ANTIDIABETIC ACTIVITY OF *CLERODENDRUM PHLOMIDIS* Linn. AND *GYMNEMA SYLVESTRE* Linn. IN ALLOXAN INDUCED DIABETIC RATS - A COMPARATIVE PRECLINICAL STUDY

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ABSTRACT

The present study was aimed to evaluate the antidiabetic potential of *Clerodendrum phlomidis* leaf extract and compare its efficacy with *Gymnema sylvestre* leaf extract, a known antidiabetic herbal drug and a standard reference drug, Glibenclamide on alloxan induced diabetic rats. The rats were divided into five groups and treated accordingly; Group I: Normal healthy control rats; Group II: Disease control-Alloxan induced diabetic rats; Group III: Diabetes induced rats treated with *Clerodendrum phlomidis* leaf extract; Group IV: Diabetes induced rats treated with *Gymnema sylvestre* leaf extract; Group V: Drug control rats - diabetes induced animals treated with Glibenclamide. Oral administration of plant extracts on alloxan induced diabetic rats showed a marked decrease in the levels of biochemical parameters such as blood glucose, liver glycogen and glycosylated hemoglobin. The levels of blood urea and serum creatinine were also brought to normal levels in the rats treated with plant drugs. The activity of glycogen synthase was reduced and the activities of glycogen phosphorylase, glucose-6-phosphatase, glucose-6-phosphate dehydrogenase were increased in diabetic rats. Profound alteration was observed in lipid profiles (total cholesterol, triglycerides, free fatty acids and phospholipids). Levels of lipoproteins (HDL, LDL and VLDL) showed a significant impairment in diabetic rats which were brought back to normal in plant drug treated and standard drug treated rats. Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phophatase (ALP) activities were increased in diabetic rats. The treatment with plant drugs brought back to normalcy of these enzymes. Reduced glutathione and lipid peroxidation levels were increased in the

diabetic condition and significantly increased after the plant drug treatment. The activity of catalase was reduced in the diabetic rats and the activity was normalized after plant drug administration. The present study evidenced that *Clerodendrum phlomidis* leaf extract is a potent antidiabetic herbal drug and its efficacy is comparable to *Gymnema sylvestre* leaf extract and Glibenclamide. Ursolic acid (a pentacyclic triterpenoid carboxylic acid) was isolated and identified as pharmacologically active compound in *Clerodendrum phlomidis* leaf extract.

KEY WORDS: Diabetes mellitus, antidiabetic activity, *Clerodendrum phlomidis, Gymnema sylvestre,* ursolic acid, animal study.

INTRODUCTION

Diabetes mellitus is a common endocrine disease, producing complex and multifarious disorders that disturb the metabolism of carbohydrate, fat and protein. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 globally [1]. Another report says that the world prevalence of diabetes among adults was 6.4%, affected 285 million adults in 2010 and it will be increased to 7.7% and 439 million adults by 2030. Between 2010 and 2030 there will be a 69% increase in adults with diabetes in developing countries and a 20% increase in developed countries [2].

Diabetes is characterized by increased fasting and post prandial blood sugar levels and abnormal fuel metabolism, which results more notably in hyperglycemia due to the defects in insulin secretion, insulin action or both. The chronic hyperglycemia in diabetes is associated with long-term damage, dysfunction and failure of various organs especially the heart, eyes, blood vessels, kidneys, and nerves. Diabetes mellitus is classified into two subtypes: type I (insulin dependent diabetes mellitus- IDDM) and type II (non-insulin dependent diabetes results- NIDDM). IDDM or juvenile-onset diabetes mellitus results due to cellular mediated autoimmune destruction of the β-cells of the pancreas[3,4], whereas NIDDM or adult-onset diabetes results from the development of insulin resistance and affects individuals usually have insulin deficiency. [5] Diabetes is a chronic disease without a cure and it is associated with significant morbidity and mortality. Though there are various approaches to reduce the ill effects of diabetes and its secondary complications, herbal formulations are preferred due to lesser side effects and low cost.[6] Many allopathic medicines are available for the treatment of diabetes, but somehow they have their own adverse effects like hypoglycemia, nausea, vomiting, hyponatremia, flatulence, diarrhoea or constipation, alcohol flush,

headache, weight gain, lactic acidosis, pernicious anemia, dyspepsia, dizziness and joint pain. So instead of allopathic drugs, herbal drugs are the best choice which is having fewer side effects and non toxic properties. From the available literature, over 400 traditional plant species are reported to have hypoglycemic activity, but only a small number of plant species have been scientifically and medically evaluated for their efficacy. Several beneficial activities are correcting altered carbohydrate metabolism, maintaining integrity and function of β-cells, insulin secreting activity and enhancing glucose up take and utilization. The antioxidant properties due to the presence of chemical constituents in traditional medicinal plant species offer excellent opportunity to develop them in to novel therapeutic agents. Phyto-constituents like glycosides, alkaloids, steroids, carotenoids, terpinoids, flavanoids, peptidoglycans, polysaccharides, inorganic ions, amino acids, glycopeptides, guanidines and galactomannans are implicated as having antidiabetic effect. The present study was aimed to evaluate the antidiabetic potential of *Clerodendrum phlomidis* leaf extract and compare its efficacy with *Gymnema sylvestre* leaf extract, a known antidiabetic herbal drug and also with a standard reference drug, Glibenclamide on alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant materials and preparation of extract

The leaves of *Clerodendrum phlomidis* Linn. (syn. *Clerodendrum multiflorum* (Burm. f) O. Kuntz., *Volkameria multiflorum* Burm. F. (Botanical Family: Lamiaceae) and *Gymnema sylvestre* Linn. (Botanical Family: Asclepiadaceae) were collected from the Sathuragiri Hills Area, Virudhunagar district of Tamil Nadu and they were authenticated by eminent professors in the department of Botany. The voucher specimens (No. 062 & 063) are deposited in the department. The leaves were shade dried for 15 days and they were coarsely powdered. The powdered plant materials were treated with petroleum ether (60-80ºC) to remove waxy matters from the leaves and then subjected to continuous hot extraction in soxhlet apparatus with 80% ethanol. The extract was filtered through hiflow powder packed in Buckner funnel with the help of vacuum. The filtered extract was concentrated and dried under reduced pressure using rotary evaporator at low temperature (40-50°C). The dried extracts were stored in 4ºC for further studies.

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. SGOT, SGPT and ALP kits were procured from Span Diagnostics, Surat, India & Transasia Bio-Medicals Ltd., India respectively.

Phytochemical studies

The extracts of *Clerodendrum phlomidis* and *Gymnema sylvestre* were subjected to analysis for preliminary phytochemicals such as alkaloids, flavonoids, terpenenoids, steroids, and tannins according to the standard methods[7,8,9,10].

Quantitative estimation of phytochemicals

Estimation of total phenolics

The total phenolic content was determined according to the method described by Siddhuraju and Becker [11]. 10µl of extract samples were taken in test tubes and made up to 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and then read at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as gallic acid equivalents per 100 mg of dry weight.

Estimation of total tannins

Using the same extract, the tannin content was estimated after treatment with polyvinylpolypyrrolidone (PVPP) [12]. 100µg/ml of PVPP was taken in test tube and to this 1 ml distilled water and then 1 ml sample extract were added. The content was vortexed and kept in the test tube at 4°C for 4 h. Then the sample was centrifuged (3000 rpm for 10 min) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The free phenolic content of the supernatant was measured and expressed as tannic acid equivalents. From the above results, the tannin content of the sample was calculated as follows: Tannin (%) = Total phenolics (%) – Non-tannin phenolics (%)

Estimation of total flavonoids

The content of total flavonoids was determined by slightly modified colorimetric method described by Zhishen *et al.*[13]. A 0.5ml aliquot was mixed with 2ml of distilled water and subsequently with 0.15ml of 5% $NaNO_2$ solution. After 6 min, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2 ml of 4% NaOH solution was added to

the mixture. Immediately, water was added to bring the final volume to 5 ml, and then it was thoroughly mixed and allowed to stand for another 15min. Absorbance of the reaction mixture was determined at 510 nm and the results were expressed as rutin equivalent per 100 g of dry weight.

Estimation of total alkaloid content

The total alkaloid contents in the samples were analyzed using 1,10-phenanthroline by the method described by Singh *et al* [14] with slight modifications. 100 mg sample was extracted in 10 ml of 80% ethanol. This was filtered through muslin cloth and centrifuged at 5000 rpm for 10 min. Supernatant obtained was used for the further estimation of total alkaloids. The reaction mixture contained 1ml of extract, 1ml of 0.025M $FeCl_3$ in 0.5M HCl and 1ml of 0.05M 1,10- phenanthroline in ethanol. The reaction mixture was incubated for 30 min in hot water bath with maintained temperature of 70 ± 2ºC. After cooling down to room temperature, the absorbance of red colour complex was measured at 510 nm against reagent blank. Alkaloid content was calculated with the help of standard curve plotted with colchicine. The values are expressed as g/100g of dry weight.

Estimation of total saponin glycosides[10].

5 g of leaf extract was dissolved in 25 ml of 90% methanol and refluxed for 1 h. Filter the methanol and repeat the reflux two more times. Combine three extracts and distill off the methanol. To the soft extract 25 ml of petroleum ether was added and refluxed for ½ h. After cooling and discard the pet ether layer. 25 ml of ethyl acetate was added to the residue and reflux for ½ h and cooled and decanted the solvent. The residue left was dissolved in 25 ml of 90% methanol Filtered and reduced the methanol content to 5 ml. To this methanol portion 25 ml of solvent ether was added drop by drop with constant stirring to precipitate the total saponins. The precipitate was filtered or decanted off the solvent and dry the residue to constant weight.

Estimation of Gymnemic acids (modified method of Singh & Dixit [15]).

Weigh accurately 3.0 gm of *Gymnema* leaf extract and dissolved it in 50 ml of distilled water in a beaker. 5% potassium hydroxide solution was added and dissolved it completely. This step was very critical and efficient trituration and maximum dissolution of Gymnemic acid was achieved. Centrifuged the solution and decanted the clear supernatant solution in to a beaker. Adjusted the pH of the solution between 1.4 and 1.6 by using 10% HCl and left it at room temperature for about 30 min. Then centrifuged and the precipitate was collected. The

precipitate was again dissolved in 20 ml of water by poking the precipitate with a clean stick. Centrifuged again and discarded the liquid. 20 ml of 95% ethanol was added to the centrifuge tube and dissolved the precipitate by poking with a stick. Transferred the solution in to 2 centrifuge tubes and centrifuged again. Transfer the clear ethanol solution in to a beaker. Again 10 ml of 95%ethanol was added and dissolved the residue. Centrifuged and transferred the ethanol and solution into the same beaker. The ethanol portion was evaporated on a water bath obtained the residue. Further drying of the residue was done at 60 to 65°C to constant weight in a hot air oven. Assay of Gymnemic acids = Residue weight X 100 X 100 / Sample weight (100-LOD) Estimation of Ursolic acid (modified method of Albuquerque[16] and Gao et al. [17])

Sample preparation

5 g of *Clerodendrum phlomidis* leaf extract was dissolved in 25 ml of 50% (v/v) methanol and heated for its complete dissolution. To this 75 ml of water wa*s* added and contents were mixed thoroughly. Then the reaction mixture was transferred to a round bottom flask and 10 ml of H_2SO_4 was added and reflux for 6-8 h. The reaction mixture was cooled and transferred into a separating funnel and then 25 ml of chloroform was added. The chloroform layer was allowed to separate and it was transferred to another separating funnel. The aqueous acidic layer was once again washed with 25 ml of chloroform. Both the chloroform washings (50 ml) were combined and washed with water till it was acid free. By the addition of anhydrous sodium sulphate, the water residue in the acid free chloroform layer was removed and after filtration, the chloroform was evaporated to get the sample.

Quantitative estimation

0.01 g of the sample was dissolved in 3 ml of absolute alcohol and detected in a UV spectrometer at 550 nm. Ursolic acid standard was dissolved in 3 ml of absolute alcohol to produce different concentrations (3.33, 6.67, 9.99, 13.32 and 16.65%) and detected in the same nm. Average mean value of three determinations was calculated. The amount of ursolic acid present in the sample was calculated by plotting the curve with standard curve (Table 2).

Experimental animals

Healthy adult Wistar strain of albino rats of both sexes, two to three months old and weighing 150-200 g were obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai. The animals were allowed to acclimatize under laboratory conditions (27±2°C with a 12 h light- 12 h dark cycle) for a period of 5 days prior to the experiment. Animals were fed

with standard rat food pellet and water *ad libitum.* All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the ethical committees (Approval No: 790/03/ac/CPCSEA).

Acute toxicity study

The acute oral toxicity studies of extracts were carried out as per the acute toxicity study method (**OECD** guidelines 425, 2001[18]) using albino rats. Animals were kept fasting for overnight providing only water. The ethanol extracts of *Clerodendrum phlomidis* and *Gymnema Sylvestre* were suspended in 1% *Accacia* solution and administered stepwise doses from 100 mg/kg-bw up to the dose of 2000 mg/kg and observed the signs of toxicity up to 72 h in the tested animals[19] . The results clearly indicated non-toxicity of the extracts at a dose of 2000 mg/kg. From this, $1/5^{th}$ (400 mg/kg bw) dose was selected for the experimental study. Hence there was no LD_{50} and the extracts tested were considered as safe and nontoxic.

Oral Glucose Tolerance Test

 After acclimation for 7 days, the oral glucose tolerance test was conducted in overnight fasted normal rats [20]. All the rats were randomly divided into five groups (n=6). Group 1 was received only vehicle 1 ml/100 g and served as control group animals; group 2, treated with 5 mg/kg of glibenclamide; the remaining two groups were treated with 400 mg/kg of aqueous methanolic extract of *Clerodendrum phlomidis* and *Gymnema Sylvestre*. The rats were fasted for 12 h (free access to water) and administered the above drugs to respective groups. Zero minute blood sugar level was determined from overnight fasted animals. After 30 min of the drug treatment (P.O.), the rats of all groups were orally fed with glucose 4 gm/kg. Blood glucose concentration was determined after 30, 60, 90 and 120 min intervals after an oral administration of glucose solution at a dose of 2 g/kg-bw. The blood samples were collected from the tail tip and measured by using glucometer and blood glucose test-strips.

Experimental design to study anti diabetic activity.

Wistar strain of albino rats (male) weighing 150-200 gm were used as the experimental models. The rats will be divided into 5 groups and each group comprising of 6 rats.

Group I: Normal Healthy Control rats (fed with normal chow and distilled water).

Group II: Diabetes induced control rats [treated with alloxan monohydrate in sterile saline (150 mg/kg-bw by i.p. injection, in a single dose)] [21].

Group III: Diabetes induced rats treated with *Clerodendrum phlomidis* leaf extract (400 mg/kg- bw/day orally for 35 days)

Group IV: Diabetes induced rats treated with *Gymnema sylvestre* leaf extract (400mg/kg- bw/day orally for 35 days)

Group V: Drug control-animals, diabetes induced rats treated with Glibenclamide (10mg/kg- bw/day orally for 35 days) [22].

Induction of diabetes in rats

Diabetes mellitus was induced in a batch of normoglycemic albino Wistar rats, starved for 16 h, 150 mg/kg body weight of alloxan monohydrate was dissolved in physiological saline and injected intra-peritoneally [21]. This dose of alloxan produced persistent hyperglycemia after 2 days as revealed by determination of urine sugar levels by BQR method. The alloxan induced diabetic rats having their blood glucose level between 300-340 mg/dl were separated and randomized in to groups (Group II, III, IV and V) each consisted six animals. The experimental rats were administered the desired drugs through oral gavages. After the experimental period, on 36th day the animals were sacrificed by cervical decapitation. The animals were anaesthetized using ether and the abdomen was opened without causing any damage to its blood supply. Samples of blood were collected from each animal kept in 4°C in refrigerator until further analysis. Liver was 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 experiment.

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), in to 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 specimen, 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 analysis of the lipid profiles.

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

Insulin was measured by using an ultrasensitive Rat Insulin Elisa Kit purchased from Mercodia AB, Sylveniusgatan, Sweden (Cat No. 10-1124-01).

Plasma - Insulin levels is expressed as IU/ml.

Estimation of glycosylated haemoglobin (HbA1c)

Isolation of erythrocyte membrane [23] and preparation of hemolysate[24].

Blood was collected with EDTA as anticoagulant. Plasma was separated by centrifugation at 1500 rpm for 15 mm. 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 method Nayak and Pattabiraman [25]. To 0.2m1 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 [26]

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 over night 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 (95% sulphuric acid containing 0.2% anthrone) 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. [27]. 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 per ml per min.

Assay of glucose- 6- phosphatase [28]

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 [29].

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 method [30]**.** 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 [31]

50 mg of wet tissue was homogenized in 10 ml of chloroform- methanol mixture using a glass homogenizer, filtered through Whatman No.1 filter paper and to this 2 ml of 0.9% NaCl solution was added. This mixture was shaken well and transferred in to a separating funnel and 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 sulphuric acid 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 [32]

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) [33]

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[34]

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 HDL supernatant were estimated for cholesterol, phospholipids and triglycerol.

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 [35]

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[36].

0.1 ml of serum sample was digested with 0.2 ml 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 blood serum was carried out by modified method using diacetyl monoxime[37].

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.

Estimation of creatinine

The colorimetric determination of creatinine was done by modified method of the Jaffe reaction[38].

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.

Assay of lipid peroxidation (TBARS) [39].

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 catalase [40].

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.

Assay of reduced glutathione (GSH)[41]

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 SGOT, SGPT and ALP

Were done with the reagents supplied in the kits (Span Diagnostic Ltd.) which were reconstituted, mixed with serum as directed. The SGOT and SGPT were measured at 340 nm and expressed as IU/L. The serum alkaline phosphatase (ALP) was estimated by mixing with the reagent (p-nitro phenyl phosphate, magnesium, buffers and stabilizers) with serum, estimated at 405 nm and expressed as IU/L.

Statistical analysis

All the results were expressed as mean ± S.D. The data were statistically analyzed by one - way analysis of variance (ANOVA). A value of $p < 0.05$ was considered to indicate a significant difference between groups.

RESULTS

Table 1: Screening of phytochemicals in *Clerodendrum phlomidis* and *Gymnema sylvestre* leaf extracts.

Pytoconstituents	*Clerodendrum phlomidis*	*Gymnema sylvestre*
Alkaloids	+	+
Flavonoids	+	+
Glycosides	+	+
Phenolic compounds	+	+
Phytosterols	+	+
Saponins	+	+

Tannins	+	+
Triterpenoids	+	+
Carbohydrates	+	+
Proteins	+	+

'+'Present, '-' Absent

*Values are means of triplicate determination.

Table 2: Quantitative analysis of phytoconstituents of leaf extracts of *Clerodendrum phlomidis* and *Gymnema sylvestre*.

Chemical constituent	*Clerodendrum phlomidis*	*Gymnema sylvestre*
Total phenolic compounds[1]	4.56±0.61	4.37±0.76
Total flavonoids[2]	0.53 ±0.96	0.38±0.66
Total alkaloids[3]	0.86±0.46	0.55±0.46
Total saponins	0.121% w/w	0.108%w/w
Gymnemic acids	--	2.05%
Ursolic acid	2.13%	--

1. g gallic acid equivalents/ 100 g dry wt of plant tissue

2. g rutin equivalents/ 100 g dry wt of plant tissue

3. g/ 100 g

Table 3: Effect of *Clerodendrum phlomidis* leaf extract and *Gymnema sylvestre* leaf extract on Blood glucose, Serum insulin, Glycosylated hemoglobin (HbA$_1$C) in alloxan induced diabetic rats.

Parameters	Group I	Group II	Group III	Group IV	Group V
Blood glucose (mg/dl)	80.46±1.45	285.35±3.67***	76.98±0.07*	80.25±1.45	85.15±1.05*
Serum insulin (ng/ml)	64.82±0.33	32.18±0.66***	68.92±0.98**	58.19±0.01*	60.19±1.08*
Glycosylated hemoglobin (HbA$_1$C) (%)	2.95±0.03	7.26±0.05**	3.12±0.09*	3.25±0.06*	3.10±0.05*
Serum protein (g/dl)	6.52±0.92	4.34±0.02**	5.86±0.05*	5.96±0.07*	4.98±0.09*
Liver Tissue protein (mg/g)	12.25±1.90	8.31±0.75**	11.83±1.12	12.11±0.98	11.55±0.97
Serum urea (mg/dl)	29.86±0.95	60.92±2.75***	33.65±0.89**	31.76±0.98	32.96±1.20*
Serum creatinine (mg/dl)	0.64±0.01	1.64±0.02***	0.81±0.01*	0.75±0.01	0.69±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 4: Effect of *Clerodendrum phlomidis* leaf extract and *Gymnema sylvestre* leaf extract on Liver glycogen, Liver glucokinase, Liver glucose-6-phosphatase, Liver glucose-6-phosphate dehydrogenase in alloxan induced diabetic rats.

Parameters	Group I	Group II	Group III	Group IV	Group V
Liver glycogen g/100 g	5.31±0.35	2.35±0.07***	4.95±0.35**	4.72±0.32*	4.95±0.32**
Liver glucokinase	139.67±2.76	82.45±1.75***	99.80±1.55***	121.12±2.86**	143.23±2.25*
glycogen synthase (μmol of UDP formed /mg protein/h	615.86±4.56	435.66±3.34***	605.35±4.85***	630.78±4.65***	635.90±3.77***
glycogen phosphorylase (μmol of UDP formed/mg protein /h	482.86±4.91	609.11±4.54***	566.88±2.25***	544.24±2.23***	566.22±3.11***
Liver glucose-6-phosphatase	8.5±0.07	19.95±0.050***	13.83±0.01**	9.78±0.08*	7.55±0.07
Liver glucose-6-phosphate dehydrogenase	12.31±0.08	6.11±0.02***	8.12±0.03***	11.95±0.07	11.25±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 5: Effect of *Clerodendrum phlomidis* leaf extract and *Gymnema sylvestre* leaf extract on tissue cholesterol, tissue triglycerides, Serum phospholipids, serum HDL cholesterol, serum HDL cholesterol, serum LDL cholesterol in alloxan induced diabetic rats.

Parameters	Group I	Group II	Group III	Group IV	Group V
Serum cholesterol (mg/dl)	82.53±1.32	163.43±2.23***	108.75±2.95***	98.45±1.54**	83.5±1.33
Serum triglycerides (mg/dl)	98.54±1.43	114.44±2.65***	103.24±2.15**	97.35±2.05	82.34±1.90*
Total lipids (mg/g)	59.54±1.45	87.65±1.56**	78.70±1.89*	50.65±0.75	60.33±0.84
Serum phospholipids (mg/dl)	60.63±1.88	119.85±3.99***	81.76±1.90**	62.35±1.65*	60.66±1.67
Serum LDL cholesterol (mg/dl)	81.20±0.99	148.85±1.25***	101.33±1.55***	84.15±1.88*	79.67±2.65
Serum VLDL cholesterol (mg/dl)	5.63±0.75	15.86±1.27***	10.65±0.05***	9.86±0.08**	12.26±0.06***
Serum HDL cholesterol (mg/dl)	69.65±1.87	31.25±0.35***	49.88±0.12**	59.96±1.89*	57.43±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 6: Effect of *Clerodendrum phlomidis* leaf extract and *Gymnema sylvestre* leaf extract on Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline phosphatase (ALP), Reduced glutathione, Lipid peroxidation and catalase.

Parameters	Group I	Group II	Group III	Group IV	Group V
Serum Glutamate Oxaloacetate Trans- aminase (SGOT-IU/L)	66.6±0.34	129.95±2.87***	91.25±2.44***	82.55±1.44*	85.32±2.21
Serum Glutamate Pyruvate Trans-aminase (SGPT-IU/L)	39.8±0.45	82.6±0.85***	37.2±0.32***	38.3±0.95	40.5±0.67**
Alkaline phosphatase (ALP-IU/L)	128.06±2.81	242.08±4.90***	129.53±3.21*	120.59±2.54**	126.54±3.33**
Reduced glutathione (GSH-nmole/g)	38.65±0.21	24.46±0.25**	30.32±1.32*	32.56±1.88*	33.83±1.11*
Lipid peroxidation (TBARS-nmoleMDA/mg) protein)	1.85±0.02	3.28±0.07**	4.08±0.05***	2.93±0.62*	2.28±0.01*
Catalase (U/mg protein)	3.85±0.08	1.98±0.05**	2.58±0.05***	2.75±0.02*	2.31±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.

RESULTS

In the present study the anti-diabetic efficacy of *Clerodendrum phlomidis* leaf extract and *Gymnema sylvestre* leaf extract on alloxan induced rats was evaluated and their potential was compared with standard drug- Glibenclamide

Acute oral toxicity

No treatment-related deaths, abnormal clinical signs or remarkable body weight changes were observed in all the experimental animals. No gross pathological observation was recorded in all the experimental animals. From the above tested condition, LD_{50} of the test drug was found to be greater than 2000 mg/kg- bw and was found to be safe when administered once orally to fasted female Wistar rats.

Phytochemical screening

Phytochemical analysis showed that alkaloids, flavonoids, glycosides, phenolic compounds, sterols, saponins, tannins, triterpenoids, carbohydrates and proteins were present in the leaf extracts of both *Clerodendrum phlomidis* and *Gymnema sylvestre* (Table 1). The quantity of phyto-constituents present in the leaf extracts of *Clerodendrum phlomidis* and *Gymnema sylvestre* is mentioned in Table 2. It was observed that 2.13% ursolic acid and 2.05% gymnemic acid were quantified in *Clerodendrum phlomidis* leaf extract and *Gymnema sylvestre* leaf extract respectively as active phytochemicals.

Antidiabetic study

Alloxan induced diabetic rats showed a significant ($p<0.001$) elevation in the level of fasting blood glucose and a significant ($p<0.001$) reduction in serum insulin level compared to normal healthy rats which might be due to the destruction of beta-cells. Oral administration of *Clerodendrum phlomidis* leaf extract (400 mg/kg- bw) and *Gymnema sylvestre* leaf extract (400 mg/kg; bw) for 35 days showed a significant ($p<0.05$) reduction in blood glucose level, when compared with diabetic control animals. Decrease in the blood glucose was more recorded in the animals treated with *Gymnema sylvestre* leaf extract than the animals treated with *Clerodendrum phlomidis* leaf extract. Treatment with both the plant drugs and the standard drug, glibenclamide almost brought back the normalcy in glucose level in diabetic rats. The level of serum insulin in alloxan induced diabetic rats was found to be significantly ($p<0.001$) reduced as compared to the healthy control rats. Oral administration of *Clerodendrum phlomidis* leaf extract and *Gymnema sylvestre* leaf extract significantly ($p<0.001$) increased the insulin levels. Diabetic rats treated with *Clerodendrum phlomidis*

leaf extract induced higher amount of insulin secretion lesser level of blood glucose than the rats treated with *Gymnema sylvestre* leaf extract and standard drug (Table 3).

Glycosylated hemoglobin (HbA1c) is a form of hemoglobin which is measured primarily to identify the average plasma glucose concentration over 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 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 alloxan induced diabetic rats of level of HbA_1C was significantly (p<0.01) increased when compared to the healthy normal rats. The rats in group III, IV and V, which were received *Clerodendrum phlomidis* and *Gymnema sylvestre* extracts and standard drug respectively for 28 days showed a significant (p<0.05) reduction in the level of glycosylated hemoglobin compared to diabetic control rats (Group II) (Table 3).

The content of serum protein and liver tissue protein significantly (p<0.001) reduced in alloxan induced diabetic rats. The oral administration of plant drugs and the standard drug brought back the normal amount of protein in diseased rats. The serum urea and serum creatinine levels in diabetic rats were significantly (p<0.001) accumulated and the levels were reduced after the treatment with plant drugs and standard drug, Glibenclamide (Table 3).

The alloxan induced diabetic rats showed significantly (p<0.001) decreased level of liver glycogen compared to the normal healthy rats. Diabetic induced rats treated with plant drugs (Group III and IV) showed a significantly (p<0.01) marked increase in glycogen level than the diabetic disease control rats (Group II). The activities of glycogenic enzymes, glycogen synthase and glucokinase were found reduced in the diabetic rats than healthy control rats and the enzyme activities were brought back to normalcy after the treatment with plant drugs and standard drug. The activities of glycolytic enzymes such as glycogen phophorylase, glucose-6- phasphatase and glucose-6- phosphate dehydrogenase were found significantly (p<0.001) elevated in alloxan induced diabetic rats than healthy control rats. The activities of all the three enzymes were brought back to decreased level after the treatment with plant drugs and standard drug for 35 days (Table 4).

The levels of lipid profiles such as total cholesterol, triglycerides, fatty acids, tissue phospholipids and lipoproteins such as LDL-cholesterol, VLDL-cholesterol were significantly ($p < 0.001$) increased in the alloxan induced diabetic rats . But levels of all these parameters were decreased after plant drug treatment of 35 days. But, there was a marked reduction in HDL cholesterol level (significant at $p < 0.001$) in diabetic rats than that of the normal control rats. After the treatment with plant drugs and standard drug, the level of HDL was found higher in group III, IV and V rats (Table 5).

The present study showed a significant ($p < 0.001$) increase in the liver marker enzymes such as serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) in alloxan induced diabetic rats. Diabetic animals treated with leaf extract of *Clerodendrum phlomidis* and *Gymnema sylvestre* showed a controlled level of SGPT, SGOT and ALP (Table 6).

The levels of reduced glutathione and lipid peroxidation were significantly ($p < 0.01$) increased in the diabetic rats and the treatment with plant drugs significantly ($p < 0.05$) reduced to restore the normal levels. The activity of antioxidant enzyme in liver such as catalase was significantly ($p < 0.001$) decreased in the diabetic rats as compared to healthy rats. Treatment with plant drugs and standard drug significantly restored the activity (Table 6).

DISCUSSION

Diabetes mellitus is a non-communicable disease and is considered to be one of the five leading causes of death worldwide and this chronic metabolic disorder which has risen dramatically over the past 2 decades. Although the prevalence of both type I and II diabetes mellitus are increasing worldwide, the prevalence of type II diabetes is expected to rise rapidly. In spite of inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have paved way to increase emphasis on the use of plant based medicines for a wide variety of human ailments and medications.

Recently the search for suitable antidiabetic agents has focused on plants used in traditional medicine. Although diabetes is being managed and treated in many developed countries exclusively by conventional medication, in many developing countries, diabetic patients have resorted to traditional medicinal herbs for treatment of this disease, largely because of these

are more accessible and less expensive for those who are living in poor socio-economic conditions. Traditional medicines from medicinal plants are used by about 60% of the world's population because of their natural origin and less side effects.

The present study was hence carried out to evaluate the antidiabetic effect of leaf extract of *Clerodendrum phlomidis* leaf extract and *Gymnema sylvestre* on Alloxan induced diabetic rats. The extraction used in the study provided an efficient method for extracting the active principles that could have brought the possible antidiabetic effect. Many plant chemical constituents have been reported earlier as to be responsible for bringing antidiabetic effect[42]. Acute oral toxicity studies performed showed no gross pathological abnormalities which provided NN extract direct relevance for protecting human and animal health. The data presented in the study demonstrated that 400 mg/kg was the minimum dose that produced an effective glucose lowering effect. The phytochemical analysis showed that the major constituents were tannins and other phenolic compounds [43], which could have been responsible for its hypoglycemic effect. More recently, phenolic compounds [44,45] and tannins [46] have been reported as active principles and as being in involved in glucose metabolism regulation.

Alloxan was used as an agent to induce diabetes mellitus in this study which acts by selectively causing cytotoxicity on pancreatic b-cells [47]. Thus it affects the endogenous insulin release and subsequent increase in blood glucose level [48] by rapid depletion of β-cells and is generally dependent upon the degree of destruction of β-cells. It is a difficult task to regenerate the β-cells and induce their function, once they are destroyed. However, a number of herbal compounds have been reported to have this effect on chemically- induced diabetic rats [49]. Under our experimental conditions, Wistar rats treated with 150 mg/kg Alloxan underwent a strong hyperglycemia (250-275 mg/dl) that remained unchanged until the study was completed due to β-cell destruction in pancreas.

Significant elevation in the level of fasting blood glucose and a significant reduction in serum insulin level were observed in the Alloxan induced diabetic rats as compared to normal healthy rats which might be due to the destruction of beta-cells. Alloxan is reduced to dialuric acid which is then auto oxidized back to alloxan resulting in the production of H_2O_2, O^2, O_2 and hydroxyl radicals and causes damages to the beta- cells of islets of Langerhans [50]. Oral administration of *Clerodendrum phlomidis* leaf extract and *Gymnema sylvestre* leaf extract

showed a significant (p<0.05) reduction in blood glucose level, when compared with healthy control animals.

The plant drug treated rats of were found to regenerate the β -cells, which in turn lead to the normal secretion of insulin compared to that of the standard drug. It is worthy to note that both the plant drugs have induced the insulin secretion. The mechanism may be due to the regeneration of beta cells and stimulation of elevated secretion of insulin, thereby increasing the utilization of glucose in various tissues [51]. Increase in insulin secretion and decline in blood glucose by plant extract treatment may be because of their beta cell regenerative property of different constituents present in the leaves. Diabetic rats treated with *Clerodendrum phlomidis* leaf extract induced higher amount of insulin secretion lesser level of blood glucose than the rats treated with *Gymnema sylvestre* leaf extract and standard drug. The phytochemicals, especially ursolic acid present in the extract of *Clerodendrum phlomidis* may be responsible for its best performance. Sun-Mi Jang and others[52] reported that ursolic acid exhibits potential antidiabetic and immunomodulatory properties by increasing insulin levels with regeneration of pancreatic β-cells and modulating blood glucose levels, T-cell proliferation and cytokines production by lymphocytes in type 1 diabetic mice fed a high-fat diet. The animal study carried out by Ullevig et al.[53] identified that ursolic acid is a potential therapeutic agent for the treatment of diabetic complications. Cao *et al.*[48] reported that ursolic acid isolated from *Cornus officinalis* is the main hypoglycemic ingredient and resulted in good hypoglycemic effect in alloxan induced diabetic mice. Ling et al. [54] reported that ursolic acid treatment in streptozotocin induced diabetic rats exhibited a protective effect on kidneys, implying that ursolic acid could be a potential treatment for diabetic nephropathy. In the present study ursolic acid was isolated from the leaves of *Clerodendrum phlomidis* and the extract was standardized for 2.0% ursolic acid. Further, an US patent was granted to Mylari and Fleming [55] for the preparation of ursolic acid salts and methods of treating diabetes and obesity in mammals.

Gymnema sylvestre possessed the anti-diabetic activity by enhancing the secretion of insulin from β-cells of pancreas. Along with that it increases the enzyme activity, which in turn increases the glucose uptake and utilization [56]. In the present study administration of leaf extract of *Clerodendrum phlomidis* showed higher amount insulin secretion in diabetic rats and also 2.13% of ursolic acid was recorded in the *Clerodendrum phlomidis* leaf extract. The

presence of ursolic acid in the *Clerodendrum phlomidis* leaf extract may be responsible for the regeneration of β-cells of pancreas and secretion of insulin in a significant level.

In alloxan induced diabetic rats the level of glycosylated hemoglobin (HbA_1C) was significantly   (p<0.01) increased when compared to the healthy normal rats. The rats in group III, IV and V, which were received *Clerodendrum phlomidis* and *Gymnema sylvestre* leaf extracts and standard drug respectively for 35 days showed a significant (p<0.05) reduction in the level of glycosylated hemoglobin compared to diabetic control rats (Group II). Glycosylated hemoglobin is produced through the glycosylation of hemoglobin. Glycosylated hemoglobin is formed progressively and irreversibly over a period of time and is stable till the life of the RBC and is unaffected by diet, insulin or exercise on the day of testing. Therefore, glycolsylated hemoglobin can be used as an excellent marker of overall glycemic control. Since it is formed slowly and does not dissociate easily, it reflects the real blood glucose level[57].

In the present investigation the group of animals such as group (III and IV) which received leaf extracts of *Clerodendrum phlomidis* and *Gymnema sylvestre* showed a significant (p<0.05) elevation in the level of glycosylated hemoglobin compared to healthy control rats (Group I). Administration of both the plant drugs brought back normality in the level of glycosylated hemoglobin in diabetic induced rats, which was comparable to the standard drug.

Renal dysfunction is another most common complication during diabetes mellitus. Decrease in protein and increase in urea and creatinine levels in serum are the markers of kidney dysfunction. In the present study urea and creatinine levels of alloxan induced diabetic rats were found higher compared to normal healthy rats. The diabetic rats treated with plant drugs showed significant decline in their serum urea and creatinine levels. These results are in accordance to the results reported by Mahalingam and Kannabiran[58]. Further, the protein content in serum and liver tissue was lesser in diabetic rats when compared to healthy control rats. The diabetic rats manifest a negative nitrogen balance related to proteolysis in muscles and other tissues. Reduced protein synthesis and increased protein catabolism accelerates urea synthesis thereby resulting in hyperuremia. On treatment with leaf extracts of *Clerodendrum phlomidis* and *Gymnema sylvestre* restored the normalcy in these renal markers indicating the renal protection afford by the plant drugs. Similar result was reported in alloxan induced diabetic rats treated with *Passiflora foetida* leaf extract [59].

The alloxan induced diabetic rats showed significantly decreased level of liver glycogen compared to the normal healthy rats. Diabetic induced rats treated with plant drugs showed a significantly marked increase in glycogen level than the diabetic disease control rats. The activities of glycogenic enzymes, glycogen synthase and glucokinase were found reduced in the diabetic rats than healthy control rats and the enzyme activities were brought back to normalcy after the treatment with plant drugs and standard drug. Glycolytic enzymes such as glycogen phophorylase, glucose-6- phasphatase and glucose-6- phosphate dehydrogenase were found significantly (p<0.001) elevated in alloxan induced diabetic rats than healthy control rats. The activities of all the three enzymes were brought back to decreased level after the treatment with plant drugs and standard drug. Glucokinase is the prime enzyme which catalyses glucose phosphorylation in liver. Impairment of glucokinase activity suggests the impaired oxidation of glucose via glycolysis leading to its accumulation in the blood, resulting in hyperglycemia. Insulin influences the intracellular utilization of glucose in a number of ways. Insulin increases hepatic glycolysis by increasing the activity of the key glycogenic enzyme such as glucokinase[60]. Rats treated with *Clerodendrum phlomidis* leaf extract and *Gymnema sylvestre* leaf extract showed a significant increase in the activity of glucokinase and glycogen synthase. The present study showed that the effect of both the plant drugs is comparable to that of the glibenclamide and the level of activity is almost equal to the healthy animals.

The increased activity of glycolytic enzymes such as glycogen phophorylase, glucose-6-phosphatase and glucose-6- phosphate dehydrogenase were observed in diabetic rats than in the normal healthy rats. Glucose-6-phosphatase plays a vital role in the homeostatic regulation of blood glucose levels. When the blood glucose level falls, then the liver is capable of rapidly releasing glucose into the circulation by glycolysis of glycogen and it serves as a fuel for other tissues that lack the ability to make glucose [61]. Increased hepatic glucose output is a major cause of the fasting hyperglycemia and that characterizes diabetes.

The activities of glucose-6-phosphatase and glucose-6- phosphate dehydrogenase were inhibited after the treatment with the plant extracts, which suggests that glucose -6-phosphate is not utilized for the synthesis of glucose in the glycogenic pathway, but may be used as a substrate for glycogenesis or in the HMP pathway. In the present study the leaf extract of both *Clerodendrum phlomidis* and *Gymnema sylvestre* inhibited the activity of glucose-6-phosphatase and the activity was better than that of glibenclamide treated rats.

The hepatic glucose-6-phosphate dehydrogenase activity in diabetic rats showed a significant reduction compared to that of normal healthy rats. During diabetes, lipogenesis is decreased while a marked increase of lipolysis in the hepatic tissues occurs. This lead to the under utilization of glucose resulting in increased lipolysis and in turn stimulate the activities of gluconeogenic enzymes[62]. In the present study it was noticed that the leaf extract of *Clerodendrum phlomidis* and *Gymnema sylvestre* significantly increased the activity of NADP-linked lipogenic enzyme, glucose-6-phosphate dehydrogenase, than the diabetic control rats. Increase in the glucose-6-phosphate dehydrogenase activity was recorded higher in the rats treated with *Clerodendrum phlomidis* leaf extract than the rats treated with *Gymnema sylvestre* leaf extract.

The alloxan induced diabetic rats showed a significant increase in the levels of lipid profiles such as total cholesterol (TC), triglycerides (TGL), fatty acids, tissue phospholipids and lipoproteins such as LDL-cholesterol (LDL-c), VLDL-cholesterol (VLDL-c). But all these parameters were decreased after the treatment with plant drugs. There was a marked reduction in HDL-cholesterol (HDL-c) in the diabetic rats compared to that of the normal healthy rats. Similar results were obtained by Gaafar et al. [63], in STZ induced diabetic rats after treatment with white and red cabbage extracts. Studies in human and animals demonstrated that the alteration of lipid profiles in diabetic conditions represents as a risk factor for cardiovascular diseases.

Elevated levels of TC and LDL-cholesterol levels in diabetes may be one of the causes for coronary heart disease. The abnormally high concentration of serum lipids in diabetes mellitus is mainly due to an increase in the mobilization of free fatty acids from the peripheral fat deposits. The marked hyperlipidemia that characterizes the diabetic condition may therefore be regarded as a consequence of the uninhibited actions of lipolytic enzymes on the fat deposits [64].

On treatment with the plant drugs, both the lipid profiles (TC and TGL) and lipoproteins (LDL-c and VLDL-c) were found to have a remarkable decrease, whereas the level of HDL-cholesterol increased significantly in the drug treated rats. Breakdown of fatty acids for energy results in increased production of acetyl CoA which may take up the pathway of cholesterol biosynthesis. It is reported that TGL is the soul energy during starvation. In diabetes mellitus as glucose is not taken up by the cells. The cells are under starvation and depend on TGL for energy. Hence there is a rapid mobilization of TGL from tissues.

In experimentally induced diabetic rats there was a marked elevation in TGL level and it was brought back to normalcy after treating the rats with plant drugs. It was observed that the treatment of *Clerodendrum phlomidis leaf* extract and *Gymnema sylvestre* leaf extract showed a significant decrease in the serum lipid profiles (TC and TGL) and level of lipoproteins (LDL and VLDL) and by elevating HDL-cholesterol level in plant drug treated rats, thus helped in retarding the secondary complications of diabetes mellitus. The anti-lipidemic potential of leaf extract of *Clerodendrum phlomidis* and *Gymnema sylvestre* may be due to increase in insulin secretion which finally led to decrease in the synthesis of cholesterol and fatty acids. Similar observation was reported in STZ induced diabetic rats treated with *Cleome rutidosperma* and *Senecio biafrae* extracts[65]. Maity *et al.* [66] reported that the lipid profile of diabetic rats was improved after the administration of ethanolic extract of *Sechium edule* fruits. Mishra *et al.* [67] reported that ethanolic extract of *Pterocarpus marsupium* heartwood was found effective in decreasing total cholesterol, triglycerides and LDL cholesterol and increasing in HDL cholesterol in the sera of STZ induced diabetic rats.

Significant elevation of tissue free fatty acids and phospholipids levels was evidenced in diabetic rats than the normal control animals. Insulin insufficiency in diabetes mellitus causes uninhibited action of lipase. This in turn increases fatty acid and hence fatty acid mobilization is also elevated. The abnormally high concentration of tissue lipids in diabetes mellitus is mainly due to an increased mobilization of free fatty acids from the peripheral fat deposits, since insulin inhibits the hormone sensitive lipase.

The marked hyperlipidemia that characterizes the diabetic condition may therefore be regarded as a consequence of the uninhibited actions of lipolytic enzymes on fat deposits. Excess of free fatty acids are produced by alloxan administration, which in turn promotes the conversion of some fatty acids to phospholipids and cholesterol. Along with these two substances the excess of TGL formation is also responsible for the elevation in the level of serum phospholipids [68].

After the treatment with *Clerodendrum phlomidis* leaf extract and *Gymnema sylvestre* leaf extract the diabetic rats showed a notable resumption of free fatty acids and phospholipids levels. The effect of *Clerodendrum phlomidis* leaf extract and *Gymnema sylvestre* leaf extract was found to be potential than the glibenclamide treatment.

The protein content in serum and tissue was reduced in diabetic animals compared to normal healthy animals. After the treatment of *Clerodendrum phlomidis* leaf extract and *Gymnema sylvestre* leaf extract of diabetic rats for 35 days the protein content was increased to near normal level. Insulin is a physiological hormone, which plays an important role in the maintenance of protein balance. Because it is not only stimulates the uptake of amino acids and protein synthesis, but also inhibits protein degradation [69].

Rats treated with *Clerodendrum phlomidis* leaf extract and *Gymnema sylvestre* leaf extract with its regenerative potential caused a profound increase in insulin secretion, thereby the tested plant drugs enhanced the protein sparing action of glucose and help to maintain serum and tissue protein levels. The present study, the maximum efficacy was shown by *Clerodendrum phlomidis* leaf extract and *Gymnema sylvestre* leaf extract in diabetic rats than the standard drug-glibenclamide. Significant increase in the activities of hepatic marker enzymes, such as serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and alkaline phosphatase (ALP) was recorded in alloxan induced diabetic rats. This profound increase in serum marker enzymes indicates an impaired liver function due to diabetic condition. Controlled levels of SGPT, SGOT and ALP were observed in diabetic rats treated with leaf extracts of *Clerodendrum phlomidis* and *Gymnema sylvestre*.

In diabetic animals, the change in the hepatic marker enzymes is directly related to changes in the metabolism in which these enzymes are involved. The increased levels of marker enzymes which are active in the absence of insulin because of increased availability of amino acids in diabetic condition [70]. In the present study, treatment with leaf extracts of both *Clerodendrum phlomidis* and *Gymnema sylvestre* significantly decreased SGPT, SGOT and ALP enzyme activities in the diabetic rats. The decrease was more pronounced in the rats treated with *Gymnema sylvestre* and the value is comparable with the standard drug.

Hence, the improvement noticed in the levels of the above marker enzymes is considered as a consequence of restoration in the carbohydrate, fat and protein metabolism. The normalization of SGPT and SGOT activity after treatment with plant drugs also indicates a revival of insulin secretion [71]. Elevation of ALP activity has been reported in diabetic rats and the increase in ALP was significantly reversed by the treatment with leaf extracts of both *Clerodendrum phlomidis* and *Gymnema sylvestre*, which is comparable with the treatment of standard drug- glibenclamide.

Reduced Glutathione (GSH) is an important antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides. Sustained hyperglycemia is associated with low cellular levels of the glutathione (GSH), which leads to tissue damage attributed to oxidative stress. Patients with uncontrolled type 2 diabetes have severely deficit of synthesis of glutathione attributed to limited precursor (amino acids) availability. Dietary supplementation with GSH precursor amino acids can restore GSH synthesis and lower oxidative stress and oxidant damage in the face of persistent hyperglycemia. In the present study GSH level was decreased in diabetic rats, whereas treatment of plant extracts restored the level of GSH. The amino acids present in the leaf extracts may be responsible for synthesis of GSH would be the possible explanation for the increased level GSH in the rats treated with plant extracts.

There is considerable evidence that hyperglycemia represents the main cause of complications of diabetes mellitus, and oxidative stress resulting from increased generation of reactive oxygen species plays a crucial role in their pathogenesis. In fact, in the absence of an appropriate response from endogenous antioxidant mechanisms, the redox imbalance causes the activation of stress-sensitive intracellular signaling pathways. The latter play a key role in the development of late complications of diabetes mellitus, as well as in mediating insulin resistance (*i.e.*, resistance to insulin-mediated glucose uptake by some cells) and impaired insulin secretion.

Lipid peroxidation, owing to free-radical activity, plays an important role in the development of complications of diabetes. Increased levels of lipid peroxidation, as a consequence of free radical activity, have been reported in diabetes. In the present study also a significant increment in the level of lipid peroxidation was noticed in diabetic rats and the treatment with plant extracts reduced it to normal levels. Further, catalase level was decreased in diabetic rats and upon treatments with plant drugs the same was increased which suggests that these alterations may be owing to the compensatory mechanisms of the antioxidant potential of the plant drugs.

Oxidative stress in the diabetes coexists with a reduction in the antioxidant capacity, which can increase the deleterious effects of free radicals. Increased oxidative stress is believed to play an important role in the etiology and pathogenesis of chronic complications of diabetes. In diabetic condition high glucose level can inactivate antioxidant enzymes such as reduced

glutathione and catalase by glycating the proteins and hyperglycemia induces oxidative stress which in turn causes lipid peroxidation. Decrease in the level of reduced glutathione and increased lipid peroxidation in the diabetic rats were restored as an effect of plant extract administration, indicating the efficacy of plant drugs in attenuating antioxidant status in diabetic liver and play an important role in prevention of diabetic complications.

Further, in diabetes mellitus secondary complications are the major cause of morbidity and mortality. These secondary complications are mainly due to the production of free radicals. These free radicals are capable of damaging cellular molecules like DNA, proteins and lipids which in turn leads to altered cellular functions. Several recent studies have proved that the treatment of animal models having diabetes mellitus with anti oxidants are capable of neutralizing these free radicals and are effective in preventing experimentally induced diabetes mellitus as well as reducing the free radical induced complications. In the present study alloxan is used to induce diabetes in the rats. Alloxan is toxic to pancreatic β- cells and resulting in the production of reactive oxygen species (ROS). The level of reduced glutathione and lipid peroxidation was increased and the level of catalase was decreased in the alloxan induced diabetic rats. The treatment with extracts of *Clerodendrum phlomidis* and *Gymnema sylvestre* restored normal level of enzyme activity in the diabetic rats. Some plants extracts are reported to exert hypoglycemic action by potentiating the insulin effect, either by stimulating the pancreatic secretion of insulin from the cells of islets of Langerhans [72] or its release from bound insulin, while others act through extra pancreatic mechanisms by inhibition of hepatic glucose production or corrections of insulin resistance [73]. In the present work phytochemicals like phenols, tannins, flavonoids, alkaloids, steroids, and glycosides were identified and quantified in the extracts of both *Clerodendrum phlomidis* and *Gymnema sylvestre* and these compounds may also be responsible for the antidiabetic activity.

It has been suggested that enhanced production of free radicals and oxidative stress is central event to the development of diabetic complications. This suggestion has been supported by demonstration of increased levels of indicators of oxidative stress in diabetic individuals suffering from complications. Therefore, it seems reasonable that antioxidants can play an important role in the improvement of diabetes. There are many reports on effects of antioxidants in the management of diabetes. A number of phenolic compounds have been identified in the leaf extracts of both the plants, which are active in quenching oxygen derived free radicals by donating hydrogen atom or an electron and thus neutralize the free radicals.

CONCLUSION

The results of the present study shows that leaf extracts of *Clerodendrum phlomidis* and *Gymnema sylvestre* possess antidiabetic and antioxidant efficacy. Phytochemical screening indicated the presence of pharmacologically active ingredients in the leaves. The improved glycemic control is evident from the results of restoration of insulin secretion. The improvement in lipid profile indicates the beneficial effect of the leaf extracts in controlling energy wasting. The leaf extract significantly normalizes the biochemical alterations that occurred during diabetic mellitus. The normalization in the activities of gluconeogenesis enzymes and renal marker enzymes indicates the non-toxic, antidiabetic nature of the plant drugs. The improved enzymatic antioxidant status indicates the antioxidant property of the plant drugs. In conclusion, the observed antidiabetic and antioxidant property could be due to the presence of biologically active ingredients in the leaves. Thus, the study provides a scientific rationale for the use of *Clerodendrum phlomidis* and *Gymnema sylvestre* leaves in the traditional system of medicine. Further, in most of the parameters leaf extract of *Clerodendrum phlomidis* showed better performance than *Gymnema sylvestre* leaf extract. Extraction, isolation and structural identification of active ingredients like ursolic acid from the leaf of *Clerodendrum phlomidis* may provide valuable lead molecules for efficient antidiabetic activity.

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