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ANTIDIABETIC EFFICACY OF MOLLUGO OPPOSITIFOLIA LINN. EXTRACT- AN ANIMAL MODEL STUDY.

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ABSTRACT

Diabetes Mellitus is a metabolic syndrome characterized by hyperglycemia resulting in complications affecting the nerves, kidneys, eyes and micro and macrovascular structures. This metabolic disease causes disturbance in carbohydrate, protein and fat metabolisms and primarily due to the lack of insulin secretion and/or resistance to insulin. Diabetes and its prevalence is growing alarmingly due to the aging, urbanization, sedentary food habits, obesity and lack of physical activity. A wide number of traditional medicinal plants are used to treat diabetes mellitus. There are more than 400 plant species which have scientifically proved for their antidiabetic activity. **Objective**: The present study was aimed to evaluate the antidiabetic potential of *Mollugo oppositifolia* extract on alloxan induced diabetic rats and compare their efficacy with the standard drug, Glibenclamide. **Experimental design**: An animal model study was carried out by using albino Wistar rats. The rats were divided into four groups Group I: Normal healthy control; Group II: Disease control-alloxan (150mg/kg-bw) induced diabetic rats. Group III: Diabetes induced rats treated with *Mollugo oppositifolia* extract (400 mg/kg-bw); Group IV; Drug control animals-diabetes induced rats treated with Glibenclamide (10 mg/kg-bw). After the experimental period of 35 days, the blood and the liver tissue samples were collected and analyzed. **Results obtained**: Administration of alloxan induced diabetes in rats and showed a significant alterations in (1) biochemical parameters such as blood glucose, serum insulin, glycosylated haemoglobin, serum protein, liver tissue protein, serum urea, serum creatinine, liver glycogen, (2) Glycogen metabolizing enzymes: liver glucokinase, glycogen synthase, glycogen phosphorylase, liver glucose-6-phosphatase, liver glucose-6-phosphate dehydrogenase, (3) Lipid profiles: total lipids,

cholesterol, triglycerides, phospholipids, LDL cholesterol, VLDL cholesterol, HDL cholesterol in serum, (4) Liver marker enzymes: serum aspartate aminotransaminase (AST), serum alanine aminotransaminase (ALT), alkaline phosphatase (ALP), (5) Biomarker antioxidants: reduced glutathione and lipid peroxidation and (6) Antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Treatment with plant extract normalized the adverse effects of diabetic condition. **Conclusion**: Treatment of diabetic rats with *Mollugo oppositifolia* extract brought back the normalization in all the parameters investigated, which indicate the antihyperglycemic and antidiabetic efficacy of *Mollugo oppositifolia* extract. The efficacy was comparable with the standard drug, Glibenclamide. In most of the parameters the performance of plant extract was better than the standard drug.

KEY WORDS: Antidiabetic activity, *Mollugo oppositifolia* extract, Glibenclamide, biochemical parameters.

INTRODUCTION

Diabetes is the major health problem in India. India with 180 million population, 15 million are reported to be with Type 2 Diabetes Mellitus. According to whose estimation for prevalence of diabetes, it is in the fifth position worldwide and by the year 2030, it will be expected to occupy second place. Type 2 Diabetes Mellitus comprises an array of metabolic dysfunctions associated with an increased incidence of micro and macrovascular complications, which are the major causes of morbidity and mortality.

Diabetes is a complex group of disease with a variety of causes in which the body cannot regulate the sugar (glucose) in the blood. The blood delivers glucose to provide with energy to perform a person's daily activities. The digestive tract breaks down starches, carbohydrates and other complex sugars found in foods into glucose, a simple form of sugar and the glucose is then released into the bloodstream. In a healthy person the blood glucose level is regulated by several hormones, primarily by insulin. The pancreas contains clusters of cells called islets of Langerhans. β- cells in the Langerhans biosynthesis insulin and release it into the blood stream. People suffered from diabetes either do not produce enough insulin (Type I diabetes) or cannot use insulin properly (type 2 diabetes), or both (which occurs with several forms of diabetes). Insulin is the key enzyme which converts the excess amount of glucose into glycogen and stored in liver and muscles. People with diabetes have high blood glucose level, which is also called hyperglycemia [1]. Hyperglycemia is the most important factor at the

onset and its uncontrolled condition leads to various biochemical disorders such as hyperlipidemia and oxidative stress. The biochemical derangements in both Type-1 and Type-2 diabetes further leads to various vascular complications, such as nephropathy, retinopathy, neuropathy and atherosclerosis.

Traditional medicines derived from medicinal plants are used by about 60% of the world populations. In the last few years, there has been an exponential growth in the field of research in herbal medicines and the herbal drugs are gaining popularity both in developed and developing countries because of their natural original, efficacy and lesser side effects. A wide number of traditional medicinal plants are being used to treat diabetes mellitus. Several beneficial role such as correcting altered carbohydrate metabolism, maintaining integrity and function of β-cells, insulin secreting activity, enhancing glucose up take and utilization and antioxidant properties present in traditional medicinal plant and their constituents offer exciting opportunity to develop them into novel therapeutics.

Mollugo oppositifolia Linn. [synonymes: *Glinus oppositifolius* (Linn.) Aug. DC., *Mollugo spergula* Linn., *Mollugo subserrata* Blanco] belongs to the Botanical Family: Molluginaceae/ Aizoaceae. It is a prostrating annual herb growing in India, Sri lanka, Pakistan, Thailand, Bangladesh, tropical Africa and Australia. The common names in India: Hindi- Jima, Sanskrit- Kapithapatra and Tamil-Thora Pooondu. Common names in other countries: English- Bitter Leaf, China- Jia fan lu, Thailand – Pak khee khuang, Bangaladesh - Gima shak, Phillipines – Sarsalida and Australia – Slender Carpet weed[2].

In India, *Mollugo oppositifolia* is traditionally used in the treatment of inflammation and various pains. The plant is used as an aperients, appetizer, uterus stimulant and antiseptic. It is used in itch and other skin diseases and for suppression of the lochia. Warmed herb with castor oil is a good cure for earache. Some tribes in south India use this plant to cure liver diseases. Evaluation of the anti-inflammatory and analgesic activity of this plant is reported, but there is no scientific investigation so far been documented for its antidiabetic activity. Hence the plant is selected to investigate its antidiabetic activity in an animal model study by using albino Wistar rats. In the present study 80% methanolic extract of *Mollugo oppositifolia extract* was used as plant drug and evaluate its antidiabetic potential and its efficacy was compared with a known standard antidiabetic drug, Glibenclamide.

MATERIALS AND METHODS

Preparation of Plant Extract

Mollugo oppositifolia was collected from the Sathuragiri Hills Area, Virudhunagar district of Tamil Nadu and the plant was authenticated by the eminent professors in the department of Botany. The voucher specimen (No. 061) was deposited in the department. 1000 gm of *Mollugo oppositifolia* dried plant (areal portion) was taken and extracted with 80% aqueous methanol. To one part of the plant material, six parts of 80% aqueous methanol was added in a suitable round bottom flask fitted with condenser and extracted for 3 h. The extract was filtered and the filtrate was evaporated to dryness in a Rotary flash vacuum evaporator at low temperature. Paste from of the extract obtained was further processed to make into a dry powder.

Chemicals and Reagents

Alloxan monohydrate and colchicine (AR-grade) were purchased from Sigma Aldrich Co., USA. Glibenclamide (2.5 mg) tablet (Aventis Pharma Ltd.) was purchased from a local medical shop. Other analytical grade chemicals, reagents and solvents were purchased from HiMedia Laboratories, Mumbai, India.

Phytochemical study

The extract of *Mollugo oppositifolia* was subjected to analysis for preliminary phytochemicals such as alkaloids, flavonoids, terpenenoids, steroids, and tannins according to the standard methods.[3,4,5,6,7]

1. S**aponins**: 300 mg of extract was boiled with 5 ml water for two minutes. The mixture was cooled and mixed vigorously and left for three minutes. The formation of frothing indicated the presence of saponins.
2. **Tannins**: To an aliquot of the extract, sodium chloride is added to make to 2% strength. Then it is filtered and mixed with 1% gelatin solution. Precipitation indicated the presence of tannins.
3. **Triterpenes**: 300 mg of extract was mixed with 5 ml chloroform and warmed for 30 minutes. The chloroform solution was then treated with a small volume of concentrated sulphuric acid and mixed properly. The appearance of red color indicated the presence of triterpenes.

4. **Alkaloids**: 300 mg of extract was digested with 2 M HCl. Acidic filtrate was mixed with amyl alcohol at room temperature, and examined the alcoholic layer for the pink colour which indicated the presence of alkaloids.
5. **Flavonoids**: The presence of flavonoids was determined using 1% aluminium chloride solution in methanol, concentrated HCl, magnesium turnins, and potassium hydroxide solution.
6. **Glycosides:** 500 mg of extract was dissolved in 2.0 ml of glacial acetic acid containing one drop of $FeCl_3$ Solution. This was then under laid with 1.0 ml of concentrated H_2SO_4. A brown ring appeared at the interface indicated the presence of glycosides.
7. **Phenols:** 500 mg of plant extract was dissolved in 2 ml of ethanol and treated with few drops of neutral ferric chloride solution 5%, intense colour developed indicated the presence of phenols.
8. **Anthroquinones:** Borntreger's test was used for the detection of anthroquinones. 5 g of plant extract was shaken with 10 ml of benzene. This was filtered and 5.0 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of violet colour in the ammoniacal (lower) phase indicated the presence of free hydroxyl anthroquinones.
9. **Terpenoids:** 500 mg of plant extract was dissolved in 2 ml of ethanol and taken in a test tube and then added few pieces of tin plus 3 drops of thionyl chloride, violet or purple colour developed indicated the presence of terpenoids.
10. **Steriods:** (Liebermann Burchard reaction): 200 mg of plant extract was dissolved in 10 ml of chloroform and filtered. 2 ml of filtrate + 2 ml of acetic anhydride + conc. H_2SO_4. Appearance of blue green ring indicated the presence of steroids.

Experimental Animals

Healthy adult Wister strain of male albino rats, about three months old and weighing 150-200g were purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai. The animals were allowed to acclimatize under laboratory conditions 25 ± 2^0C and RH 55±5% with a 12:12 light and dark cycle in polypropylene cages for a period of 5 days prior to the experiment. Animals were fed with standard rat chow pellet and provided water *ad libitum.* All procedures involving laboratory animal use were in accordance to the Institute Animal Ethics Committee regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental Design to Study Antidiabetic Activity

The rats were divided into 4 groups and each group comprising of 6 rats.

1. Group I: Normal Healthy Control animals (fed with normal chow and water).
2. Group II: Diabetes induced control animals treated with alloxan monohydrate in sterile Saline (150 mg/kg-bw by i.p. injection, in a single dose) [8].
3. Group III: Diabetes induced rats treated with *Mollugo oppositifolia* leaf extract (400 mg/kg- bw/day orally for 35 days)
4. Group IV: Drug control-animals, diabetes induced rats treated with Glibenclamide (10mg/kg- bw/day orally for 35 days) [9].

After the experimental period, the rats were sacrificed by cervical decapitation. Blood was collected; liver and pancreas were dissected out and washed in ice-cold saline. Liver tissues were homogenized, in 0.1 M phosphate buffer, pH 7.4 and used for various analyses.

Induction of Diabetes

Diabetes was induced in overnight fasted rats by single intraperitoneal injection of alloxan (150 mg/kg-bw) dissolved in freshly prepared saline[10]. Since, alloxan is capable of inducing fatal hypoglycemia due to massive pancreatic insulin deficiency and persistent hyperglycemia, the rats will be provided with 10% glucose solution after 6 h of alloxan administration for the next 24 h to overcome drug induced hypoglycemia. After 48 h, rats with moderate diabetes (i.e. fasting blood glucose concentration, >250 mg/dl) were selected for further studies.

Acute toxicity study

The acute oral toxicity study of selected plant extracts was carried out as per method of the acute toxicity study (OECD guidelines 425, 2001) using albino Wistar rats. Adult and healthy rats (100-150 g) were selected and divided into three groups of 3 animals each. Animals were kept fasting for overnight and provide only water. The *Mollugo oppositifolia* extract was suspended in 1% acacia solution and it was administered once orally via gastric intubation at stepwise doses from 100 mg to 2000 mg/kg-bw. Lethality and abnormal clinical signs were observed up to 72 h in the tested animals [11].

Statistical Analysis

The data of results obtained were subjected to and expressed as mean ± SD. The data were statically analyzed by one way analysis of various (ANOVA) and to compare the means of

the studied groups with *post hoc* Duncan multiple range tests at 5% and 1% for those results where significant difference was indicated. Values are expressed as mean ± S.E.M (n=6) *p<0.05, **p<0.01, ***p<0.001 Compared with control.

BIOCHEMICAL STUDIES

Collection of Blood for Estimation of Biochemical Parameters

The blood was collected from the rat tail vein for the estimation of blood sugar by using glucometer and blood glucose test-strips, supplied by Ascensia Entrust of Bayer Health Care. For estimation of other biochemical parameters, blood was drawn from the retro-orbital plexus of the rats (fasted for 12 h), into sterilize eppendorf tubes. The blood samples were allowed to coagulate for 30 min at room temperature and then they were centrifuged at 3000 rpm for 10 min. The serum used as sample, should be free from haemolysis and must be separated from the clot promptly. The resulting upper serum layer was collected in the properly cleaned, dried, and labeled eppendorf tubes and they were stored at -8°C for further analyses.

Biochemical Analysis

Blood Glucose

Fasting blood glucose was estimated by using a commercial glucometer and test strips (Accucheck Sensor test meter)

Estimation of Serum Insulin

Serum insulin levels were assayed using a standard Mercodia Rat Insulin ELISA enzyme immunoassay kit from Mercodia, Sweden (Cat. No. 10-1124-01). Quantitative estimation of serum insulin was done by rat insulin ELISA Kit (Mercodia). The sensitivity of the kit is 0.025µg/l. It is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation, insulin in the sample reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to micro titration well. A simple washing step removes unbound enzyme loaded antibody. The bound conjugate was detected by reaction with 3,3',5,5'- tetramethylbenzidine. The reaction was stopped by adding acid and read using a spectrophotometer at 450 nm. Plasma - Insulin levels is expressed as ng/ml.

Estimation of Glycosylated Haemoglobin (HbA1c)

Isolation of Erythrocyte Membrane [12] and Preparation of Hemolysate[13].

Blood was collected with EDTA as anticoagulant. Plasma was separated by centrifugation at 1500 rpm for 15 min. The packed cells were washed well with isotonic saline solution. After washing, the packed cells were lysed by suspending them in hypotonic Tris-HCl buffer for one hr. The lysed cells were centrifuged at 15,000 rpm for 30 min. The supernatant was subsequently used as hemolysate, which was used for the analyses.

Glycosylated hemoglobin content was estimated by the method of Nayak and Pattabiraman [14]. To 0.2 m1 of hemolysate, 1.8 ml of 0.3M oxalic acid was added and the mixture was hydrolyzed for 2 hr, cooled and added 1.0 ml of 40% TCA. After centrifugation at 1400 rpm for 20 min, 1.5 m1 of the supernatant was treated with 0.5 m1 of 0.05M- thiobarbituric acid. After incubated at 37°C for 40 min the colour developed was read at 443 nm. Standard fructose in the concentration of 10-40 µg was processed similarly. Values were expressed as glycosylated hemoglobin present in l00 ml of blood.

Estimation of Liver Glycogen [15]

The samples of liver tissue in test tubes were subjected to alkali digestion with 5 m1 of 30% KOH in a boiling water bath for 20 min. The tubes were cooled and 3 m1 of ethanol and a drop of ammonium acetate were added. The tubes were then placed in a freezer overnight to precipitate glycogen. The precipitated glycogen was collected after centrifugation at 3000 rpm for 10 min. The precipitate was washed twice with alcohol and dissolved in 3 m1 of water, 4 m1 of anthrone reagent (0.2% anthrone in 95% sulphuric acid) was added to the tubes heated in a boiling water bath for 20 min. The green colour developed was read at 640 nm and compared with standard glucose solutions (10 –100 µg/ml).

Glycogen concentration was calculated by using the following formula:

(OD of the sample x volume of extract / OD of the standard x g of tissue) x concentration of standard x 100 x 0.9, where 0.9 is the factor for converting glucose value to glycogen

Assay of Glucokinase

Glucokinase (ATP: D-hexoses-6-phosphotransferases) was assayed by the method of Brandstrup *et al.*[16]. 1 ml and 2 m1 of 0.2M Tris buffer (pH 7.4) was pipette out into test tube and control test tube respectively. Then l ml of substrate was mixed with the buffer taken in the test tubes. To this, 1 ml of liver homogenate was mixed with the substrate taken in the

'test' test tube. Then 0.5 ml 0.1% magnesium chloride, 0.5 m1 of 0.5% ATP solution and 0.5 m1 of 0.1% sodium fluoride were added and acclimatized at 37°C for 3 min. Then the test tubes were incubated at 37°C for 30 min. The enzymatic reaction was arrested by the addition 2 m1 of alkaline copper sulphate solution and test tubes were heated in the boiling water bath for 8 min. Then 2 ml of phosphomolybdic acid was added and the developed colour is read at 620 nm against control. From the absorbance, the activity of glucokinase was calculated using standard graph. The activity of glucokinase is expressed as micromole of glucose phosphorylated / ml / min.

Assay of Glycogen Synthase

Liver glycogen synthase activity was assayed by the modified method of Gold and Sega l[17]. 1.0 g of liver tissue was homogenized in three volumes of 0.1 M glycylglycine buffer (pH 7.4) at 0ºC for 30 sec, and the homogenate was centrifuged at 2ºC at 8,000 rpm for 10 min. The resultant supernatant solution was held at 0ºC, and the first assay for the glycogen synthase activity was made 30 min after removal of the liver from the animal. Enzyme activity was determined by measuring the amount of radioactivity incorporated into glycogen from "4C-labeled UDP-glucose. The assay mixture contained 20 mg of rat liver glycogen prepared as follows: 1 Mmol of UDP-glucose, 0.02 mCi (38,600 counts / min) of UDP-glucose-U-14C (final specific activity = 0.02 mCi/mmol), 264 umol of glycylglycine, pH 7.4, were added with 4 Mmol of glucose-6-phosphate in a volume of 3.8 ml. The reaction was initiated by adding to the assay mixture with 0.2 ml of the supernatant solution of the liver homogenate. The assay incubations were made in screw-capped 40-ml glass centrifuge tubes at 37ºC for 15 min with gentle shaking. The reaction was stopped by the addition of 6 ml of hot 30% (wt/vol) KOH to each tube, and the mixture was heated for 10 min at 100ºC. Saturated Na_2SO_4 (0.5 ml) was added to the mixture, and glycogen was precipitated with 1.1 vol of absolute ethanol. The mixture was again heated to boiling, cooled for 30 min in an ice bath, and centrifuged. The glycogen pellet was dissolved in 5 ml of distilled water and precipitated with 6.25 ml of absolute ethanol. The mixture was heated, cooled, and centrifuged as before. The glycogen pellet was dissolved in distilled water to a final volume of 5 ml. 1ml sample of the glycogen solution was added to 10 ml of Phase Combining System solubilizer and counted. Another 0.02-ml sample was assayed for glycogen content. Enzyme activities were expressed as nanomoles of glucose transferred from uridine diphosphate glucose (UDPG) to glycogen per minute per gram of liver (dry weight) under the conditions of the assay corrected for the 2 to 5% loss of glycogen in the isolation procedure.

Assay of Glucose- 6- Phosphatase [18]

1.0 ml of reaction mixture containing 0.3 m1 of 0.1 M citrate buffer (pH 6.5), 0.5 m1of substrate (0.01M glucose-6-phosphate in distilled water) and 0.2 m1 of enzyme extract was incubated at 37°C for 1 h. The reaction was terminated by adding 1 ml of 10% TCA. The suspension was centrifuged and the phosphorous content of the supernatant was assayed by colorimeter.

Assay of Glucose-6- Phosphatase Dehydrogenase [19]

The reaction mixture, containing 0.1 ml each of Tris HCl buffer, 0.1M magnesium chloride, 2mM $NADP^+$ and 0.l ml of homogenate. To this 0.5 m1 of water was added and allowed to stand at room temperature for 10 min. The reaction was initiated by the addition of 0.2 m1 of 6mM glucose-6-phosphate. The change in OD was read at an interval of 30 sec for 3 min at 340 nm. The enzyme activity is expressed as micromole of NADP oxidized/ min/ mg protein.

Estimation of Protein in Serum and Liver Tissue

Protein content was estimated by Biuret reagent [20]**.** In a test tube 0.5 ml of plasma was mixed with 1.5 ml of 0.85% sodium chloride solution and 8 ml of Biuret reagent was added to it. To prepare standard, 1 ml of standard protein (Bovine serum albumin) was mixed with 1 ml of 0.85% sodium chloride solution. Then 8 ml of Biuret reagent was added to this. To prepare the blank 2 ml of 0.85% sodium chloride solution was mixed with 8 ml of Biuret reagent. All the test tubes were shaken well and allowed to stand for 30 min. Read the test and standard samples against blank at 520 nm.

Extraction of Total Lipids [21]

50 mg of wet tissue was homogenized in 10 ml of chloroform- methanol mixture using a glass homogenizer, filtered through What man No.1 filter paper and to this 2 ml of 0.9% NaCl solution was added. This reaction mixture was shaken well and transferred into a separating funnel and it was allowed to stand overnight at 4°C. A clear biphasic layer was formed and lower phase containing all the lipids. The lipid layer was separated and the volume was made up to 10 ml by the addition of chloroform. This was transferred to a 50 ml beaker and the solvent was allowed to evaporate at 50-60°C for 5 h. Then 5 ml of concentrated $H_2SO_4$was added to it, mixed well, placed in a boiling bath for 10 min and was then cooled to room temperature. 0.2 ml of this was taken in a test tube and 5 ml of phosphovanillin reagent was added, mixed well and allowed to stand for 30 min. Standard

was prepared by mixing 0.2 ml of standard cholesterol and 5 ml of phosphovanillin reagent, mixed well and allowed to stand for 30 min. Blank was prepared by adding 0.2 ml of chloroform to 5 ml of phosphovanillin reagent. Read test samples and standard against blank at 520 nm. The content of total lipids was expressed as µg/mg.

Estimation of Serum Cholesterol [22]

To 0.1ml of serum 10 ml of ferric chloride reagent was added in a test tube. Mixed well and kept for 10 min at room temperature. It was then centrifuged at 3000 rpm for 30 min. 5 ml of the supernatant was pipette out into a test tube and 3 ml of concentrated sulphuric acid was added and mixed well. To prepare standard, 10 ml of working standard (200 mg of cholesterol dissolved in 10 ml of ferric chloride reagent and made up to 100 ml with glacial acetic acid) was mixed with 0.1 ml of sodium chloride and kept for 10 min and centrifuged. 5 ml of supernatant was taken and to this 3 ml of concentrated sulphuric acid was added. Both the tubes were kept for 30 min at room temperature. To prepare the blank, 5 ml of ferric chloride solution was mixed with 3 ml of concentrated sulphuric acid. This was kept for 30 min. Read the test samples and standard against blank at 560 nm.

Estimation of Triglycerol (TGL) [23]

0.1 ml of serum was added to 4 ml of isopropanol, mixed well and to this 0.4 g of alumina was added and shaken well for 15 min. Centrifuged at 2000 rpm for 10 min and then 2 ml of the supernatant was transferred to appropriately labeled tubes. The tubes were placed in a water bath at 65° C for 15 min for saponification after adding 0.6 ml of the saponification reagent (0.5g of potassium hydroxide dissolved in 6 ml of distilled water and then 44 ml of isopropanol was added to it). After cooling down to room temperature 1 ml of sodium metaperiodate regent followed by 0.5 ml of acetyl acetone reagent were added. After mixing, the tubes were incubated in a water bath at 65°C for 30 min. The contents were cooled and read at 430 nm. Against appropriate blank. Tripalmitin (10 mg of tripalmitin was dissolved in 100 ml of isopropanol) was used as standard.

Assay of HDL Cholesterol [24]

To 1.0 ml of lipid extract, 0.18 ml of heparin- manganese chloride reagent (3.167g of manganese chloride was added to 1 ml solution of heparin containing 20,000 units. The mixture was made up to 8 ml with distilled water) was added and mixed. This was allowed to stand in an ice bath for 30 min and then centrifuged in a refrigerated centrifuge at 2500 rpm

for 30 min. The supernatant contained HDL fraction. Aliquots of the supernatant were estimated for LDL and HDL cholesterol and phospholipids.

Aggregation of VLDL

1 ml of lipid extract was added to 0.15 ml of SDS solution (sodium dodecyl sulphate - 10% in 0.15 M NaOH (pH-9). The contents were mixed well and incubated at 37°C for 2 h. The reaction mixture was centrifuged in a refrigerated centrifuge at 10,000 rpm for 30 min. VLDL aggregated as a pellicle at the top. The supernatant was a mixture containing HDL and LDL fractions. The fractions of lipoproteins were assayed after heparin manganese chloride and SDS precipitation. The values are expressed in mg/dl plasma. After precipitation the cholesterol levels in supernatant was measured to get HDL cholesterol. SDS precipitated VLDL and the cholesterol content in the supernatant was measured for HDL cholesterol, LDL cholesterol and VLDL cholesterol.

LDL cholesterol = Total serum cholesterol - Total serum TGL- HDL Cholesterol / 5

VLDL =Total Serum TGL / 5

Estimation of free Fatty Acid [25]

0.1 ml of lipid extract was evaporated to dryness. 0.1 ml of phosphate buffer, 6.0 ml of extraction solvent (chloroform: methanol - 5:1) and 2.5 ml of copper reagent were added. All the tubes were shaken vigorously and then 200 mg of activated silicic acid was added and left aside for 30 min. The tubes were centrifuged and 3 ml of the upper layer was transferred to another tube containing 0.5 ml of diphenylcarbazide solution and mixed carefully. The absorbance was read at 550 nm. Palmitic acid was used as standard

Estimation of serum phospholipids [26]

0.1 ml of serum sample was digested with 0.2 m1 of perchloric acid over a sand bath. Digestion was continued till it was colorless. The liberated phosphorus was estimated. 4.3 ml of deionised water was added to the digested sample followed by 0.5 ml molybdic acid. After 10 min, 0.2 ml of ANSA was added. Tubes were well shaken and kept aside for 20 min. Blue colour developed was read at 620 nm.

The total phospholipids were estimated by multiplying the value of Pi by 25 and expressed as mg/g wet tissue.

Estimation of Blood Urea

Estimation of urea in serum was carried out by modified method using diacetyl monoxime [27]**.** Preparation of acid reagent: 1 g of ferric chloride hexahydrate was mixed with 50 ml of 40% orthophosphoric acid. 0.3 ml of this reagent was mixed with 40 ml of 50% sulphuric acid. Preparation of color reagent: (A) Dissolve 0.4 g of diacetyl monoxime in 20 ml distilled water. (B) Dissolve 0.05 g of thiosemicarbazide in 20 ml of distilled water. Mix (A) and (B) 3.5 ml each and made up to 50 ml with distilled water. Preparation of urea standard: 3 mg of analytical grade urea was dissolved in distilled water. Preparation of test sample: 0.02 ml of serum in a test tube was mixed with 2 ml of distilled water, 2 ml of acid reagent, 2 ml of color reagent. Preparation of standard: 0.02 ml of standard solution in a test tube was mixed with 2 ml of distilled water, 2 ml of acid reagent and 2 ml of color reagent. Preparation of blank: 0.02 ml of distilled water in a test tube was mixed with 2 ml of distilled water, 2 ml of acid regent and 2 ml of color reagent. Mixed the contents in the test tubes thoroughly and kept them in water bath for 10 min. Cool down the tubes in ice cold water for 5 min and read at 520 nm against blank. The content of urea was expressed as mg/dl.

Estimation of Creatinine

The colorimetric determination of creatinine was done by modified method of the Jaffe reaction [28]. Preparation of test sample: 2 ml of serum in a centrifuge tube was added with 2 ml of 2/3N sulphuric acid and 2 ml of 10% sodium tungstate and centrifuged at 3000 rpm for 5 min. 3 ml of supernatant in test tube was mixed with 1 ml of 0.04N picric acid and 1 ml of 0.75N sodium hydroxide. Preparation of standard: 3 ml of standard solution (0.05 mg/dl) in a test tube was mixed with 1 ml of 0.04N picric acid, 1 ml of 0.75N sodium hydroxide. Preparation of blank: 3 ml of distilled water in a test tube was mixed with 1 ml of 0.04N picric acid, 1 ml of 0.75N sodium hydroxide. Mixed the contents in the test tubes thoroughly and waited for 15 min and read at 510 nm against blank. The content of creatinine was expressed as mg/dl.

Assay of Lipid Peroxidation (TBARS) [29]

To 0.1ml of tissue homogenate, 4 ml of 0.85N H_2SO_4 and 0.5 ml of 10% phosphotungstic acid were added and stirred well. The content was centrifuged at 5000 rpm for 10 min. The supernatant was discarded and the sediment was mixed with 2.0 ml of H_2SO_4 and 0.3 ml of 10% phosphotungstic acid. The mixture was centrifuged for 10 min. The sediment was suspended in 4.0 ml of distilled water and 1 ml of TBA reagent (mixture of equal volumes of

0.67% TBA aqueous solution and glacial acetic acid). The tube was kept in a boiling water bath for 1 hr, after cooling, 5 ml of butanol was added and the colour of the extract in the butanol phase was read at 532 nm. The reaction mixture without tissue homogenate was used as blank. The enzyme activity was expressed as nano moles/mg tissue protein.

Assay of Reduced Glutathione (GSH) [30]

To 0.5 ml of tissue homogenate, 20% TCA was added and precipitated. The contents were mixed well for complete precipitation of protein and centrifuged. To aliquots of clear supernatant, 2 ml of DTNB reagent (0.6mM DTNB in 0.2 M phosphate buffer, pH 8.0) was added and 0.2 M phosphate buffer was added to make a final volume of 4 ml. The absorbance was read at 412 nm against a blank containing TCA instead of sample. A series of standard solutions (prepared from 10 mg of reduced glutathione dissolved in 100 ml of water) were treated in a similar way to determine glutathione content. The amount of glutathione was expressed as nano moles of GSH oxidized/mg protein.

Estimation of aspartate transaminase (AST) [31]

The assay mixture containing 1 ml of substrate (1.33 g of aspartic acid and 15mg of keto glutarate dissolved in 100 ml of 0.1 M phosphate buffer (pH 7.5) containing 0.5mM NaOH) and 0.2 m1 of serum was incubated for 1 h at 37°C. To the control tubes serum was added after the reaction was arrested by the addition of 1ml of 0.02% DNPH. The tubes were kept at room temperature for 30 min added 0.5 m1 of 0.4 N NaOH and the colour developed was read at 540nm. The activity of AST was expressed as moles of pyruvate formed/min/mg of protein.

Estimation of Alanine Transaminase (ALT) [31]

The assay mixture containing 1 ml of substrate (l.78 g of alanine and 30 mg of keto glutarate were dissolved in 100 ml of phosphate buffer containing 0.5mM NaOH) and 0.2 ml of serum was incubated for 1 h at 37°C. To the control tubes serum was added after the reaction was arrested by the addition of 1ml of 0.02% DNPH. The tubes were kept at room temperature for 30 mm. added 0.5 ml of 0.4N NaOH and the colour developed was read at 540 nm. The activity of ALT was expressed as moles of pyruvate formed/min/mg of protein.

Estimation of Serum Alkaline Phosphatase (Alp) [31]

The reaction mixture containing 1.5 ml of 0.1M carbonate buffer (pH 10), 1 ml of 0.1M disodium phenyl phosphate, and 0.1 ml of 0.1M magnesium chloride and 0.l ml of serum was

incubated at 37°C for 15 min. The reaction was arrested by the addition of Folin's Ciocalteau phenol reagent Control tubes were also treated similarly but serum was added after the reaction was arrested with Folin's Ciocalteau phenol reagent. Finally 1 ml of 15% Sodium carbonate was added. The colour developed was read after 10 min at 640 nm. The activity of ALP was expressed as moles of phenol liberated/min/mg of protein.

Activity of Superoxide Dismutase [32]

To 0.1ml of tissue homogenate, 0.75 ml of ethanol and 0.15 ml of ice cold chloroform were added and centrifuged. The supernatant was taken and 0.5 ml of 0.6nM EDTA solution and 1 ml of buffer (0.1 M pH 10.2) were added and mixed well. The reaction was initiated by the addition of 0.5 ml of fresh epinephrine (1.8nM) and the increase in absorbance was measured at 480 nm. The reaction mixture without tissue homogenate was used as blank. The enzyme activity was expressed as U/mg of protein.

Assay of Catalase [33]

0.2 ml serum was incubated in 1.0 ml substrate (65 µmol per ml hydrogen peroxide in 60 mmol/l sodium-potassium phosphate buffer, pH 7.4) at 37ºC for 60 sec. Serum catalase activity is linear up to100 kU/l. If the catalase activity exceeded 100 kU/l the serum was diluted with the phosphate buffer (2 to 10-fold) and the assay was repeated. One unit catalase decomposes 1 µmol of hydrogen peroxide/1 min under these conditions. The enzymatic reaction was stopped with 1.0 ml of 32.4 mmol/l ammonium molybdate ((NH4) 6 Mo_7O_{24}.4 H_2O) and the yellow complex of molybdate and hydrogen peroxide was measured at 405 nm against blank 3. Serum catalase activity (kU/l) = A(sample) - A(blank 1) / A(blank 2) _ A(blank 3) X 271. Blank 1 contained 1 ml of substrate, 1 ml of molybdate and 0.2 ml of serum; blank 2 contained 1 ml of substrate, 1 ml of molybdate and 0.2 ml of buffer; blank 3 contained 1 ml of buffer, 1 ml of molybdate and 0.2 ml of buffer. The activity of catalase was expressed as µmoles of H_2O_2 consumed /min/mg protein

Assay of Glutathione Peroxidase [34]

The reaction mixture (consisted of 0.2ml each of 0.8M EDTA, 0.2M reduced glutathione, 10mM sodium azide, 2.5mM H_2O_2, 0.4 ml 0.2M phosphate buffer (pH 7.0) and 0.1ml tissue homogenate) was incubated at 37ºC at different time interval. The reaction was arrested by adding of 0.5 ml of 10% TCA and the tubes were centrifuged at 3000 rpm for 10 min. To 0.5ml of supernatant, 4ml of 3mM disodium hydrogen phosphate and 0.5ml of DNTB

solution were added and the colour developed was read at 420 nm immediately. The activity of glutathione peroxidase was expressed as µmoles of glutathione oxidized/min/mg protein.

Statistical Analysis

The data of results obtained were subjected to statistical analysis and expressed as mean ± SD. The data were statically analyzed by one way analysis of various (ANOVA) and to compare the means of the studied groups with *post hoc* Duncan multiple range tests at 5% and 1% for those results where significant difference was indicated. Values are expressed as mean ± S.E.M (n=6) *p<0.05, **p<0.01, ***p<0.001 Compared with control

RESULTS AND DISCUSSION

Diabetes is a growing health problem worldwide and now it is emerging as an epidemic problem. The management of diabetes is still a major challenge. Plants have always been a source of drugs for human ailments since time immemorial. The Indian traditional systems of medicine are replete with the use of plants for the management of diabetic conditions. Thus there is a great demand for research on natural products with anti-diabetic properties to evaluate the traditional claim. Numerous studies have confirmed the benefits of medicinal plants with anti-hyperglycemic effects in the management of diabetes mellitus.

The present study was aimed to evaluate the antidiabetic effect of *Mollugo oppositifolia* plant extract on alloxan induced rats and the efficacy was compared with a known antidiabetic drug, Glibenclamide.

Table- 1: Screening of phytochemicals in Mollugo oppositifolia extract.

Phytoconstituents*	Qualitative determination
Alkaloids	++
Flavonoids	+++
Glycosides	++
Phenolic compounds	+++
Phytosterols	+
Saponins	+
Tannins	++
Triterpenoids	+
Carbohydrates	++
Proteins	+

'+'Present,'-'Absent. *Values are means of triplicate determination.

Table-2: Effect of Mollugo oppositifolia extract on some biochemical parameters in alloxan induced diabetic rats.

Parameters	Group I	Group II	Group III	Group IV
Blood glucose (mg/dl)	81.65±1.45	276.35±3.67***	77.98±0.07*	83.15±1.05*
Serum insulin (ng/ml)	65.82±0.33	35.18±0.66***	71.92±0.98**	62.19±1.08*
Glycosylated haemoglobin (HbA_1C) (%)	3.95±0.03	8.16±0.05**	4.12±0.09*	3.90±0.05*
Serum protein (g/dl)	7.52±0.92	3.94±0.02**	6.76±0.05*	5.76±0.09*
Liver Tissue protein (mg/g)	11.75±1.90	7.81±0.75**	10.80±1.12	11.25±0.97
Serum urea (mg/dl)	30.86±0.95	62.92±2.75***	34.65±0.89**	33.96±1.20*
Serum creatinine (mg/dl)	0.74±0.01	1.84±0.02***	0.91±0.01*	0.76±0.02

Values are expressed as mean±S.D (n=6). Statistical significance: *p<0.05, **p<0.01, ***p<0.001 as compared with control group.

Table-3: Effect of Mollugo oppositifolia extract on Liver glycogen and glycogen metabolizing enzymes in alloxan induced diabetic rats.

Parameters	Group I	Group II	Group III	Group IV
Liver glycogen g/100 g	6.61±0.35	3.55±0.07***	5.95±0.35**	4.66±0.32**
Liver glucokinase	149.67±2.76	92.45±1.75***	99.80±1.55***	143.23±2.25*
glycogen synthase (µmol of UDP formed /mg protein/h	633.86±4.56	555.66±3.34***	625.35±4.85***	611.90±3.77***
glycogen phosphorylase (µmol of UDP formed/mg protein /h	475.86±4.91	612.11±4.54***	575.88±2.25***	586.22±3.11***
Liver glucose-6-phosphatase	9.5±0.07	20.95±0.05***	15.83±0.01**	8.55±0.07
Liver glucose-6-phosphate dehydrogenase	13.31±0.08	7.15±0.02***	9.20±0.03***	12.25±0.03

Values are expressed as mean±S.D (n=6). Statistical significance: *p<0.05, **p<0.01, ***p<0.001 as compared with control group.

Table-4: Effect of Mollugo oppositifolia extract on lipid profiles in alloxan induced diabetic rats.

Parameters	Group I	Group II	Group III	Group IV
Serum cholesterol (mg/dl)	85.53±1.32	170.43±2.23***	112.75±2.95***	84.5±1.33
Serum triglycerides (mg/dl)	100.54±1.43	125.44±2.65***	106.24±2.15**	91.34±1.90*
Total lipids (mg/g)	61.54±1.45	79.65±1.56**	88.70±1.89*	60.33±0.84
Serum phospholipids (mg/dl)	60.63±1.88	119.85±3.99***	81.76±1.90**	60.66±1.67
Serum LDL cholesterol	83.20±0.99	155.85±1.25***	98.33±1.55***	80.67±2.65

(mg/dl)				
Serum VLDL cholesterol (mg/dl)	7.23±0.75	16.86±1.27***	12.55±0.05***	12.25±0.06***
Serum HDL cholesterol (mg/dl)	72.35±1.87	43.45±0.35***	57.88±0.12**	65.43±1.35*

Values are expressed as mean±S.D (n=6). Statistical significance: *p<0.05, **p<0.01, ***p<0.001 as compared with control group.

Table-5: Effect of *Mollugo oppositifolia* extract on the activity of liver function enzymes and biomarker antioxidants in alloxan induced diabetic rats.

Parameters	Group I	Group II	Group III	Group IV
Aspartate aminotransferase (AST-IU/L)	72.6±1.34	262.95±11.87***	88.32±2.21**	93.25±2.44***
Alanine aminotransferase (ALT)-IU/L)	45.8±1.45	180.6±9.85***	38.2±0.32***	41.8±0.57**
Alkaline phosphatase (ALP-IU/L)	132.06±2.81	265.08±4.90***	125.54±3.33**	133.53±3.21*
Reduced glutathione (GSH-nmole/g)	37.65±0.21	26.46±0.25**	32.32±1.32*	30.83±1.11*
Lipid peroxidation (TBARS-nmoleMDA/mg) protein)	2.65±0.02	3.54±0.07**	2.23±0.05***	4.98±0.01*

Values are expressed as mean±S.D. (n=6). Statistical significance: *p<0.05, **p<0.01, ***p<0.001 as compared with control group.

Table-6: Effect of Mollugo oppositifolia plant extract on the activity of antioxidant enzymes in alloxan induced diabetic rats.

Parameters	Group I	Group II	Group III	Group IV
Superoxide dismutase (SOD) (U/mg protein)	26.43±2.3	14.27±0.1***	25.70±0.5*	22.30±3.9*
Catalase (CAT) (nmoles/ min/mg protein	8.58±0.9	3.16±0.6***	7.66±0.7**	5.41±0.9
Glutathione peroxidase (GPx) (nmoles/min/mg protein)	12.55±0.9	5.48±0.9***	16.60±1.6*	11.68±1.1

Values are expressed as mean±S.D. (n=6). Statistical significance: *p<0.05, **p<0.01, ***p<0.001 as compared with control group.

The qualitative phytochemical analysis of *Mollugo oppositifolia* extract showed presence of alkaloids, flavonoids, glycosides, and phenolic compounds, phytosterols, saponins, tannins, triterpenoids, carbohydrates and Proteins. Phenolic compounds and flavonoids were recorded higher in the extract (Table-1). Over 150 plants extracts and some of their active principles

including flavonoids are known to be used for the treatment of diabetes [35, 36, 37]. Moreover, tannin-containing drugs are demonstrated for their antidiabetic activity by Iwu [38], and Klein *et al.*, [39]. Similarly, several phenolic compounds of plant origin possess marked anti-diabetic activity [1, 40, 41]. Possibly the insulin-like activity of these bioactive compounds present in *Mollugo oppositifolia* (Table-1) may be responsible for its hypoglycemic effects.

The acute toxicity study clearly indicated the non-toxicity of the extract at a dose of 2000 mg/kg and the 1/5th dose (400 mg/kg-bw) was selected for further studies. Since there was no LD_{50} observed, the extract tested was considered as safe and nontoxic. Administration of alloxan significantly ($p<0.001$) showed an elevated level of blood glucose in disease control rats when compared to healthy rats. Oral administration of *Mollugo oppositifolia* extract and standard drug for 35 days showed a significant ($p<0.001$) reduction in blood glucose level in drug treated animals. Decrease in the blood glucose level was more recorded in the animals treated with *Mollugo oppositifolia* extract than animals treated with standard drug (Table-2). The elevated level of blood glucose in diabetic control rats might be due to the destruction of β-cells by alloxan. Alloxan is a cytotoxin, and it is widely used in animal model studies to induce diabetes. Alloxan damages the pancreatic β-cells by producing H_2O_2 and other reactive species [42, 43]. Consequently, there is a reduced secretion of insulin leading to clinical conditions such as hyperglycemia, polyphagia, polydypsia and weight loss [44, 45, 46]. Serum insulin level was significantly ($p<0.001$) decreased in disease control rats when compared to healthy rats. Oral administration of plant drug and glibenclamide increased the serum insulin levels. The increase in serum insulin was found higher in the plant extract treated animals than the standard drug treated animals (Table-2). Administration of *Mollugo oppositifolia* extract in diabetic rats influence a significant increase in insulin levels and lowering the blood glucose and in this results correlates well with the observation of Anitha *et al.*, [47]. This observation suggests that both pancreatic and ex-pancreatic mechanism might be involved in the antidiabetic and antihyperglycemic action of plant extract. The possible mechanism by which the extract reduced the blood glucose level in diabetic rats might be stimulation of surviving β-cells to increase the insulin secretion and also there may be possibilities of regenerating the β- cells by the action of phytoconstituents (particularly flavonoids and other phenolic compounds) present in the plant extract.

Glycosylated hemoglobin (HbA1c) is a form of hemoglobin which is measured primarily to identify the average plasma glucose concentration over a prolonged period of time. It is

formed in nonenzymatic glycation pathway by hemoglobin exposure to plasma glucose. Normal level of glucose produce normal amount of HbA1c. As the average amount of plasma glucose increases, the fraction of HbA1c increases in a predictable way. This serves as a marker for identifying average blood glucose levels over the previous months prior to the measurement. In diabetes mellitus, higher amounts of HbA1c indicating poorer control of blood glucose levels. Assay on the level of HbA1c in type 1 diabetic patients may be helpful in monitoring blood glucose. In the present study, the glycosylated haemoglobin level was significantly ($p<0.001$) increased in disease control rats when compared to healthy rats. After the treatment of plant drug and standard drug the level of glycosylated haemoglobin was significantly ($p<0.001$) decreased. The increase in glycosylated haemoglobin was comparatively higher in the plant drug treated animals than with standard drug treated animals (Table-2).

Glycosylated haemoglobin is produced through the glycosylation of haemoglobin. Glycosylated haemoglobin is formed progressively and irreversibly over a period of time, stable till the life of the RBC and unaffected by diet or insulin or exercise on the day of testing. Therefore, glycosylated haemoglobin can be used as a reliable marker of overall glycemic control. Since it is formed slowly and does not dissociate easily, it reflects the renal blood glucose level. Glycosylated haemoglobin is an easily measurable biochemical marker that strongly correlates with the level of ambient glycemic during a period of 2 to 3 months and is more accurate and reliable measure than fasting blood glucose level [48].

Serum protein and liver tissue protein levels were significantly ($p<0.01$) decreased in disease control rats when compared to healthy ones. Oral administration of plant drug and standard drug significantly ($p<0.05$) increased the serum and liver tissue protein levels. The levels of protein in serum and liver were increased to near normal levels after the treatment with drugs for 35 days. The increment in the protein levels were significantly ($p<0.05$) higher in plant drug treated rats than standard drug treated rats. The decreased level of total protein may due to the changes in circulating amino acid level and hepatic amino acids uptake by muscles during diabetic condition [49]. In the present study, the elevated levels of serum and liver proteins in drug treated rats may be related with the increased level of plasma insulin in diabetic rats treated with the extract of *Mollugo oppositifolia*. Because it is not only stimulates the uptake of amino acids and protein synthesis, but also inhibits the degradation of protein [50].

Serum urea content was significantly ($p<0.001$) increased in disease control rats when compared to healthy rats. After the treatment with plant drug and standard drug the serum urea content was decreased. The reduction in the content of serum urea was comparatively higher in the plant drug treated animals than the animals treated with standard drug (Table-2). Significant elevations in blood urea level indicate the impaired renal function of diabetic animals. Urea is the main end product of protein metabolism. Amino acid de-amination takes place in the liver, which is also the site of urea cycle, where ammonia is converted into urea and excreted through urine. The level of urea varies directly with protein intake and inversely with the rate of excretion. Some of the urea is bound to haemoglobin so its concentration in red blood cells is greater than in the plasma. Renal diseases which diminish the glomerular filtration lead to urea retention and decrease in urea level is seen in severe liver disease with destruction of β- cells leading to impairment of the urea cycle [51].

Serum creatinine level was significantly ($p<0.001$) increased in disease control rats when compared to healthy rats. After the treatment of plant drug and standard drug the serum creatinine level was decreased. The decrease in the serum creatinine level was significantly higher in the plant drug treated animals than the animals treated with standard drug (Table-2). Serum creatinine level was enhanced in the diabetic rats due to failure in the renal function which is generally impaired in diabetic condition. Creatinine is a waste product formed in muscle by creatinine metabolism. Creatinine is synthesized in the liver, passes into the circulation and is taken up almost entirely by skeletal muscle. Its retention in the blood is evidence of kidney impairment. In the present study the treatment with *Mollugo oppositifolia* extract was effective in preventing the increase in serum creatinine level when compared to the disease control animals. The results of present study indicates that the plant extract ameliorate the renal function. Similar result was observed by others in different animal model antidiabetic studies [1,8,9,10,52,53].

Reduction in protein in plasma and elevation in urea and creatinine levels in the serum were observed in diabetic animals. This condition is existed in the diabetic animals because of renal dysfunction and also due to increased protein breakdown [54]. The decrease in serum urea and creatinine levels on treatment with *Mollugo oppositifolia* extract prevented the progression of renal damage in diabetic rats. Liver glycogen content was significantly ($p<0.001$) decreased in disease control animals when compared to healthy rats. A significant ($p<0.01$) increase in the liver glycogen content was observed after oral administration of plant

drug and standard drug. Liver glycogen content was found higher in plant drug treated animals than standard drug treated animals (Table-3). Glycogen deposition in the liver is mainly regulated by insulin. The decreased amount of liver glycogen in diabetic rats have been observed earlier by other researchers also [1,55,56]. The decrease in liver glycogen observed in this study may be due to lack of insulin secretion in the diabetic condition and also due to the inactivation of glycogen synthesis enzymes. Insulin promotes deposition of glycogen in the liver by stimulating glycogen synthase and inhibiting the glycogen phosphorylation [57]. The hepatic glycogen level was increased in the diabetic rats after treatment with *Mollugo oppositifolia* extract, which may be due to the improvement in the insulin activity and induction of glycogen synthesis process in the liver. Maiti *et al.,*[58] reported a significant improvement in glycogen level in the diabetic rats after 30 days of treatment with *Spirulina platensis* extract.

The activity of liver glucokinase was significantly ($p<0.001$) decreased in disease control rats when compared to healthy rats. After the treatment of plant drug and standard drug the liver glucokinase enzyme activity was significantly ($p<0.001$) increased (Table-3). In the present study, the glucokinase activity was decreased in alloxan induced diabetic rats which may be due to insulin deficiency. Insulin stimulates and activity of glucokinase in the liver. Lanjhiyana *et al.,* [59] reported that ethanolic leaf extract of *Phyllanthus amarus* and glibenclamide elevated the activity of glucokinase in the liver of diabetic rats. In the present study, *Mollugo oppositifolia* extract and glibenclamide might stimulate the insulin secretion which may activate glucokinase activity thereby increasing the utilization of glucose subsequently influenced the decrease in the blood glucose level [1].

Glycogen synthase enzyme was significantly ($p<0.001$) decreased in disease control rats when compared to healthy rats. After the treatment with plant drug and standard drug the level of the glycogen synthase activity was significantly ($p<0.01$) increased. More amount of glycogen synthase enzyme activity was recorded in the plant drug treated animals than the animals treated with standard drug (Table-3). Glycogen synthase, a highly phosphorylated protein is the rate limiting enzyme of glycogen synthesis. Decreased activity of glycogen synthase in liver of diabetic mice has been reported in other animal model diabetic studies also [1,10,56,60]. Glycogen phosphorylase enzyme was significantly ($p<0.001$) increased in disease control animals when compared to healthy rats. Oral administration of plant drug and standard drug the glycogen phosphorylase enzyme activities were significantly ($p<0.01$)

decreased. Glycogen phosphorylase enzyme activity was comparatively lesser in the plant drug treated animals than standard drug treated animals (Table-3). Glycogenolysis (breakdown of glycogen) usually occurs to provide glucose when there is a decrease in the glucose available in the blood, which promptly mediated by the activity of glycogen phosphorylase. Many enzymes of glycogen breakdown and of glucose catabolism are thiol enzymes and they are affected by tissue redox systems as well as by the available free thiols in the tissue[61]. Glycogen content of liver as well as glycogen breakdown during diabetic condition significantly decreased because of lack of insulin. Since alloxan effectively damaged the β-cells and there was no biosynthesis of insulin, which trigger the glycogen utilization and thus its content was lower in alloxan diabetic rats.

Liver glycose-6- phosphatase enzyme was significantly ($p<0.01$) increased in disease control rats when compared to healthy ones. Oral administration of plant drug and standard drug significantly ($p<0.01$) decreased the liver glycose-6- phosphatase enzyme activity. The decline in the liver glucose-6- phosphatase enzyme activity was found higher in plant drug treated animals than standard drug treated animals (Table-3). Thus administration of plant extract enhanced the prevention of high glycose-6- phosphatase activity in diabetic rats. Glucose 6 phosphatase a key enzyme in glucanogenesis in which it catalyzes the hydrolysis of glucose 6 phosphate and thus it plays an important role in glucose homeostasis for the treatment of diabetes [62,63].

Liver glucose-6- phosphatase dehydrogenase enzyme was significantly ($p<0.001$) decreased in disease control rats when compared to healthy rats. Administration of plant drug and standard drug increased the liver glucose-6- phosphatase dehydrogenase activity. The enzyme activity was significantly higher in standard drug treated animals than the plant drug treated animals (Table-3). This enzyme is active in the pentose phosphate pathway (a metabolic pathway) that supplies reducing energy to cells by maintaining the level of the co-enzyme, nicotinamide adenine dinucleotide phosphatase (NADPH) . The NADPH in turn maintains the level of glutathione in the cells that helps protect the red blood cells against oxidative damage. Higher quantitative of production of NADPH is recorded in the tissues actively engaged in biosynthesis of fatty acids and/or isoprenoids, such as the liver, mammary glands, adipose tissues and the adrenal glands. Glucose-6- phosphatase dehydrogenase reduces NADPH while oxidizing glucose-6-phosphate.

The administration of alloxan significantly ($p<0.001$) increased the levels of the lipid profile constituents, such as serum cholesterol, triglycerides, total lipids, phospholipids and LDL and VLDL cholesterol in diabetic rats when compared to healthy rats. After the treatment with plant drug and standard drug the lipid profiles' levels were significantly ($p<0.001$) decreased. The reduction in the serum cholesterol level was comparatively higher in the plant drug treated animals than the animals treated with standard drug (Table-4). It showed that apart from carbohydrate metabolism, drugs played an important role in lipid metabolism also. The possible mechanism for decreased lipid levels could be either insulin releasing or insulin proved to inhibit the activity of hormone sensitive lipases in adipose tissue and suppress the release of lipids [64]. The decline in the level of serum triglycerides in hamsters treated with ethanolic extract of *Mollugo oppositifolia* was reported which indicates the antilipidemic property of *Mollugo oppositifolia*. Glucose transport is the rate-limiting step in glucose utilization in insulin targeted skeletal muscle cells. This transport is medicated by major glucose transporter proteins present in skeletal muscle cells [65]. Alloxan induced diabetes lead to increase in the levels of total lipids, this result correlates with the result of Parimala and Shoba [66]**.** In diabetic rats, the increased synthesis of very low density lipoproteins and/ or decline in the removal of it from the blood may be the causes of increased total lipid levels. Phospholipids were increased in alloxan induced diabetic rats. Phospholipids are present in cell membrane and make up vast majority of the surface lipoprotein forming a lipid bilayer that acts as an interface with both polar and non-polar lipoprotein or lipoprotein core [67][68]. Lipids play a vital role in the pathogenesis of diabetes mellitus and the most common lipid abnormalities in diabetes are hypercholesterolemia. The reductions in serum triglycerides, total cholesterol and low density lipoprotein (LDL)-cholesterol concentrations and increase in HDL observed in the treated groups could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics [69].

Serum HDL cholesterol level was significantly ($p<0.001$) decreased in disease control rats when compared to healthy rats. After the treatment with plant drug and standard drug the serum HDL cholesterol level was significantly ($p<0.01$) increased. The decrease in serum HDL cholesterol level was recorded higher in the plant drug treated animals than with standard drug treated animals (Table-4). It was observed from the present investigation that there was an increase in the components of lipid profile such as total lipids, phospholipids, triglycerides, cholesterol, LDL and VLDL Cholesterol levels along with decrease in HDL cholesterol level in the alloxan induced diabetic rats. *Mollugo oppositifolia* extract was found

effective in decreasing total cholesterol, triglycerides and LDL and VLDL and increasing HDL cholesterol levels. The anti-hyperlipidemic efficacy of *Mollugo oppositifolia* extract may be due to increase in insulin secretion which finally led to a decrease in the synthesis of cholesterol and fatty acids.

In general, diabetes is associated with profound alterations in the plasma lipid, triglycerides and lipoprotein profile and with an increased risk of coronary heart disease [70]. High level of total cholesterol and triglycerides is one of the major factors for coronary heart disease and arthrosclerosis [71], which are the secondary complications of diabetes. Diabetic rats treated with *Mollugo oppositifolia* extract showed reduction in the serum lipid level. From this study, it is conclusively recorded that the *Mollugo oppositifolia* extract could modulate the abnormalities of lipid profiles in the diabetic condition and subsequently reduces the other secondary complications.

The liver marker enzymes such as alanine amino transferase (ALT), aspartate amino transferase (AST) and Alkaline phosphates (ALP) levels were found significantly ($p<0.001$) increased in disease control rats when compared to healthy rats. Oral administration of plant drug and standard drug significantly ($p<0.01$) decreased the levels of these enzymes. The decreased levels in liver marker enzymes were significantly ($p<0.01$) higher in plant drug treated animals than standard drug treated animals (Table-5). Elevated levels of liver marker enzymes observed in diabetic rats is the indicative of liver damage. Liver is the vital organ of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites [72]. ALT, AST and ALP are reliable marker enzymes of liver function. Marked elevations in ALT, AST and ALP in diabetic animals indicated the hepatocellular damage [73]. Further, Gosh *et al.* [74] reported that the diabetic complications such as increased glucanogenesis and ketogenesis may be due to the elevated activity of transaminase enzymes. In alloxan induced diabetic rats, the liver cells were necrotized and the integrity of plasma membrane was decreased which causes an increase in the activities of ALT, AST and ALP. Treatment with *Mollugo oppositifolia* extract reduced the activity of ALT, AST and ALP in diabetic rats, which indicates that the extract tends to prevent the damage of liver cells in diabetic condition by maintaining integrity of plasma membrane, thereby suppressing the leakage of enzymes through membrane. This effect may be due to the phytoconstituents present in the plant extract (Table-1). ALP activity was significantly ($p<0.001$) increased in disease control animals when compared to healthy rats. Oral administration of plant drug and standard drug

the ALP activity was significantly ($p<0.01$) decreased in diabetic rats. The decrease in ALP was comparatively more in plant drug treated rats than standard drug treated rats. The activity of ALP has clinical and toxicological importance, since changes in its activity is indicative of tissue damage by toxicants. The elevated level of ALP in the diabetic rats may be due to the damage of liver tissues by alloxan. AST, ALT and ALP are the makers of liver dysfunction whose plasma concentration above homeostatic limit could be associated with various forms of disorders which affect the functional integrity of the liver [75, 76]. Considerable decrease in the level of hepatic marker enzymes by *Mollugo oppositifolia* extract could be correlated to its hepatoprotective activity. Reduced glutathione (GSH) and lipid peroxidation (TBARS) are the biomarker antioxidants. GSH level was significantly ($p<0.001$) decreased in disease control rats when compared to healthy rats. After the treatment with plant drug and standard drug the level of reduced glutathione was significantly ($p<0.01$) increased. The increase in reduced glutathione level was comparatively higher in the plant drug treated animals than the animals treated with standard drug (Table-5). GSH, a tripeptide present in all the cells is an important antioxidant [77]. Decreased GSH level in diabetes have been considered to be an indicator of increased oxidative stress. GSH also functions as free radical scavenger and in the repair of radical caused biological damage. A decrease was observed in GSH in liver and kidney during diabetes. The decrease in GSH level represents increased utilization due to oxidative stress. Administration of plant extract and glibenclamide increased the content of GSH in liver and kidney of diabetic rats.

Lipid peroxidation (TBARS) level was significantly ($p<0.001$) increased in disease control rats when compared to healthy rats. After the treatment of plant drug the lipid peroxidation level was significantly ($p<0.001$) decreased. The decrease in the lipid peroxidation level was found higher in plant drug treated animals than the animals treated with standard drug (Table-5). Similar trend was reported by Priyadharshini and Anuradha [10] and Okoro et al. [78]. The elevated cytotoxic and highly reactive oxidative stress markers such as lipid peroxides causes oxidative damages to proteins and DNA and the reduced cellular non enzymatic and enzymatic antioxidant levels in diabetic conditions further increases the severity of tissue dysfunction resulting in β- cells death and resulting in decreased insulin synthesis and secretion[56]. Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) are the antioxidant enzymes, which scavenge the free radicals. The activities of SOD, CAT and GPx were significantly ($p<0.001$) decreased in the diabetic rats when compared to

healthy rats. Oral administration of *Mollugo oppositifolia* extract and standard drug Glibenclamide significantly ($p<0.001$) increased the activities of these enzymes (Table-6)

Reactive oxygen species (ROS) are generated in all living organisms mainly during mitochondrial metabolism. ROS may include superoxide anion ($O_2^{\cdot -}$), hydroxyl radical ($OH^{\cdot -}$), hydrogen peroxide (H_2O_2) and nitric oxide (NO). Disturbance between the production of reactive oxygen species and antioxidants defenses against them produces a oxidative stress, which amplifies tissue damage and promoting DNA, lipid and protein oxidative damage and oxidative stress, which may lead to cell injury and death. Long term oxidative stress has been associated with numerous diseases and disorders in higher organisms. Oxidative stress plays a major role in the causation of diabetes. Free radicals are generated in disproportionate manner in diabetes mellitus cause lipid peroxidation [79, 80]. In the present study significant increase in the levels of lipid peroxidation observed in liver and kidney of diabetic control mice might be due to reduction in antioxidant defense or due to the increase in free radicals generation. Treatment with ethanolic extract of IS and its fractions significantly reduced levels of lipid peroxidation in liver and kidney. SOD, CAT and GPx are enzymatic antioxidants which prevent cells from being exposed to oxidative damage by direct elimination of reactive oxygen species (ROS) [81]. In diabetes mellitus, antioxidant enzymes SOD, CAT and GPx are inactivated due to high concentration of glucose and thus increase the availability of superoxide anion ($O2^-$) and hydrogen peroxide in the biological systems, which in turn generate hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation[82, 83]. After the oral treatment with *Mollugo oppositifolia* extract, the activities of SOD, CAT and GPx were increased indicating the efficacy of the extract in attenuating the oxidative stress in diabetic liver and kidney. Oberly *et al.*,[84] reported that the level of catalase activity was significantly reduced in alloxan induced diabetic rats. The activity was reversed to near normal values in aqueous extract of *Psidium guajava* flowers treated rats.

CONCLUSION

This study shows that the *Mollugo oppositifolia* extract has beneficial effects on blood glucose level and insulin secretion. The extract restored the altered total cholesterol, triglycerides, and other lipid components, liver marker enzymes (AST, ALT and ALP), total protein and liver glycogen levels to near normal and also enhanced the activities of endogenous antioxidant enzymes such as SOD, CAT and GPx. The efficacy of *Mollugo oppositifolia* extract was comparable with the effect of standard drug, Glibenclamide on

alloxan induced diabetic rats. In some parameters *Mollugo oppositifolia* extract performed better than Glibebclamide. This effect may be due to the phytochemicals present in the *Mollugo oppositifolia* extract. Further studies are required to explore the antidiabetic mechanisms of *Mollugo oppositifolia* extract by isolation and identification of active molecules responsible for antiulcer activity.

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Conflicts of Interest

The authors report no conflict of interest.

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