± SEM.
*** p < 0.001, ** p < 0.01, * p < 0.05, n = 6 animals in each group.

MDA Levels

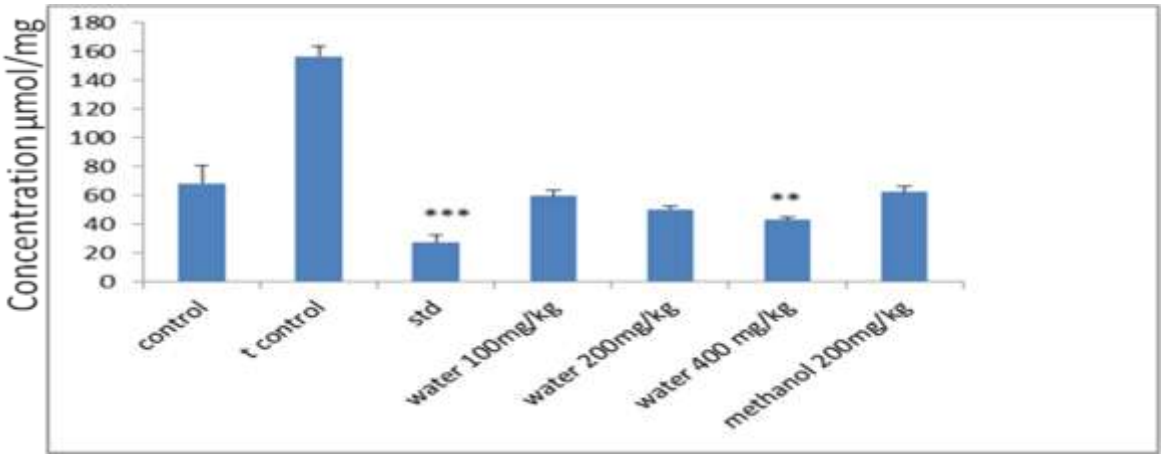

Figure 1.8: Figure depicting MDA levels

A significant decrease in MDA levels was observed with GSWE (400mg/kg), when compared to the standard group as depicted in the Figure 5.9. GSWE was found to exhibit significant antioxidant activity.

GSH LEVELS

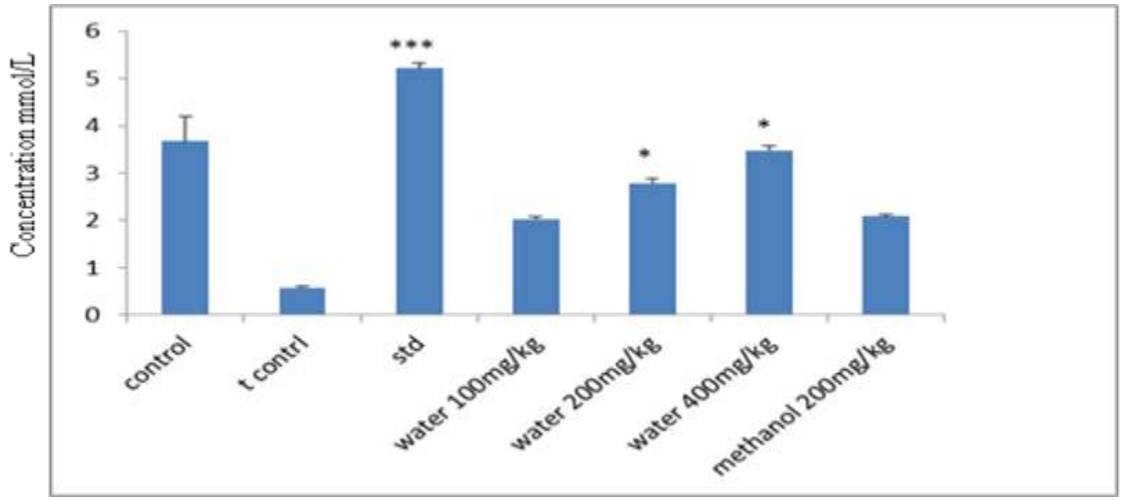

Figure 1.9: Figure depicting GSH levels

A significant increase in GSH levels was observed with GSWE (200mg/kg, & 400 mg/kg) when compared to the standard group as depicted in the Figure 5.10. GSWE (100mg/kg), was found to exhibit significant antioxidant activity.

CONCLUSION

Gomphrena serrata commonly known as "Neervada vallie", belongs to the family Amaranthaceae. This plant is mainly distributed in hilly areas during rainy to winter but doesn't survive in extreme winters.

Microscopic study revealed the presence of numerous rosette shaped calcium oxalate crystals and pitted xylem vessels were one of the characteristic features which are helpful for the standardisation and authentication of the plant.

This plant was chosen for its Antidiabetic & anti-oxidant activity because it was claimed to possess its use as an Antidiabetic in folklore medicine.

In oral acute toxicity test, method described in OECD-420 guidelines was used. This method consumed a fewer animals than conventional method. Mortality, signs of toxicity and abnormalities were observed during the experimental period. The estimated LD_{50} of GSE was more than 2000 mg/kg, po since this dose failed to produce any clinical signs of toxicities such as convulsion, hyperactivity, sedation, respiratory depression and loss of wrighting reflex. This study indicated that GSE was practically non-toxic or non-lethal.

In Antidiabetic testing of the extract of *G. serrata* plant, experimental models used were OGTT (1 day) and Subacute (15 days) study were used. The results of this study indicated that GSWE (200mg/kg & 400 mg/kg) markedly exhibited a dose related Antidiabetic activity. The potency of GSWE (400 mg/kg, p.o) was comparable to the reference standard (Gliblenclamide). The GSE clearly demonstrated Antidiabetic activity in all experimental animal models used in this study. Therefore, these results implied that the GSE possesses Antidiabetic activity.

In the Antioxidant activity of the extract of *G.serrata* plant, experimental models used were DPPH assay, and MDA & GSH levels. The results of this study indicated that GSME & GSWE both exhibited significant free radical scavenging activity thus, lowering the free radical levels in DPPH solution. The antioxidant activity of GSWE was comparable to the standard (Ascorbic acid).

MDA levels in a tissue can serve as markers of the toxicity and an increase in the MDA levels shows damaged tissues, the GSWE and GSME of *G. serrata* decreased the level of MDA from the tissue which had been damaged previously by streptozotocin. The GSWE (400mg/kg & 200 mg/kg) lowered the MDA levels significantly and was comparable to the standard (Glibenclamide).

Similarly, the GSH levels can also serve as markers of tissue toxicity, their decreased levels indicate toxicity in the tissue, and the GSWE and GSME of *G. serrata* increased the levels of GSH in the tissue which had been damaged by streptozotocin. GSWE (400 mg/kg &200 mg/kg) caused significant increase in the GSH levels which was comparable to the standard (Glibenclamide).

Therefore, these results implied that *Gomphrena serrata* plant extract possesses significant Antidiabetic and antioxidant

potential and hence, it may serve as a safe and effective herbal therapy for the treatment of diabetes.

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