Research Article

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EVALUATION OF ANTIDIABETIC ACTIVITY OF METHANOLIC EXTRACTS OF ROOT AND STEM OF *GMELINA ARBORIA* ROXB. IN STREPTOZOCIN INDUCED DIABETIC RATS

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ABSTRACT

An anti-diabetic activity of *Gmelina arborea* Roxb. (Family- Verbenaceae) was carried out on healthy adult wistar rats weighing between 250-300 gm. Methanolic extract of powdered root (MER) and stem (MES) of *G. arborea* were given orally in dose of 250 and 500 mg/kg. Glibenclamide (0.25mg/kg) was also given orally. Diabetes was induced in rats by the intravenous (i.v.) injection of STZ in tail vein at a dose of 45 mg/kg dissolved in citrate buffer (1 M, pH 4.5) (1 ml/kg). The sugar level of the treated and the untreated animals were determined by withdrawing the blood at regular intervals and evaluated for various parameters such as the estimation of glucose by GOD-POD method, estimation of triglycerides by GPO-PAP method in blood and serum of each animals. On streptozotocin induced diabetic rats, S500 showed higher % decrease in blood glucose level (74.41 %). The phytochemical analysis of MER revealed the presence of flavonoids, phenolics, lignan, fixed oil, fats, and also showed presence of alkaloids, glycosides, tannins, sterols, carbohydrate etc. while MES was found rich in flavonoids and phenolics and coumarins. MER in dose of 500 mg (TC-74.32 mg/dL, TG-70.63 mg/dL, HDLC- 36.47 mg/dL), MES in dose of 500 mg (TG-68.03 mg/dL, HDLC-26.66 mg/dL) and MES in dose of 250 mg (TG- 60.04 mg/dL) and Glibenclamide treatment resulted in normalization of serum lipids which may contribute to the beneficial effect on pancreatic β cells by releasing insulin.

Keywords: anti-diabetic, *Gmelina arborea*, streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is a chronic disorder of carbohydrate, lipid, and protein metabolism characterized by persistent elevation of fasting blood glucose above 200 mg/dL, due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action. It is the most prevalent disease in the world affecting 25% of population and afflicts 150 million people and is set to rise to 300 million by 2025[1]. According to WHO only in USA it is estimated that 17 million people (6.2% of the population) have diabetes out of which 11.1 millions are diagnosed and 5.9 millions are undiagnosed. In India it is estimated that 33 million peoples are diabetic and it is projected that by year 2025 there will be 60-80 million people suffering from diabetes. The WHO expert committee on diabetes mellitus recommended further evaluation of the folkloric methods of managing the disease because of high mortality and morbidity arising from its attendant complications and draw-backs associated with the use of conventional antidiabetic drug. In pursuit of this goal, several medicinal plants are being investigated for possible hypoglycemic activity based on several approaches including ethnobotanical survey.

In India, several indigenous plants used in the local treatment of DM. *Gmelina arborea* Roxb. (Family: Verbenaceae) which is locally known as 'Gambhari'and well reputed plant in Ayurveda. Roots of this plant are used in many Ayurvedic formulations like Dashmula and Chyawanprasha. The stem bark and heartwood possess hypoglycemic activities[2]. According to literature survey, roots and whole stems of *G. arborea* not evaluated for its antidiabetic activity. In this present study an attempt was made, to find the potency of methanolic extracts of roots and stems of *G. arborea* for anti-diabetic purpose.

MATERIAL AND METHODS

Plant Material

Fresh roots and stem were collected from fully-grown trees from fields near the outskirts of Ankleshwar city, Dist. Bharuch (Gujarat, India) in October 2010. The Plant material was authenticate at Botanical Survey of India, Gujarat and given a voucher number BSI/AZC/2010/Tech/570. Roots and stem were dried under shade and powdered (Passed through 40#) for further use. The powder was stored in air tight container and used for the further study.

Chemical and Reagents

Streptozotocin and Glibenclamide were acquired from Sisco Research Laboratory, Mumbai (India) and Aventis Pharma Ltd., (India) respectively. Streptozotocin (STZ) at a dose of 45 mg/kg was given after dissolving in citrate buffer (1 M, pH 4.5) (1 mL/kg) intravenously into tail vein. Methanolic extract of powdered roots (MER) and

powdered stem (MES) of *G. arborea* were suspended in 0.5% CMC. MER and MES of *G. arborea* were given orally in dose of 250 and 500 mg/kg. Glibenclamide (0.25mg/kg) was also given orally.

Preparation of Extract

The measured amount of the powder was taken and subjected to continuous and sequential, hot solvent extraction using methanol in a Soxhlet apparatus. Filtrates were evaporated and dried at 40^0C in hot air oven and stored in air tight container. Suspension of methanolic extract of roots (MER) and stems (MES) of *G. arborea* were prepared by using 0.5% CMC in normal saline.

Analytical TLC Profile of *G. Arborea*

1 gm of powdered roots and stems of *G. arborea* were extracted with 10 ml methanol using reflux for 15 min., filtered and concentrated to 2-3 ml. This methanolic extract of roots (MER) and stems (MES) were spotted for different classes of Phytochemical like alkaloids, flavonoids, Phenolics, lignans, terpenoids etc[3] using different mobile system.

Flavonoids: Mobile phase: Ethyl acetate: Formic acid: Glacial acetic acid: Water (100:11:11:26) Detection: UV 364 (Blue- green spot), Vanillin- Phosphoric acid (Brown spot)

Alkaloids Mobile phase: Chloroform: Methanol (95:5) Detection: UV 364 (No fluorescent spot), Iodine vapours (Dark yellow spot)

Phenolics Mobile phase: Chloroform: Acetic acid (9:1) Detection:UV 364 (Green florescence), Vanillin-Sulphuric acid (Brown colour)

Lignans Mobile phase: Ethyl acetate: Methanol (9:1) Detection: UV 364 (Yellow green florescence), Sulphuric acid reagent (Brown Purple colour spot)

Terpenoids Mobile phase: Chloroform: Methanol (18:1) Detection: UV 364 (yellow green florescence), Sulphuric acid reagent (Brown color spot) mentioned in Table 1 and Figure 1.

Estimation of Phenolic Substances

1g of air-dried powdered roots as well as stems of *G. arborea* were extracted with 100ml methanol, water and ethyl acetate by maceration for 24 hours and filtered. The final volume of the filtrate was adjusted to 100ml using methanol. 5ml of this extract was diluted with an equal volume of methanol and was used for the estimation of phenols. 10ml of the each extract were added 10ml of distilled water and 1.5ml of diluted (1:2) Folin Ciocaulteu reagent and the mixture was kept for 5min. After adding 4ml of 20% Na_2CO_3 solution, the final volume was adjusted to 25ml using distilled water. The absorbance was measured at 765nm at an interval of 30 min. up to 2 hours using distilled water as a blank[4]. The data were compared with similarly prepared set of standard substance- Gallic acid, in concentration range of 50 µg to 300 µg per 25ml. C = 0.001X – 0.04

Estimation of Flavonoids

1g of air-dried powdered roots as well as stems of *G. arborea* were extracted with 100ml methanol, water and ethyl acetate by maceration for 24 h and filtered. The final volume of the filtrate was adjusted to 100ml using methanol. One ml of this extract was diluted up to 10ml with methanol and was used for the estimation of flavonoids[5]. To 3ml of the each extract, 3ml of methanolic $AlCl_3$ was added. After 10 min., absorbance was read at 430nm. C = 0.004X – 0.083 (Figure 2a,2b,2c and 2d).

Pharmacological activity

The pharmacological activity on healthy adults' wistar rats was explored. They were kept for 1 week in laboratories before the experiments for acclimatization to the laboratory conditions. Prior to experimental treatments, animals were fasted overnight but were allowed free access to water. Six animals were used for each group of study. All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Institute Of Pharmacy, Nirma University, Ahmedabad and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Protocol number for the present study was given IPS/PCOG/MPH1011/2016 and Item number given was 16. After that treatment with extract of *G. arborea* collected the Blood samples by retro-orbital plexus puncture method using glass capillary under light ether anaesthesia without any anticoagulant, light anaesthetic condition using solvent ether. Then each blood sample were centrifuged at 10,000 rpm for 30 min at 5^0C. The serum was kept at 5^0C until used. Blood glucose (mg/dL) was measured using Glucose SLR enzymatic kit (Labcare Diagnostics (India) Pvt.Ltd)[6].

Experimental procedure

Effect of MER and MES of *G. arborea* on Oral Glucose Tolerance Test (OGTT) on Glucose Loaded Rats

Rats were divided randomly into different six groups (n = 3) and samples were administered as described below. Fasting blood sugar level of each rats were determined at zero time, after overnight fasting with free access to water. All samples and glucose were given orally using oral gastric gavages. Glucose solution was administered 30 min after the administration of the MER and MES. Blood samples were collected before (-30min) and after oral administration of vehicle, standard and MES and MER at 30, 60, 120 and 240 min and total blood glucose levels of each animal were estimated[6]. (Table 2)

Effect of MER and MES of *G. arborea* on Normal Rats (Normoglycemic Activity)

Rats were divided randomly into different six groups (n = 3) and samples were administered as described below. All samples were given orally using oral gastric gavages. Fasting blood sugar level of each rats were determined at zero time, after overnight fasting with free access to water. Blood samples were collected after oral administration of vehicle, standard and MES and MER at 0, 1, 2, 3, 5 and 24 hr and total blood glucose levels of each animal were estimated [6]. (Table 3)

Induction of Diabetes

Diabetes was induced in rats by the intravenous (i.v.) injection of STZ in tail vein at a dose of 45 mg/kg dissolved in citrate buffer (1 M, pH 4.5,1 ml/kg) [7-8]. In order to stave off the hypoglycaemia during the first day after the STZ administration, diabetic rats were given 5% glucose solution orally. Three days after the injection, blood glucose levels were measured and the animals with blood glucose levels above 250 mg/dl were confirmed as diabetic rats. Rats were fasted for 16 h prior to STZ injection[6-8].

Evaluation of Antidiabetic Activity

The diabetic rats were randomly divided into different seven groups with six rats (n = 6) in each group (Table 3). All test samples and standard drug were administered orally using oral gastric gavages tube for 21 days consecutively. Blood glucose levels were determined at day 1, 7, 14 and 21 of the study. Finally on day 21, blood were collected from all animals and evaluated for various parameters in blood and serum of each animal and for estimation of insulin blood sample collected on 30th day [9-10]. (Table 4)

Biochemical Estimations

The serum level of Glucose, Creatinine, Total Protein (TP), Triglyceride (TG), Total Cholesterol (TC), Insulin and High Density Lipoprotein cholesterol (HDLC) were determined spectrophotometrically using enzymatic colorimetric assay kits (Labcare Diagnostics (India) Pvt. Ltd). While Low Density Lipoprotein Cholesterol (LDLC), Very Low Density Lipoprotein Cholesterol (VLDLC) and antiartherogenic index (AAI) were calculated using Friedewald's equations. The whole blood level of Glycosylated Haemoglobin was determined spectrophotometrically using enzymatic colorimetric assay kits ((Lab care Diagnostics (India) Pvt. Ltd)[11-16].

Estimation of Glucose (GOD-POD Method)

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoeimine dye as indicator mix & incubate for 5 min at 37°C or 15 min at room temperature Measure absorbance of serum sample (AT) and standard (AS) against Reagent Blank (Glucose SLR reagent) at 505 nm. The colour is stable for 30 min at room temperature Calculation: Total Glucose (mg/dL) = (AT/AS) x conc. of standard

Estimation of Triglyceride (Enzymatic Colorimetric Test – GPO PAP method)

Triglycerides are determined after enzymatic hydrolysis with lipases. The quinonemine indicator is formed from hydrogen peroxide, 4- aminophenazone, and 4-chlorophenol under the catalytic influence of peroxidise. Mixed well, incubate for 5 mins at 37°C (or 10 min at 20 - 25° C). Measure absorbance of Serum Sample (AT) and Standard (AS) against reagent blank at 505 nm. The colour is stable for 30 min at 20 - 25° C. Calculation: Triglycerides (mg/dL) = (AT/AS) x conc. Standard

Estimation of Total Cholesterol (Enzymatic Colorimetric Test)

Cholesterol esters are hydrolyzed to produce cholesterol. Hydrogen Peroxide is then produced from oxidation of cholesterol by cholesterol oxidase. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxide. The absorption of the red quinoneimine dye is proportional to the concentration of cholesterol in the Serum sample. Mixed well, Incubate for 5 min at 37°C (or 10 min at 20 - 25°C) Measure absorbance of Serum sample (AT) and Standard (AS) against reagent blank (Cholesterol-SLR Reagent) at 505nm. The colour is stable for 30 min. at 20 - 25°C. Total Cholesterol (mg/dL) = (AT/AS) x conc. Standard

Estimation of High Density Lipoprotein Cholesterol (HDL Cholesterol-Direct Enzymatic Colorimetric Method)

The method depends on the properties of a detergent which solubilises only the HDL so that HDL-c is released to react with the cholesterol esterase, cholesterol oxidase and chromogens to give colour. The non HDL lipoprotein LDL, VLDL and chylomicrons are inhibited from reacting with the enzymes due to absorption of the detergents on their surfaces. The intensity of the color formed is proportional to the HDL concentration in the Serum sample. Reagent Composition is mentioned in Table 5. Calculation: HDL Cholesterol in the Serum (mg/dL) = (Δ A) Serum sample/(Δ A) Calibrator x Calibrator conc. mg/dL

Estimation of Creatinine (Without Deproteinisation Method)

Creatinine in alkaline solution reacts with picrate to form a coloured complex which gives absorbance at 500 - 520 nm. The amount of complex formed is directly proportional to the creatinine concentration in sample. Mix and after 30 sec at room temperature, read initial absorbance and start timer simultaneously. Read again after 1 min. Determine ΔA min. of standard (As) and Serum sample (Ac) against reagent blank. Calculation:

Creatinine mg/dL Serum = Δ Ac / Δ As x Conc. of standard

Estimation of Total Protein (Colorimetric test – Biuret test)

Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a color complex. Mix well, Incubate for 10 min at 20-25°C. Measure absorbance of the Serum Sample (Ac) and Standard (As) against reagent blank. Calculation: g/dL Protein = Ac/As x Conc. Standard

Estimation of Low Density Lipoprotein Cholesterol (LDL Cholesterol)

The concentration of LDL Cholesterol was calculated mmol/L using Friedewald's equation as stated below[16]. LDLC = TC – (HDLC + TGL/2.2)

Estimation Very Low Density Lipoprotein Cholesterol (VLDL Cholesterol)

The concentration of VLDL Cholesterol was calculated mmol/L using Friedewald's equation as stated below[16]. VLDLC = TGL / 2.2

Estimation of Antiatherogenic Index (AAI)

The Antiatherogenic Index was calculated according to the method of Guido and Joseph. AAI was calculated from total cholesterol and HDL Cholesterol using the formula below. The values are expressed as a percentage[17]. AAI=HDLC/TC-HDLC X 100

Estimation of Glycosylated Haemoglobin

Whole blood was mixed with lysing reagent to prepare a hemolysate. This was then mixed with a weakly binding cation exchange resin. The non-glycosylated haemoglobin (GHb) binds to the resin leaving GHb free in the supernatant. The GHb percentage was determined by measuring the absorbance of the GHb fraction and of the total haemoglobin (Hb). Calculation: GHb% = A of GHb/ AofTHb X 7.2

Estimation of Insulin

The immunoradiometric assay of insulin was a "sandwich" type assay. In the kit, mouse monoclonal antibodies directed against two different epitopes of insulin and hence not competing were used. Serum and plasma samples (pre-treated or not with precipitation reagent), controls (pre-treated or not with precipitation reagent) and calibrators were incubated in test tubes coated with the first monoclonal antibody in the presence of the second monoclonal antibody which was labelled with iodine 125. After incubation, the content of tubes was rinsed so as to remove unbound ^{125}I- labelled antibody. The bound radioactivity was then determined in gamma counter. The insulin concentrations in the samples were obtained by interpolation from the standard curve. The concentration of insulin in the samples was directly proportional to the radioactivity.

RESULTS AND DISCUSSION

Oral Glucose Tolerance Test in Rats (OGTT)

MER and MES in two different doses, 250 and 500 mg/kg, administered in glucose loaded animal for the determination of effect on blood glucose concentration. Change in blood glucose level in animals of all groups, before and after 30 min interval were taken and compared with control and standard. Results showed that decrease in blood glucose level after 60 min in all groups of animal. (Figure 3)

Effect in Normoglycemic Rats (NG)

In normoglycemic study, no glucose load was given to the animals. Results indicated that MES and MER were showed slightly increase in the blood glucose level and further there was decrease in blood glucose level after 5 h. (Figure 4)

Evaluation of Antidiabetic Activity on Streptozotocin Induced Diabetic Rats

In order to determine chronic effect, two doses of MES and MER were administered to the rats for 21 days consecutively. The blood glucose level monitored on 1, 7, 14 and 21 days after administration of sample. Change in blood glucose level was followed after 7 days in all groups. S500 showed higher % decrease in blood glucose level (74.41 %) while other three groups S250, R250 and R500 also showed remarkable decrease in blood glucose level. Body weights of animal were decreased in all groups. (Figure 5a,5b and 5c)

Biochemical Estimations

Estimation of serum profile and whole blood (GHb), were done for MER and MES of powdered roots and stems of *G. arborea* at two dose level, 250 and 500 mg/kg, Total cholesterol (TC), triglyceride (TG), LDLC, HDLC, VLDLC, Creatinine, level were decreased, as described in table while antiatherogenic Index, Glycosylated haemoglobin and total protein level were increased in comparison of induced control group while these have very low level in control group of animals. Group R500 showed highest decrease in TG (60.04 mg/dL), TC (70.63 mg/dL), HDLC (36.47 mg/dL) and AAI (88.56 mg/dL) and also showed decrease in LDLC (19.52) level .Group S250 showed significant decrease in VLDLC (27.45 mg/dL), GHb (3.969 %), while group R250 showed significant decrease in TP (3.9 g/dL) and S500 group showed significant decrease in Creatinine (0.875 mg/dL) level. All test and standard grouped showed significant increase serum insulin level amongst which S500 group showed highest increased in insulin (32.52µU/ml) compared to induced control group. (Figure 6 and 7)

Table 1: TLC profile of powdered roots and stems of *G. arborea* for different phytoconstituents

Sr. No.	Phytoconstituents				
1.	**Alkaloids**				
	Mobile phase	Chloroform: Methanol (95:5)			
	Detection	UV364		Vanillin-Phosphoric acid followed by heating at 110^0C for 10 min	
	Sample	MES	MER	MES	MER
	R_f	--	--	0.88 (Dark yellow)	0.79 (Dark yellow)
2.	**Flavonoids**				
	Mobile phase	Ethyl acetate: Formic acid: Glacial acetic acid: Water (100:11:11:26)			
	Detection	UV364		Vanillin-Sulphuric acid followed by heating at 110^0C for 10 min	
	Sample	**MES**	**MER**	**MES**	**MER**
	R_f	0.8 (Blue florescence)	0.9, 0.56 (Blue florescence)	0.19, 0.71 (Brown color)	0.89 (Brown color)
3.	Phenolics				
	Mobile phase	Chloroform: Acetic acid (9:1)			
	Detection	UV364		Vanillin-Sulphuric acid followed by heating at 110^0C for 10 min	
	Sample	**MES**	**MER**	**MES**	**MER**
	R_f	0.8, 0.6 (Green florescence)	0.9, 0.65 (Green florescence)	0.87 (Brown color)	0.9 (Brown color)
4.	**Terpenoids**				
	Mobile phase	Chloroform: Methanol(18:1)			
	Detection	UV364		Sulphuric acid reagent followed by heating at 110^0C for 10 min	
	Sample	**MES**	**MER**	**MES**	**MER**
	R_f	0.8, 0.6, 0.5, 0.2 (yellow green florescence)	0.78, 0.6, 0.5 (yellow green florescence)	0.6, 0.25 (Brown color spot)	0.6 (Brown color spot)

Table 2: Treatment protocol for Oral Glucose Tolerance Test (OGTT) on Glucose loaded rats

Sr. No.	Groups	Treatment
1.	Normal control	0.5% CMC, p.o. + Glucose solution, 2 g/kg, p.o.
2.	Standard	0.25 mg/kg, p.o. + Glucose solution, 2 g/kg, p.o.
3.	R250	MER in dose of 250 mg/kg, p.o.+ Glucose solution, 2 g/kg, p.o.
4.	R500	MER in dose of 500 mg/kg, p.o. + Glucose solution, 2 g/kg, p.o.
5.	S250	MES in dose of 250 mg/kg, p.o. + Glucose solution, 2 g/kg, p.o.
6.	S500	MES in dose of 500 mg/kg, p.o. + Glucose solution, 2 g/kg, p.o.

Table 3: Treatment protocol for effect of MER and MES of *G. arborea* on normal rats

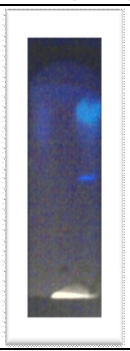
Sr. No.	Groups	Treatment
1.	Normal control	0.5% CMC, p.o.
2.	Standard	Glibenclamide, 0.25 mg/kg, p.o.
3.	R250	MER in dose of 250 mg/kg, p.o.
4.	R500	MER in dose of 500 mg/kg, p.o.
5.	S250	MES in dose of 250 mg/kg, p.o.
6.	S500	MES in dose of 500 mg/kg, p.o.

Table 4: Treatment protocol for evaluation of antidiabetic activity of MES and MER of *G. arborea* on STZ induced diabetic rats

Sr. No.	Groups	Treatment
1.	Normal control	0.5% CMC
2.	Diabetic control	45mg/kg of streptozotocin (STZ), i.v
3.	Standard	STZ + Glibenclamide, 0.25 mg/kg, p.o. per day
4.	R250	STZ + MER in dose of 250 mg/kg, p.o. per day
5.	R500	STZ + MER in dose of 500 mg/kg, p.o. per day
6.	S250	STZ + MES in dose of 250 mg/kg, p.o. per day
7.	S500	STZ + MES in dose of 500 mg/kg, p.o. per day

Table 5: Reagent composition for Cholesterol Estimation

R1	GOOD pH 7.0 Cholesterol oxidase, < 1000 U/L Peroxidase, < 1300 U/L Dimethoxyaniline (TOOS), < 1 mM
R2	GOOD pH 7.0 Cholesterol esterase < 1500 U/L 4- Amino antipyrine (4-AP) <1 mM Detergent <2% Ascorbate oxidase <3000 U/L
HDL/LDL Calibrator	Standard, Lyophilized human serum




364 nm	Vanillin Phosphoric Acid	364 nm	Vanillin Phosphoric Acid
Identification of Alkanoids		**Identification of Flavonoids**	




364 nm	Vanillin Phosphoric Acid	364 nm	Vanillin Phosphoric Acid
Identification of Phenolics		**Identification of Terpenoids**	

Figure 1: Identification of Alkanoids, Flavonoids, Phenolics, Terpenoids by TLC method in *G.arboria* MES & MER

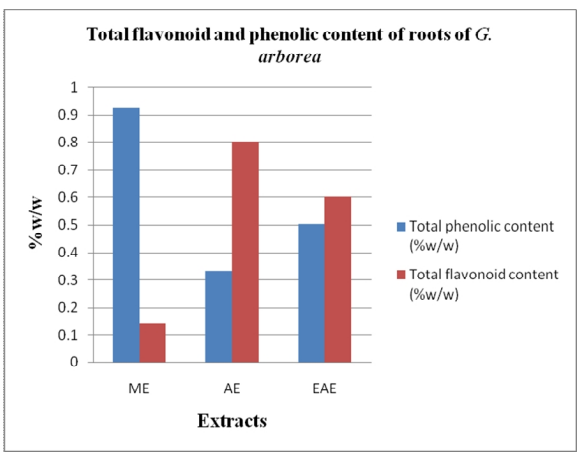


Figure 2a: Total flavonoid and phenolic content of roots of *G. arborea*

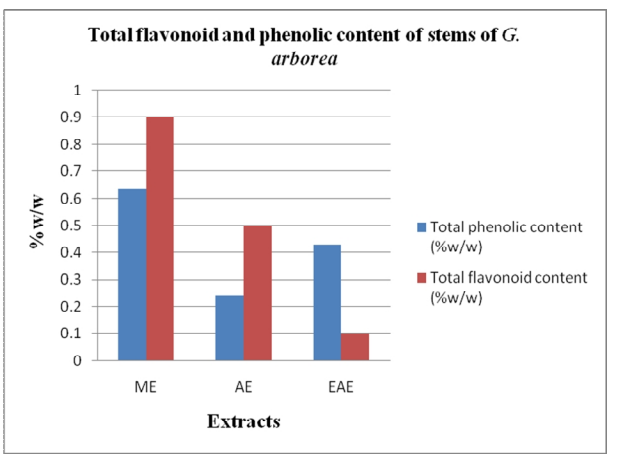


Figure 2 b: Total flavonoid and phenolic content of stems of *G. Arborea*

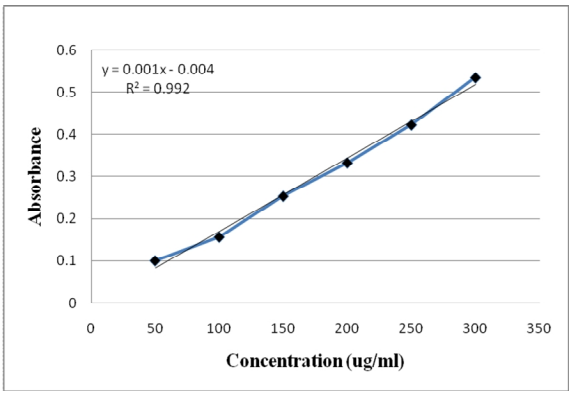


Figure 2c: Standard curve for total phenolic content estimation (Qurcetin)

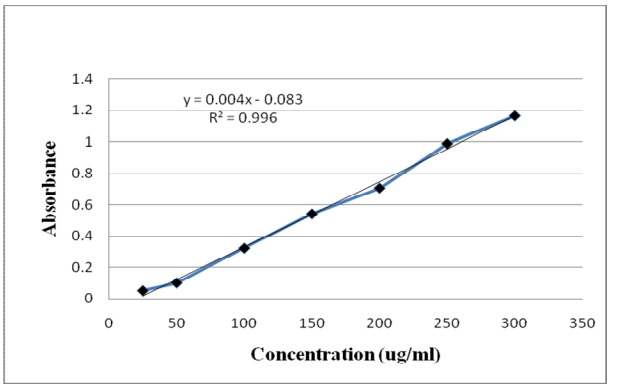


Figure 2d: Standard curve for total flavonoid content estimation (Ellagic acid)

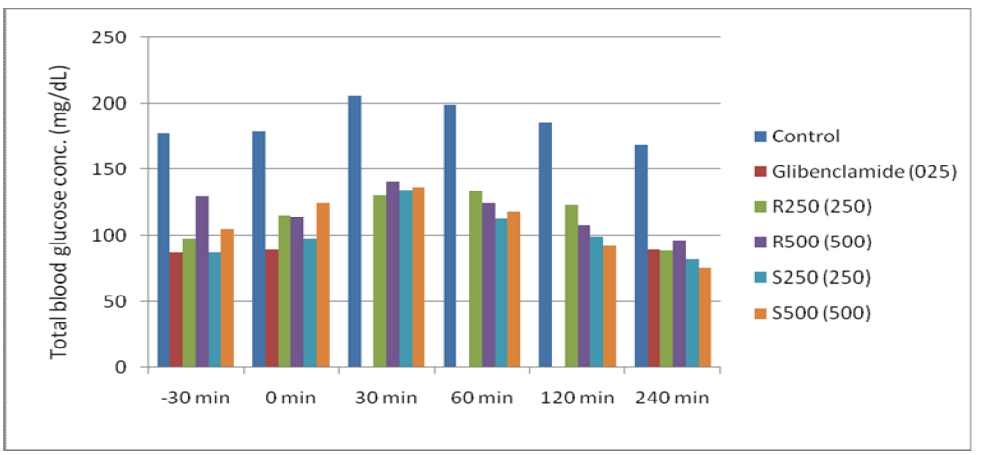


Figure 3: Effect of different concentrations of methanolic extract of powdered roots and stems of *G. arborea* on Oral Glucose Tolerance Test (OGTT)

N = 3, all values are mean ± SEM. Control: 0.5% CMC, (1 ml/kg) p.o., Glibenclamide as standard (0.25mg/kg, p.o.), R250: methanolic extract of root (250mg/kg p.o.), R500: methanolic extract of root(500mg/kg p.o.), S250: methanolic extract of stem(250mg/kg p.o.), S500: methanolic extract of stem(5000mg/kg p.o.). *P<0.001, significant from control group.

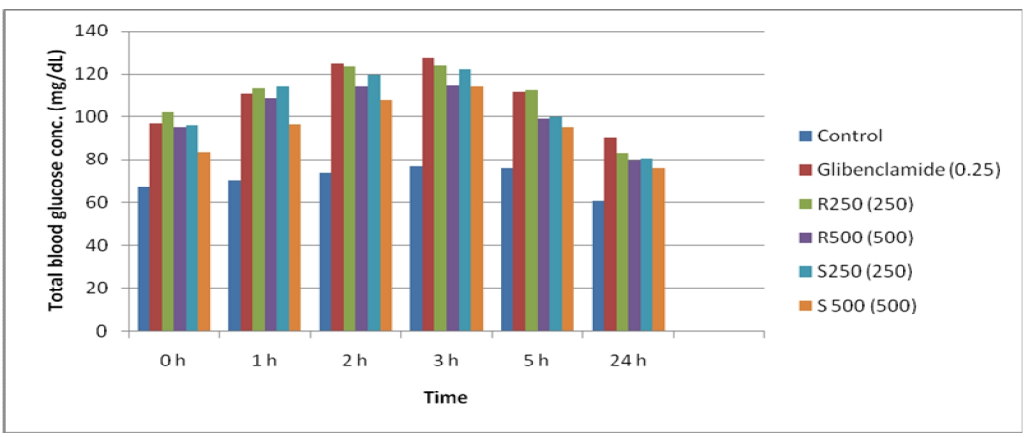


Figure 4: Effect of different concentrations of methanolic extract of powdered roots and stems of *G. arborea* on normoglycemic animals

N = 3, all values are mean ± SEM. Control: 0.5% CMC, (1 ml/kg) p.o., Glibenclamide as standard (0.25mg/kg, p.o.), R250: methanolic extract of root (250mg/kg p.o.), R500: methanolic extract of root (500mg/kg p.o.), S250: methanolic extract of stem(250mg/kg p.o.), S500: methanolic extract of stem(5000mg/kg p.o.). *P<0.001 significant from control group

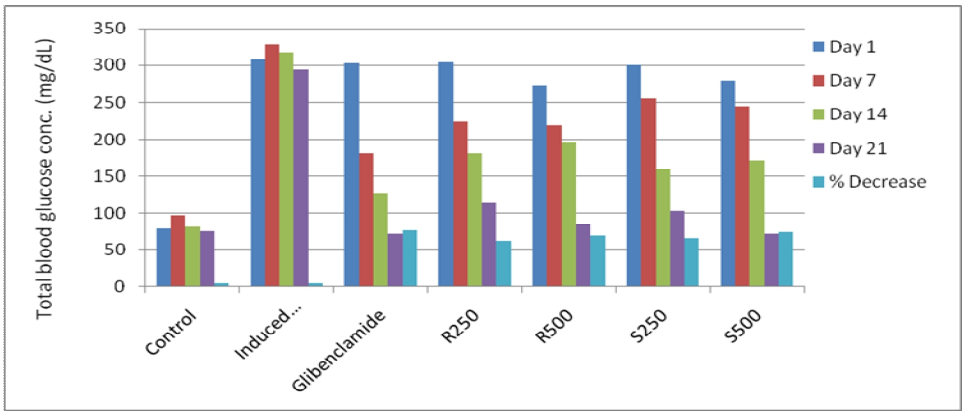


Figure 5a: Effect of different concentrations of methanolic extract of powdered roots and stem of *G. arborea* on total blood glucose level in STZ induced diabetic rats

N = 6 in each group, all values are mean ± SEM. Control: 0.5% CMC, (1 ml/kg) p.o., Induced control: Streptozotocin (STZ), 45 mg/kg, i.v.Glibenclamide as standard (0.25mg/kg, p.o.), R250: methanolic extract of root (250mg/kg, p.o.), R500: methanolic extract of root (500mg/kg p.o.), S250: methanolic extract of stem (250mg/kg p.o.), S500: methanolic extract of stem(5000mg/kg p.o.). *significantly different from control group P<0.001, ** significantly different from induced control group P<0.001

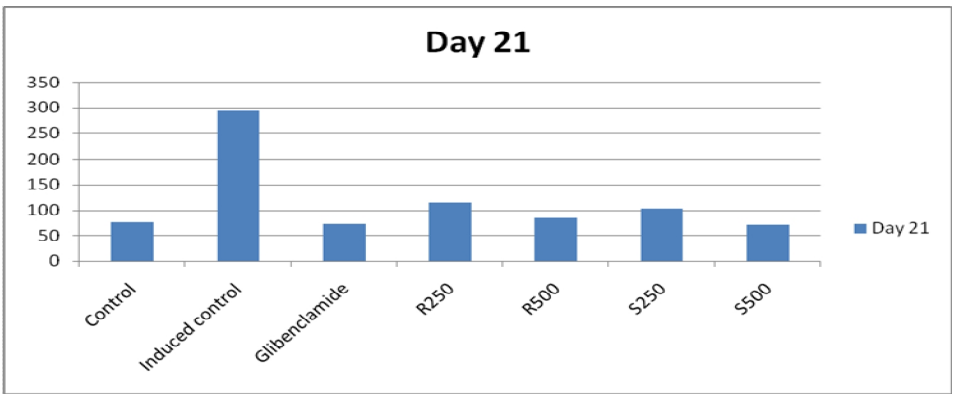


Figure 5b: Effect of different concentrations of methanolic extract of powdered roots and stem of *G. arborea* on total blood glucose level on day 21 in STZ induced diabetic rats

Each bar represents Mean ± SEM, N = 6 Control: 0.5% CMC, (1 ml/kg) p.o., Induced control: Streptozotocin, 45 mg/kg, i.v.Glibenclamide as standard (0.25mg/kg, p.o.), R250: methanolic extract of root (250mg/kg p.o.), R500: methanolic extract of root(500mg/kg p.o.), S250: methanolic extract of stem(250mg/kg p.o.), S500: methanolic extract of stem(5000mg/kg p.o.). *significantly different from control group P<0.001, ** significantly different from induced control group P<0.001

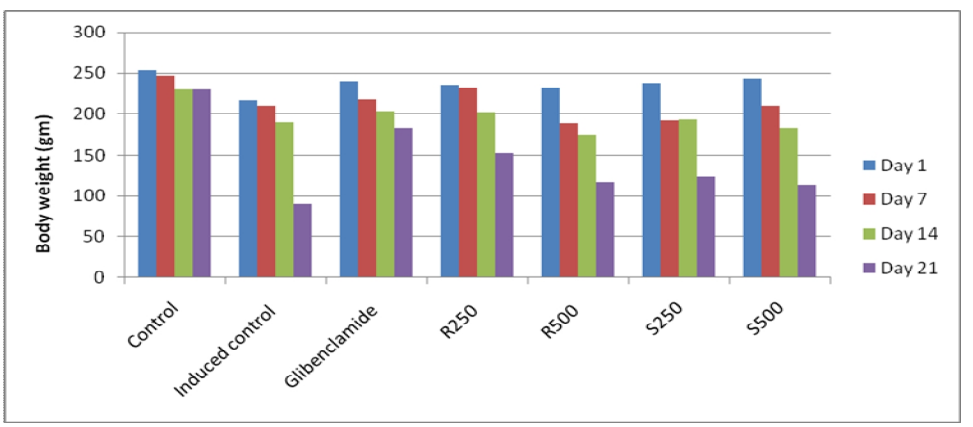


Figure 5c: Effect of different concentrations of methanolic extract of powdered roots and stem of *G. arborea* on body weight of STZ induced diabetic rats

N = 6 in each group, all values are mean ± SEM. Control: 0.5% CMC, (1 ml/kg) p.o., Induced control: Streptozotocin (STZ), 45 mg/kg, i.v.Glibenclamide as standard (0.25mg/kg, p.o.), R250: methanolic extract of root (250mg/kg p.o.), R500: methanolic extract of root(500mg/kg p.o.), S250: methanolic extract of stem(250mg/kg p.o.), S500: methanolic extract of stem(5000mg/kg p.o.).

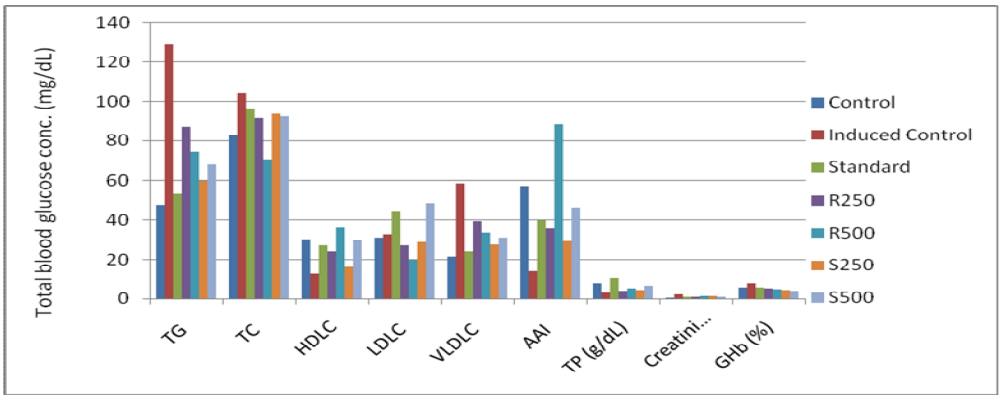


Figure 6: Effect of methanolic extract of powdered roots and stems of *G. arborea* on serum profile and whole blood (GHb) in streptozotocin induced diabetic rats

Control: 0.5% CMC, (1 ml/kg) p.o., Induced control: Streptozotocin, 45 mg/kg, i.v.Glibenclamide as standard (0.25mg/kg, p.o.), R250: methanolic extract of root (250mg/kg p.o.), R500: methanolic extract of root (500mg/kg p.o.), S250: methanolic extract of stem(250mg/kg p.o.), S500: methanolic extract of stem(5000mg/kg p.o.). *significantly different from control group P<0.01, ** significantly different from induced control group P<0.01

TG: Triglyceride; TC: Total cholesterol; HDLC: High density lipoprotein cholesterol; LDLC: Low density lipoprotein cholesterol; VLDLC: Very low density lipoprotein cholesterol; AAI: Antiatherogenic Index, TP: Total protein; GHb: Glycosylated Haemoglibin.

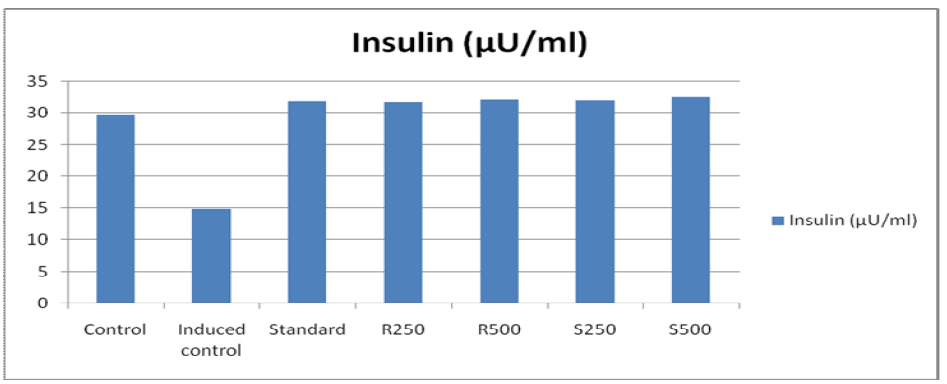


Figure7: Effect of methanolic extract of powdered roots and stems of *G. arborea* on insulin in streptozotocin induced diabetic rats

Control: 0.5% CMC, (1 ml/kg) p.o., Induced control: Streptozotocin, 45 mg/kg, i.v.Glibenclamide as standard (0.25mg/kg, p.o.), R250: methanolic extract of root (250mg/kg p.o.), R500: methanolic extract of root (500mg/kg p.o.), S250: methanolic extract of stem(250mg/kg p.o.), S500: methanolic extract of stem(5000mg/kg p.o.). *significantly different from control group P<0.05, ** significantly different from induced control group P<0.05

CONCLUSION

Regulation of blood glucose level in diabetes can prevent the various complication associated with the disease. Medicinal plants are used in several countries to manage diabetes mellitus which are thought to be less toxic than allopathic hypoglycaemic drugs, plant medicine are also easily available and affordable to many people. Selection of scientific and systemic approach for the biological evaluation of plants products based on their use in the traditional system of medicine forms the basis for an ideal approach in the development of new drugs from the plant. The experimental results of the present study conclude that the methanolic extracts of *G.arborea* root and stem are endowed with antidiabetic potential.

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