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ANTIDIABETIC AND ANTIHYPERLIPIDEMIC ACTIVITIES OF *FERONIA ELEPHANTUM* GUM IN STREPTOZOTOCIN INDUCED DIABETIC RATS.

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ABSTRACT

ORIGINAL RESEARCH ARTICLE

Gum of the plant *Feronia elephantum* Correa (Rutaceae) is useful in the treatment of diabetes in primitive books and conventional medicine. The present study was aimed to evaluate antidiabetic effect of *Feronia elephantum* gum extract in different doses along with antihyperlipidemic activity. In vitro antidiabetic activity was studied by inhibition of alpha amylase and alpha glucosidase enzymes. We have tried to separate some of fatty material from the feronia gum and assessed the pharmacological activity. Polyethylene glycol was converted to PEG-COOH using succinic anhydride by using conjugation chemistry technique and then was allowed to react with NHS (n- hydroxyl succinimide) to synthesize PEG-NH2 complex. Conjugation of plant compound containing COOH terminal was synthesized in the final reaction. The presence of Nitrogen and oxygen was identified in elemental CHN analysis. The conjugated compound was studied by NMR spectroscopy to find out the compounds conjugated with PEG complex. Single intraperitoneal injection of STZ at 55 mg/kg was used for induction of diabetes. Feronia gum aqueous extract was studied for pharmacological activities at a dose of 200 mg/kg, 300 mg/kg and 400 mg/kg and was compared with diabetic control group using Metformin as a standard drug. Real Time Polymerase chain reaction was carried out to study the mRNA expression of IRS2 gene, PPARα gene and GRIA2 gene. FGE 400 showed significant inhibition for alpha amylase and alpha glucosidase enzymes. Increased blood glucose level, glycosylated hemoglobin, body weight and urine volume was significantly decreased by treatment with FGE 400. Antidiabetic activity of FGE 400 was supported by insulin content, liver glycogen, antioxidant enzymes (CAT, SOD, GPx , GST);

hepatic glucose metabolic enzymes (Glucokinase, Glucose 6 phosphate dehydrogenase, glucose 6 phosphatase, fructose 1, 6 bisphosphatase), liver damage markers (SGOT, SGPT, ALP), histopathology study of pancreas and liver. FGE400 has positive effects for carbohydrate metabolism in liver (IRS2 gene), lowers the hepatic inflammation (PPARα gene) and increases insulin secretion (GRIA2 gene). From the study it can be concluded that *Feronia elephantum* gum extract is having prominent antidiabetic and antihyperlipidemic activities in dose dependent manner. Further studies are required for isolation and characterization of active chemical constituents.

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1. INTRODUCTION

Diabetes is reaching to pervasive extension admissible in developing countries, attacking more than 400 million people universally[1]. Type 2 diabetes mellitus (T2DM) is found to be the most widespread extensive form amongst diabetic patients[2].

In current years, extensive progress has done in diabetic sector for prohibition and treatment with glucose level control but the frequency of cardiovascular complexity is the main issue related to T2DM victims[3]. Now a days T2DM patients are undergoing from the treatment of antidiabetics, healthy food with everyday physical activities, examination of lipid profile parameters and arterial pressure monitoring. As T2DM is a treatable disease, fruitful prohibition and treatment is required to control diabetes.

Therefore, antidiabetic investigations are giving promising results by using medicinal herbs to provide various chemical constituents having effectiveness with less side effects with low cost[4]. Ethnopharmacological plants are having an important role in the investigation of novel remedies as they are rich source of bioactive chemical constituents effective as antioxidants, hypoglycemic, and hypolipidemic agents[5].

Feronia elephantum Correa, the wood apple (Synonyms: Feronia limonia Swingle; Limonia acidissima L.; Schinus limonia L.) family Rutaceae. *Feronia* elephantum tree is native in wild and dry plains of India and Ceylon and cultivated along roads and edges of fields. It is also called as monkey fruit, kath bel, curd fruit, elephant apple etc. It is a slow growing erect tree having fissured, scaly and ridged bark with zigzag twigs. Leaves are alternate having dark green color. Fruit is round to oval having grayish hard rind. Stem bark, trunk and branches oozes a yellowish white, transparent gum in the rainy season. It is used as a substitute or adulterant for gum arabic, and also used for artistic work. It consists of 35.5% arabinose and xylose, 42.7% d-galactose, and traces of rhamnose and glucuronic acid[6]. The stem bark when cut and broken oozes transparent gum which can be used in bowel diseases, dysentery and diarrhoea[35]. The gum is demulcent and constipating, and is useful in diarrhoea, dysentery, gastropathy, haemorrhoids and diabetes[36].

The Feronia gum, contained in the trunk and branches of the bel tree, counteracts diabetes by reducing the severity of the condition and helps to manage the flow, secretion, and balance of sugars in the bloodstream[7]. Feronia Gum extracted from its bark will control diabetes[8]. After the rains, the trunk and branches give off a gum called 'Feronia gum', which counteracts diarrhoea, dysentery and diabetes[9]. The bark of *Feronia elephantum* has something called Feronia gum, which can balance sugar levels in the blood by adjusting glucose and insulin levels[10].

Instead of these ethnomedicinal considerations, no any biological and chemical investigation have been carried out on *Feronia elephantum* gum for its use in diabetes. Hence the present study has carried out to find out ethnomedicinal utility of *Feronia elephantum* gum in diabetes and hyperlipidemia using streptozotocin model in rats as well as to isolate and analyze the bioactive chemical constituents from the gum responsible for the activities.

2. MATERIALS AND METHODS:

2.1. Drug and chemicals:

Streptozotocin from Biogenuix Medsystems Pvt. Ltd., New Delhi, metformin from Merck, Germany, α amylase and α glucosidase enzyme, starch, Dinitrosalisilic acid(DNS),p-nitrophenyl-β-D-glucopyranoside (p-NPG) and Acarbose from Sigma Aldrich (Merck KGaA Life science, India), solvents like petroleum ether (60-80^{o}C) AR, chloroform AR, ethanol AR, methanol AR, ethyl acetate AR, tween 80 AR, polyethylene glycol, sodium citrate dihydrate, benzene AR, citric acid, succinic anhydride, hydrochloric acid, sodium chloride, potassium chloride, calcium chloride were purchased from PCL, India. GOD/POD kit (Accu check glucometer) was procured from Roche diabetes care Inc.

2.2. Plant Material

Gum of Feronia elephantum was procured from Rahata taluka local area, Maharashtra and was authenticated by Dr. Wabale Anil Sopanrao, Head of Department of Botany, PVP college of Arts, Science and Commerce, Pravaranagar, vide letter number PVPC/ 2018-19-HD-71 dated 20/09/2018, Specimen no. SRV 789.

2.3. Separation of alcoholic and amino compounds by chemical reaction[11, 12]

Polyethylene glycol-COOH (PEG-COOH) was synthesized with slight modification in the method using PEG (MW 2000 g/mol; 2 g, 1 mmol), succinic anhydride (0.12 g, 1.2 mmol), were dissolved in 50 mL of anhydrous acetone[11]. The mixture was reflux for 12 h at 60 ^{0}C. The PEG-COOH was precipitated by diethyl ether, it was filtered and then dried under a vacuum (Reaction 1). Thin layer chromatography was carried out to monitor the reaction with Benzene: ethyl acetate (8:2). Carboxylic acid test was carried out for the product in dil. NaOH which shows product is soluble that confirms formation of PEG-COOH.

The PEG-COOH was converted to PEG-NH_2 by n- hydroxyl succinimide using DCC and DMAP chemistry. Briefly, PEG-COOH (1 g, 1.1 mmol), NHS (115 mg, 1mM) were taken in 10 mL ethyl acetate, DCC (206 mg), were added to a stirred solution in which DCC was used as a condensation agent added to remove the water formed during esterification[13]. The reaction mixture was stirred overnight and recovered (Reaction 2).

The PEG-COOH was subjected to react with plant extract (1gm) using DCC (206mg) in 10 mL ethyl acetate by the same way as reaction 2 to produce product (Reaction 3).

2.4 In vitro antidiabetic activity

2.4.1. Alpha amylase inhibitory activity[14]

The alpha amylase inhibitory activity of Feronia gum extract was carried out by performing the method of Nambirajana et al., 2018 with little modifications using acarbose as a standard. The experiment was repeated in thrice. Samples of Feronia gum extract (50 −300 µg/mL) was reacted with 20 mM, 50 µl phosphate buffer at pH 6.8, 10 µl α–amylase; 2 U/ml in 20 mM PBS buffer and were allowed to incubate at 25 °C for 30 min. After it, 20 µl of 1% soluble starch was added as a substrate (dissolved in 20 mM phosphate buffer, pH 6.8) and was allowed to incubate at 37°C for 30 min. 100 µl of colour reagent Dinitrosalicylic acid (DNS) was added and the reaction mixture was allowed to react at 95 °C for 10 min. Absorbance of final mixture was measured using UV-spectrophotometer at 540 nm. Control was devoid of plant extract and was treated same as above.

% inhibition = [(A540control – A540sample /

A540control) × 100]

Where, A540control: Absorbance value of control solution at 540 nm, A540sample - absorbance of reaction solution containing enzyme and buffer.

2.6.2. Alpha glucosidase inhibitory activity[15]

The experiment was carried in thrice using acarbose as a standard. Different concentrations (50 −300 µg/mL) of gum extract were added to tube containing 50 µl of 20 mM phosphate buffer at pH 6. 8, 10 µl alpha-glucosidase, 1 U/ml of 20 mM PBS and were allowed to incubate at 37 °C for 15 min. The catalytic reaction was initiated by addition of 20 µl of 5mM 4-Nitrophenyl α-D-glucopyranoside (p-NPG) and was allowed to incubate at 37 °C for 20 min. The reaction was stopped with addition of 50 µl of 0.1M sodium carbonate (Na_2CO_3) and the absorbance was recorded at 405 nm using UV spectrophotometer.

IC50 value of each sample was calculated as follows:

% of inhibition = (A405control – A405sample / A405control) × 100)

Where, A405control - Absorbance of control at 405 nm, A405sample - absorbance value of reaction solution containing enzyme and buffer.

2.7. In vivo antidiabetic activity

2.7.1. Animals

For assessment of in vivo antidiabetic activity of FGE, male wistar rats (250-300 gm) were used. Animals with their feed was procured from LACSMI BIOFARMS PVT LTD, Pune. The experimental protocol was approved by institutional animal ethical committee; Proposal number: ARCMR/PIMS/IAEC/1801. Constant temperature (22 ± 2°C), humidity (55%) and light-dark condition (12/12 h light/dark) was kept for animals.

2.7.2. Induction of diabetes

STZ 55 mg/kg body weight, single intraperitoneal injection (dissolved in 0.1 M sodium citrate buffer pH 4.5) was used for induction of diabetes in overnight fasted rats. After STZ injection, animals were given free access for food and water with 5 % glucose solution for 18 hrs was provided for drinking to control hypoglycemic shock. After 5 days of STZ injection, animals with fasting blood glucose level greater than 250 mg/dl were observed as diabetic and were used for experiment. Rats were equally distributed into seven groups of six in each group and were provided with treatment for next 20 days as follows: NC: Normal Control Group; DC: Disease Control Group, diabetic rats maintained on regular rat food and drinking water ad libitum (STZ 55 mg/kg); PC: Positive control group, Diabetic rats were treated with standard drug Metformin (150 mg/kg); FGE200 (Diabetic rats + feronia gum extract 200 mg/kg) p.o.; FGE300 (Diabetic rats + feronia gum extract 300 mg/kg) p.o.; FGE400 (Diabetic rats + feronia gum extract 400 mg/kg) p.o.

2.7.3. Determination of blood glucose level, body weight changes and urine volume

Parameters as fasting blood glucose level, body weight changes and urine volume in ml/5 hr was checked for all experimental groups during the study. Blood glucose level was evaluated using glucometer (Accu check glucometer, Roche diabetes care Inc.) by tail flick method.

2.7.4. Determination of lipid profile, liver damage markers, insulin level

Blood was taken in a dry test tube and coagulated for 30 min. At 2000 rpm, 10 min, serum was centrifuged, and the clear supernatant was used to study TC (total cholesterol), TG (triglycerides), HDL (high density lipoprotein cholesterol), LDL (low density lipoprotein cholesterol), VLDL (very low density lipoprotein cholesterol), ALP (Alkaline phosphatase), SGOT (Serum Glutamic Oxaloacetic Transaminase), SGPT (Serum Glutamic Pyruvic Transaminase) by performing procedure as per manufacturer instructions (Sigma Aldrich Merck KGaA Life

science, India). Plasma insulin level was evaluated utilizing the insulin radioimmunoassay with ELISA kit (Linco Research, Inc., St. Charles, MO).

2.7.5. Estimation of activities of hepatic glucose metabolic enzymes and glycogen content

Liver tissue was separated on last day of experiment and integrated in ice-cold isotonic physiological saline and at 3000 rpm, 4 °C centrifuged for 10 min. Supernatant was used to study enzymes of glucose metabolism. Procedures of Glucokinase, Glucose 6 phosphate dehydrogenase, glucose 6 phosphatase, fructose 1, 6 bisphosphatase and Glycogen content were carried out as per the manufacturer's guidelines (Sigma Aldrich Merck KGaA Life science, India).

2.7.6. Evaluation of Glycosylated hemoglobin

On day 1 and 20 of treatment, blood of experimental animals from the retro-orbital sinus was gathered in EDTA tubes and studied for glycosylated hemoglobin content on fully automated HbA1c analyzer (Merilyzer GluQuantA1c diabetes machine, Germany) working on ion exchange chromatography with HPLC principle.

2.7.7. Assay of circulatory and pancreatic antioxidant enzymes[16, 17, 18]

Pancreas was separated and integrated in Tris HCl buffer at pH 7.4 and at 12000 rpm, 4 °C centrifuged for 30 min. The activities of antioxidant enzymes were performed according to standard procedures of Kakkar et al., 1984; Rotruck et al., 1973 and Habig et al., 1974 by collecting supernatants from plasma and pancreas to perform assays and actions of antioxidant enzymes CAT (catalase), SOD (superoxide dismutase), GPx (glutathione peroxidase) and GST (Glutathione-S-transferase).

2.7.8. Histopathological studies

Animals were sacrificed by decapitation under ether anesthesia. Pancreas and liver tissues were immediately dissected and washed in ice-cold saline solution. A part of pancreas and liver was fixed in 10% neutral formalin. After fixation, tissues were submerged in paraffin; 4-5 um thick solid sections were made using rotary microtome. Hematoxylin-eosin was used for staining the sections and light microscope was used for histopathological observations.

2.7.9. Real Time Polymerase chain reaction to study the mRNA expression of IRS2 gene, PPARα gene and GRIA2 gene

According to instructions of manufacturer on kit (Qiagen RNA isolation kit, Germany), liver tissue total RNA was isolated. 1 µg of RNA was utilized for cDNA synthesis with oligo dTs to final volume 20 µL (Qiagen cDNA synthesis kit, Germany) as per the instructions with kit. IRS2, PPARα and GRIA2 primers were commercially obtained from Qiagen, Germany. qPCR (Roche, USA) was used to study gene expression by diluting 1 µl of cDNA (10 µL reaction with 1X SYBR green, 0.5 µL of each primer) on an Mx3000P instrument (Stratagene, Cedar Creek, TX, USA). Standard control was Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Parameters used to study single step real time PCR were, primary denaturation at 95° C for 3 min, followed by 50 cycles of denaturation at 95°C for 5 s, 60°C for 40 s, and 72°C for 40 s. For quantification of results of qPCR, the ΔΔCt method was used.

2.8. Statistical analysis

The results were reported as mean ± standard error mean (SEM) or standard deviation (SD). Statistical analysis of all data obtained was evaluated using Graph Pad Prism 8.02; one-way ANOVA followed by student t-test. Dunnett's comparison test was utilized to calculate statistical significance between drug-treated groups and negative control group. $p < 0.05$ was considered significant.

3. RESULTS

3.1 Separation of alcoholic and amino compounds by chemical reaction

The product of reaction 2 is PEG-NH2 was subjected to react with plant extract and followed reaction 3 to get another product (Reaction 4). Products from reaction 3 and reaction 4 was recovered and subjected to purification by dialysis membrane in methanol solvent. About 10-15 cm dialysis membrane tube was soaked in water for 1hr and tie one end with thread. Products from reaction 3 and reaction 4 was added separately in to the tube and tied another end properly keeping small space (air bubble) and tie another end of dialysis membrane using thread. The samples were kept in a beaker containing methanol on magnetic stirrer continuously stirring for 24 hrs and changed the methanol with fresh methanol and repeated the same procedure for next 24 hrs. The dialysis bags were removed and separated products and recovered.

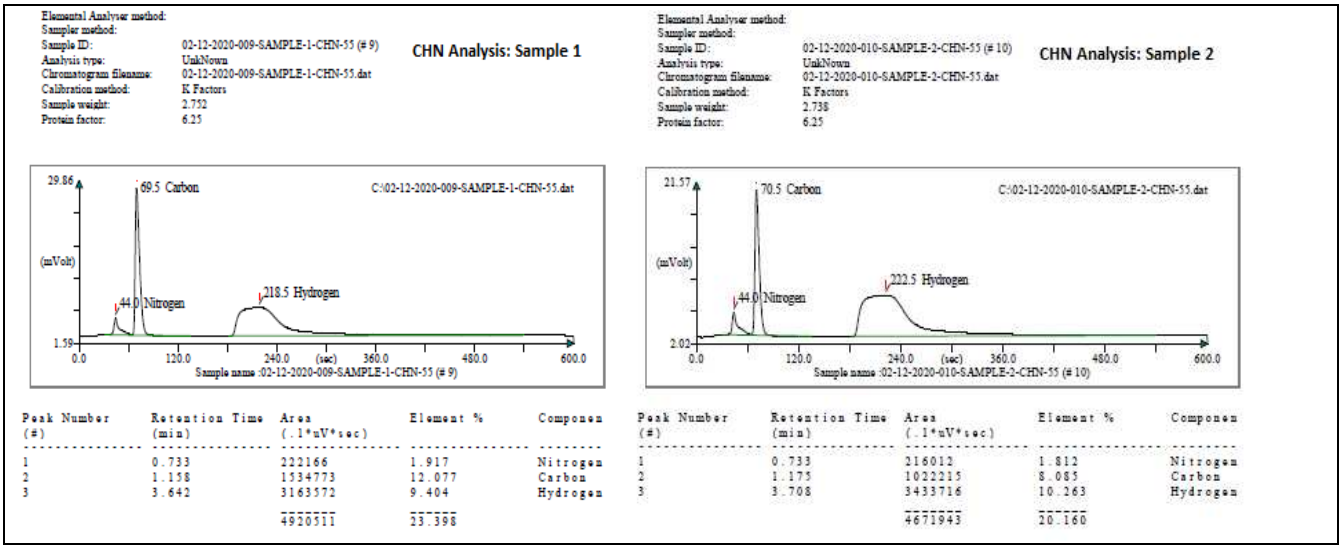

Fig 1: CHNSO Analysis of Sample 1(Product of Rea 3) and Sample 2 (Product of Rea 4).

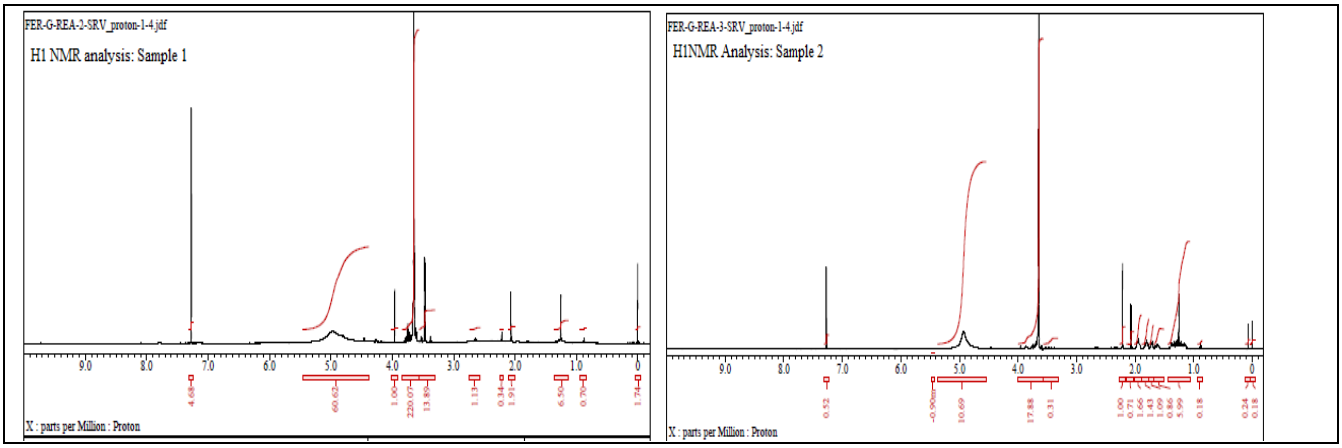

Fig 2: H1NMR Analysis of Sample 1(Product of Rea 3) and Sample 2 (Product of Rea 4).

NMR peaks of Compound from reaction 3 showed that there is formation of conjugate of unsaturated fatty acids with PEG. The oleic acid, linoleic acid and also linolenic acid is found in the mixture as there are number of small peaks near 1.2 ppm of unsaturation in the compound.

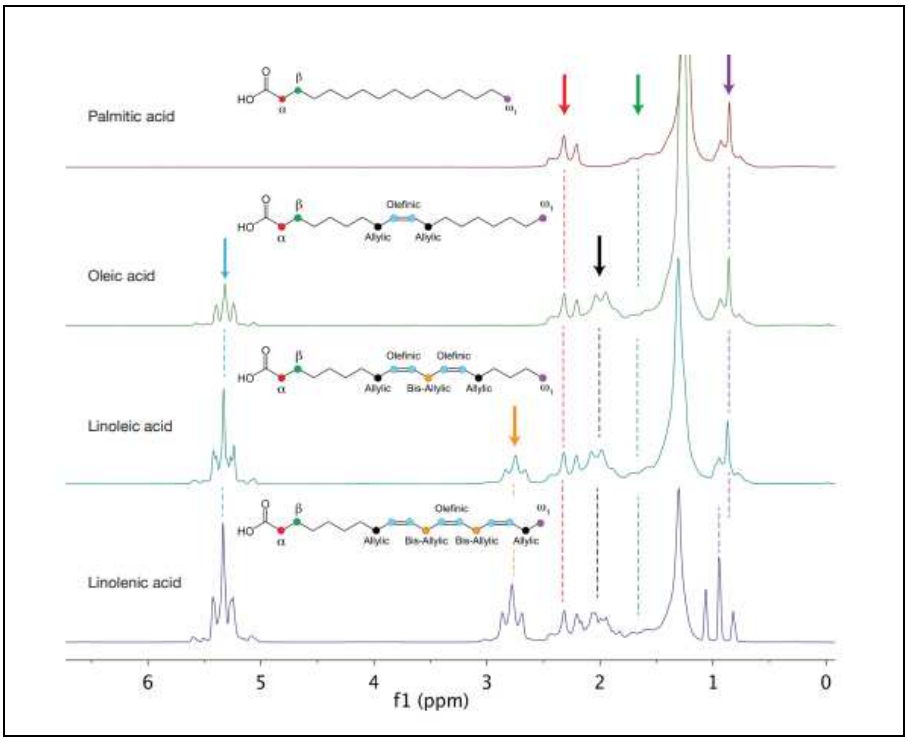

Fig 3: Reference fatty acid peaks

3.3. In vitro antidiabetic activity

3.3.1. Alpha amylase inhibitory activity

FGE shows 50% inhibition of alpha amylase enzyme at a concentration of 162.00 µg. IC50 value standard acarbose was 118.50 µg. The percentage inhibition was increased with concentration (Fig. 4 A).

3.3.2. Alpha glucosidase inhibitory activity: Carbohydrate digestion is decreased by inhibition of alpha glucosidase. The ratio of inhibition percentage is found to be concentration dependent. IC50 value of FGE was found to be 149.20 µg. The standard acarbose inhibits the enzyme at IC50 value of 107.50 µg. Alpha glucosidase inhibition activity predicts that among FGE possess highest inhibition activity (Fig. 4 B).

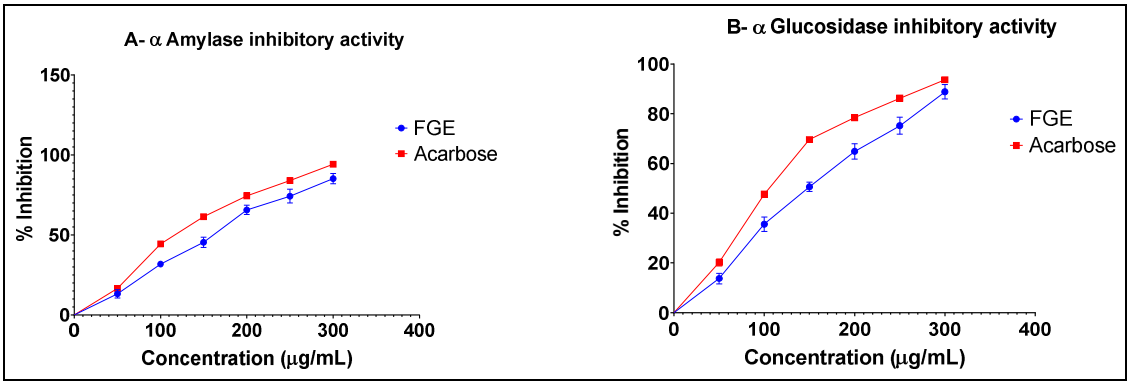

Fig. 4 In vitro antidiabetic activity**;** (A) α amylase inhibitory activity, (B) α glucosidase inhibitory activity. Data are expressed as "mean + SD" (N=3). FGE: *F. elephantum* gum extract.

3.5. In vivo antidiabetic activity

3.5.1. Effect of FGE on blood glucose level, body weight changes and urine volume

Fig. 5A, 5B and 5C illustrates blood glucose level, body weight changes and urine volume analysis on treatment day 1, 5, 10, 15 and 20 respectively. During study period diabetic control rats showed significant increase in blood glucose level, urine volume and significant decrease in body weight. Treatment with FGE 400 and metformin resulted significant decrease ($p < 0.001$) in blood glucose level, urine volume and significant increase ($p < 0.001$) in body weight of rats.

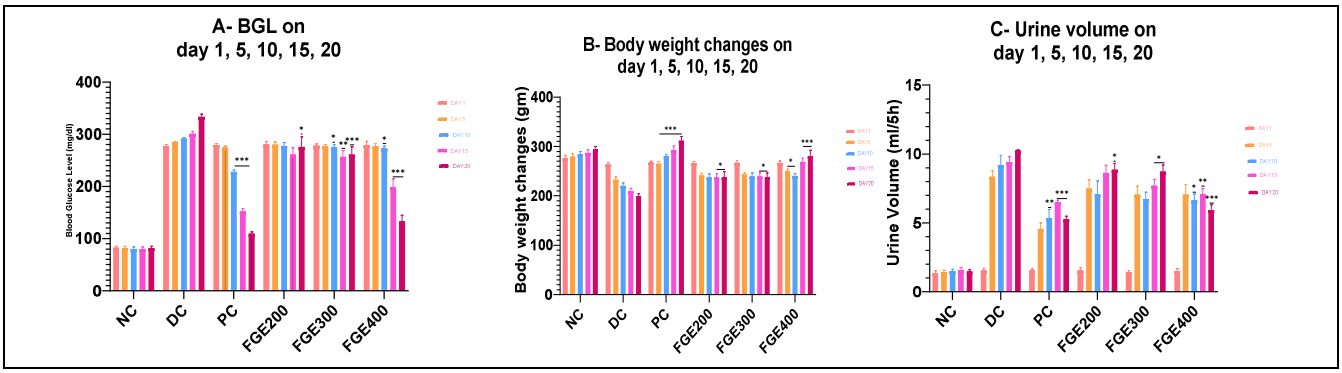

Fig. 5 Changes in blood glucose level, body weight and urine volume of experimental animal groups (A) Changes in blood glucose level of experimental animals, (B) Changes in body weight of experimental animals, (C) Changes in urine volume of experimental animals. Values are expressed as mean ± SEM, n=6. *P< 0.05, **P<0.01, ***P <0.001 indicates the significant difference between disease control and treatment groups by using one-way ANOVA followed by Dunnette's multiple comparison test. NC: Normal Control Group; DC: Disease Control Group, diabetic rats maintained on regular rat food and drinking water ad libitum (STZ 55 mg/kg); PC: Positive control group, Diabetic rats were treated with standard drug Metformin (150 mg/kg); FGE200 (Diabetic rats + feronia gum extract 200 mg/kg) p.o.; FGE300 (Diabetic rats + feronia gum extract 300 mg/kg) p.o.; FGE400 (Diabetic rats + feronia gum extract 400 mg/kg) p.o.

3.5.2. Effect of FGE on lipid profile, liver damage markers and insulin level

All groups were tested for lipid profile parameters TC, TG, HDL, LDL and VLDL (Fig. 6A and Fig. 6B) on day 20 of study. In disease control group increased levels of total cholesterol, triglycerides, low density lipoproteins and very low density lipoprotein whereas decreased level of high density lipoprotein level was observed. Significant results were obtained by treatment with FGE 400 ($p < 0.001$). Levels of liver damage markers was checked by performing hepatic function test (Fig. 6C). Increased level of liver enzymes ALP, SGOT, SGPT was observed in disease control group. This level was significantly decreased by treatment with FGE400 ($p < 0.001$). All treatment groups were checked for insulin level on day 20 (Fig.6D). Treatment with FGE400 and metformin showed significant increase ($p < 0.001$) in insulin level. Dose dependent increase in insulin level was observed for FGE treated groups. FGE400 treated group showed significant results for the studied parameters as lipid profile, liver damage markers and insulin level.

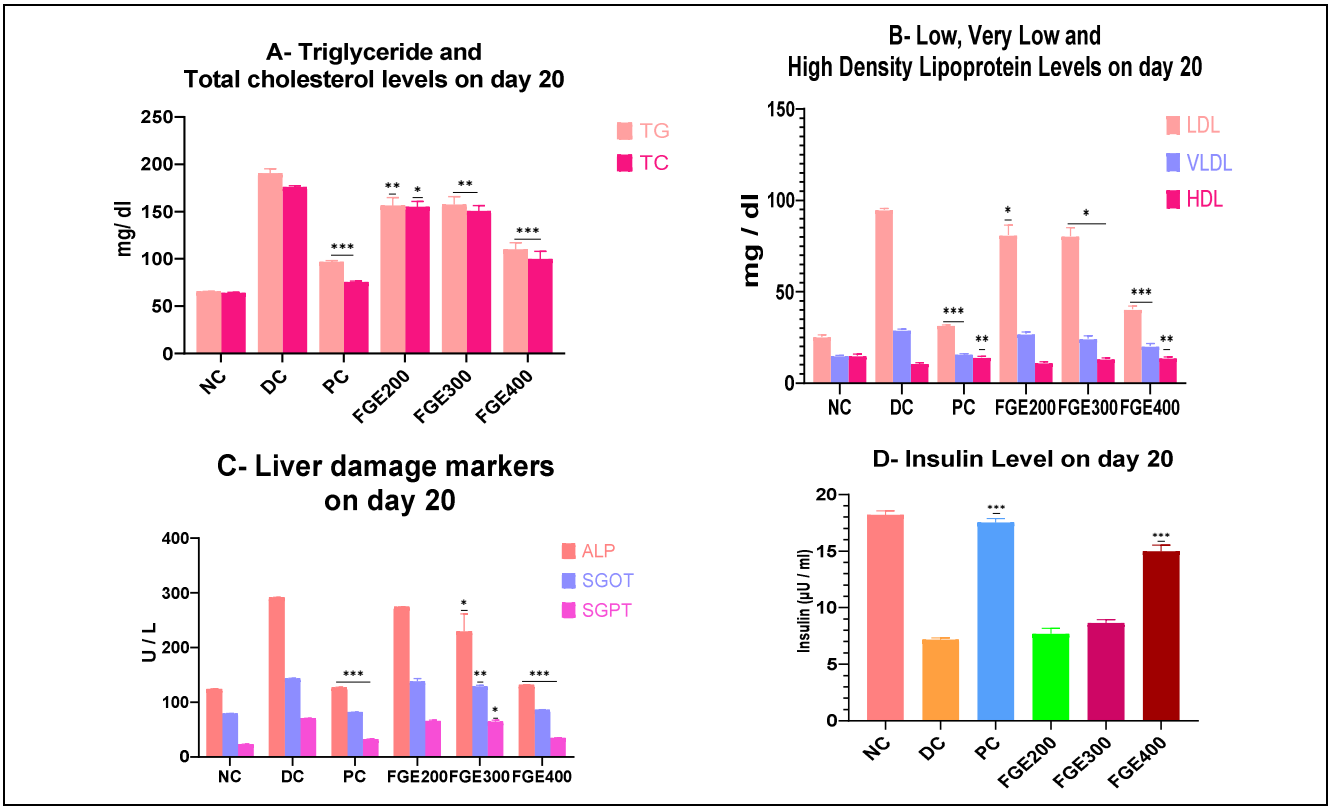

Fig. 6 Changes in lipid profile levels, liver damage markers and insulin level of experimental animal groups

(A) Changes in triglyceride and total cholesterol level of experimental animals, (B) Changes in low density lipoprotein level, very low density lipoprotein level and high density lipoprotein level on experimental animals, (C) Changes in liver damage markers levels of experimental animals, (D) Changes in insulin level of experimental animals. Values are expressed as mean ± SEM, n=6. *P< 0.05, **P<0.01, ***P <0.001 indicates the significant difference between disease control and treatment groups by using one-way ANOVA followed by Dunnette's multiple comparison test. NC: Normal Control Group; DC: Disease Control Group, diabetic rats maintained on regular rat food and drinking water ad libitum (STZ 55 mg/kg); PC: Positive control group, Diabetic rats were treated with standard drug Metformin (150 mg/kg); FGE200 (Diabetic rats + feronia gum extract 200 mg/kg) p.o.; FGE300 (Diabetic rats + feronia gum extract 300 mg/kg) p.o.; FGE400 (Diabetic rats + feronia gum extract 400 mg/kg) p.o.

(B)

3.5.3. Effect of FGE on hepatic glucose metabolic enzymes and glycogen content

The levels of hepatic glucose metabolic enzymes and glycogen content is depicted in Table 1. Decreased levels of hepatic glucose metabolic enzymes and glycogen content was found in disease control group. Treatment with FGE showed dose dependent significant increased levels of glycogen, activities of glucokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase and fructose-1, 6-biphosphatase. Treatment with metformin and FGE400 showed highly significant ($p < 0.001$) results than that of other treatment groups.

Table 1: Effect of FGE on hepatic glucose metabolic enzymes and glycogen content

Parameters	**NC**	**DC**	**PC**	**FGE200**	**FGE300**	**FGE400**
1.Glycolytic enzymes						
Glucokinase (u/h/mg protein)	0.65 ± 0.04	0.19 ± 0.02	0.58 ± 0.03***	0.24 ± 0.01	0.34 ± 0.04*	0.55 ± 0.04***
Glucose-6-phosphatedehydrogenase (lU/L)	4.82 ± 0.15	1.89 ± 0.19	4.26 ± 0.08***	1.92 ± 0.25	2.94 ± 0.33*	3.86 ± 0.21***
2. Gluconeogenic enzymes						
Glucose-6-phosphatase (µmoles of Pi liberated/min/mg of protein)	0.179 ± 0.01	0.309 ± 0.01	0.205 ± 0.01***	0.277 ± 0.02	0.241 ± 0.02*	0.216 ± 0.02**
Fructose 1,6 bis phosphatase (µmoles of Pi liberated/h/mg of protein)	0.354 ± 0.01	0.696 ± 0.02	0.398 ± 0.01***	0.679 ± 0.02	0.555 ± 0.05*	0.407 ± 0.04***
3. Hepatic glycogen						
Glycogen (mg/g liver)	21.56 ± 0.74	10.47 ± 0.29	19.14 ± 0.19***	12.26 ± 0.42*	12.13 ± 0.41*	17.14 ± 0.56***

Values are expressed as mean ± SEM, n=6. *P< 0.05, **P<0.01, ***P <0.001 indicates the significant difference between disease control and treatment groups by using one-way ANOVA followed by Dunnette's multiple comparison test. NC: Normal Control Group; DC: Disease Control Group, diabetic rats maintained on regular rat food and drinking water ad libitum (STZ 55 mg/kg); PC: Positive control group, Diabetic rats were treated with standard drug Metformin (150 mg/kg); FGE200 (Diabetic rats + feronia gum extract 200 mg/kg) p.o.; FGE300 (Diabetic rats + feronia gum extract 300 mg/kg) p.o.; FGE400 (Diabetic rats + feronia gum extract 400 mg/kg) p.o.

3.5.4. Effect of FGE on Glycosylated hemoglobin

On day 1 and 20, glycosylated HB content was examined for all treatment groups (Fig. 7). High percentage of glycated Hb was observed on day 1 of study. After treatment, the glycated Hb content was significantly reduced ($p < 0.001$) in metformin treated group and FGE400 group. FGE400 recovered glycated Hb percentage level in more amount than FGE200 and FGE300 when compared with disease control rats.

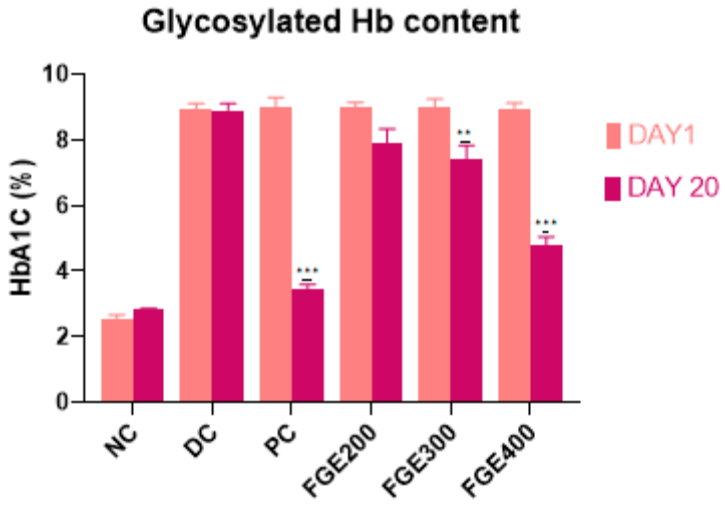

Fig. 7 Glycated hemoglobin percentage of experimental animal groups
Values are expressed as mean ± SEM, n=6. *P< 0.05, **P<0.01, ***P <0.001 indicates the significant difference between disease control and treatment groups by using one-way ANOVA followed by Dunnette's multiple comparison test. NC: Normal Control Group; DC: Disease Control Group, diabetic rats maintained on regular rat food and drinking water ad libitum (STZ 55 mg/kg); PC: Positive control group, Diabetic rats were treated with standard drug Metformin (150 mg/kg); FGE200 (Diabetic rats + feronia gum extract 200 mg/kg) p.o.; FGE300 (Diabetic rats + feronia gum extract 300 mg/kg) p.o.; FGE400 (Diabetic rats + feronia gum extract 400 mg/kg) p.o.

3.5.5. Effect of FGE on circulatory and pancreatic antioxidant enzymes

Table 2 illustrates the effect of FGE on CAT, SOD, GST and GPx in plasma and pancreas. It can be revealed that disease control group showed lower levels of activities of enzymatic antioxidants in circulation and pancreas, whereas after treatment with FGE400 and metformin, activities of enzymatic antioxidants was significantly increased ($p < 0.001$). Dose dependent results were observed in FGE groups. FGE400 increased the activities of antioxidant enzymes.

Table 2: Effect of FGE on pancreatic antioxidant enzymes.

Parameter	NC	DC	PC	FGE200	FGE300	FGE400
SOD						
Plasma (U^1/mg protein)	9.32±0.21	4.23±0.17	8.06±0.14***	4.38±0.17	5.66±0.52*	7.63±0.22***
Pancreas (U^1/mg protein)	5.34±0.15	2.42±0.24	4.43±0.2***	2.44±0.21	3.52±0.32*	4.28±0.18***
CAT						
Plasma (U^2/mg protein)	71.83±1.24	41.9±1.6	60.24±1.22***	42.35±1.67	47.48±1.47*	57.4±1.56***

Pancreas (U^2/mg protein)	6.86±0.24	2.48±0.17	5.89±0.22***	2.01±0.18	3.68±0.39*	5.31±0.27***
GST						
Plasma (U/mg protein)	35.37±0.53	15.84±0.31	29.25±0.55***	17.39±0.9	20.68±1.76*	26.88±1.23***
Pancreas (U/mg protein)	3.96±0.34	1.84±0.04	2.99±0.28***	1.97±0.09	2.38±0.11*	2.77±0.08***
GPx						
Plasma (U^3/mg protein)	46.69±0.84	22.22±0.85	37.83±0.59***	22.88±1.3	28.11±1.9*	34.07±1.08***
Pancreas (U^3/mg protein)	0.49±0.01	0.18±0.01	0.4±0.01***	0.21±0.01	0.23±0.02*	0.35±0.01***

Values are expressed as mean ± SEM, n=6. *P< 0.05, **P<0.01, ***P <0.001 indicates the significant difference between disease control and treatment groups by using one-way ANOVA followed by Dunnette's multiple comparison test. NC: Normal Control Group; DC: Disease Control Group, diabetic rats maintained on regular rat food and drinking water ad libitum (STZ 55 mg/kg); PC: Positive control group, Diabetic rats were treated with standard drug Metformin (150 mg/kg); FGE200 (Diabetic rats + feronia gum extract 200 mg/kg) p.o.; FGE300 (Diabetic rats + feronia gum extract 300 mg/kg) p.o.; FGE400 (Diabetic rats + feronia gum extract 400 mg/kg) p.o. U^1 – The amount of enzyme required to inhibit 50% NBT reduction/min for SOD. U^2 – Micromoles of H2O2 utilized/per mg of protein for catalase. U^3 – Micromoles of glutathione oxidized/mg of protein for GPx.

3.5.6. Histopathological analysis

3.5.6.1. Effect of FGE on histopathological analysis of pancreatic tissue samples

Histopathological studies of pancreas (Fig. 8) revealed normal arrangement of islets and beta cells for normal control group. Disease control group showed astonishing draining of islets and beta cells with dispersed pancreas. Metformin treated group showed general features of pancreas with large development of islets and beta cells. FGE200 showed minute growth of beta cells while islets were not seen. FGE300 showed minute evolution of beta cells with islets. FGE400 showed broad ducts with acinus cells and normal growth of islets with beta cells.

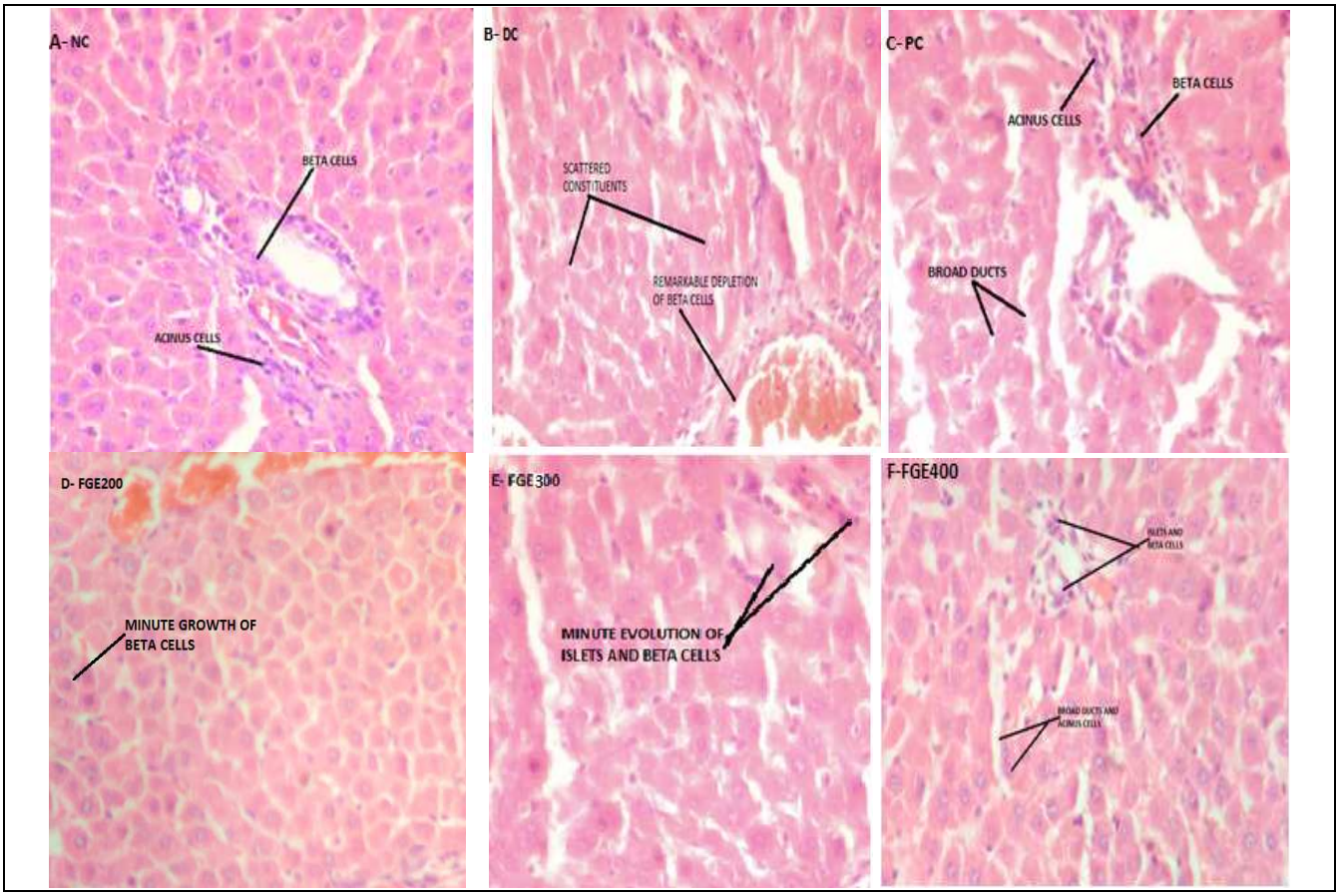

Fig. 8 Microscopic images for histopathology of pancreas tissue samples
NC: Normal Control Group; DC: Disease Control Group, diabetic rats maintained on regular rat food and drinking water ad libitum (STZ 55 mg/kg); PC: Positive control group, Diabetic rats were treated with standard drug Metformin (150 mg/kg); FGE200 (Diabetic rats + feronia gum extract 200 mg/kg) p.o.; FGE300 (Diabetic rats + feronia gum extract 300 mg/kg) p.o.; FGE400 (Diabetic rats + feronia gum extract 400 mg/kg) p.o.

3.5.6.2. Effect of FGE on histopathological analysis of liver tissue samples

Fig. 9 reveals histopathological observations of liver tissues for all groups. Normal aspects of liver with central vein and hexagonal lobules were observed in normal control group. Disease control group revealed awful destruction of liver tissue with high swelling. Treatment with metformin revealed normal structure of hepatic cells with normal liver tissues. FGE200 showed very less cure of hepatocyte inflammation. FGE300 showed less protection for hepatic swelling. FGE400 revealed recovery of hepatocytes with central vein and renewed liver tissues.

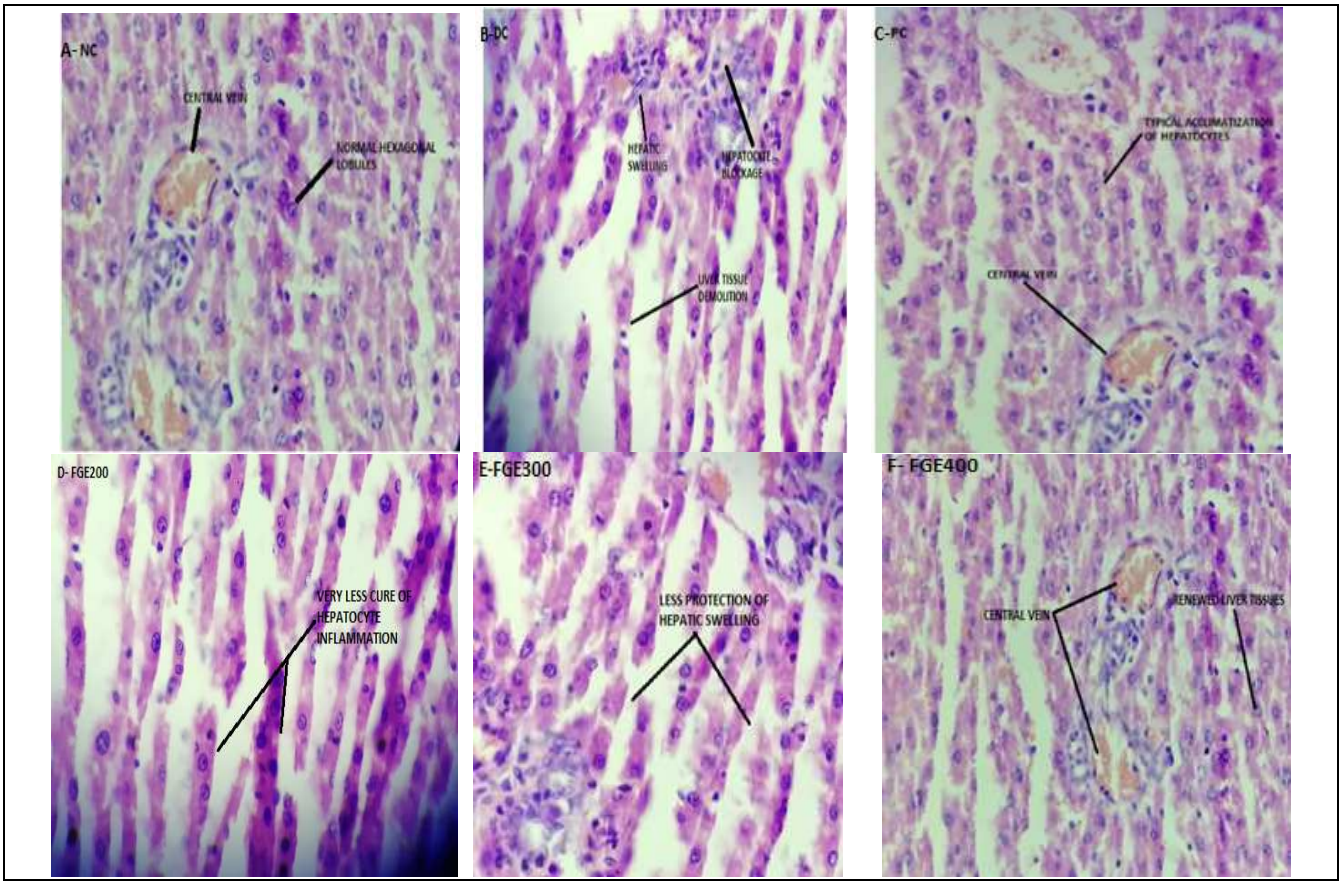

Fig. 9 Microscopic images for histopathology of liver tissue samples
NC: Normal Control Group; DC: Disease Control Group, diabetic rats maintained on regular rat food and drinking water ad libitum (STZ 55 mg/kg); PC: Positive control group, Diabetic rats were treated with standard drug Metformin (150 mg/kg); FGE200 (Diabetic rats + feronia gum extract 200 mg/kg) p.o.; FGE300 (Diabetic rats + feronia gum extract 300 mg/kg) p.o.; FGE400 (Diabetic rats + feronia gum extract 400 mg/kg) p.o.

3.5.7. mRNA expression of IRS2 gene, PPARα gene and GRIA2 gene in rat liver

Fig. 10 illustrates the outcome of energy percentage obtained from FGE on mRNA expression of IRS2 gene (Fig. 10A), PPARα gene (Fig. 10B) and GRIA2 gene (Fig. 10C) in rat liver. Experiment illustrates that the gene expression of IRS2, PPARα and GRIA2 gene mRNA was decreased in disease control group which after treatment was increased and was found to be significantly high ($p< 0.001$) in FGE400 group than that of other groups. Hence, FGE400 has positive effects for carbohydrate metabolism in liver (IRS2 gene), lowers the hepatic inflammation (PPARα gene) and increases insulin secretion (GRIA2 gene).

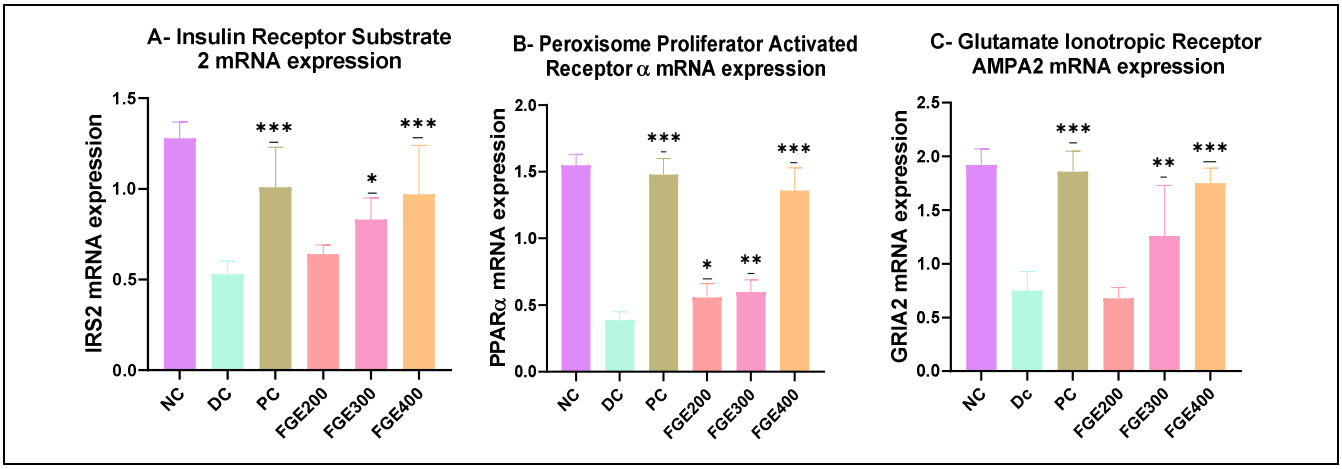

Fig. 10 Effect of FGE on liver tissue IRS2, PPARα and GRIA2 mRNA expression level (A) Expression pattern of IRS2 gene, (B) Expression pattern of PPARα gene, (C) Expression pattern of GRIA2 gene. Values are expressed as mean ± SEM, n=6. *P< 0.05, **P<0.01, ***P <0.001 indicates the significant difference between disease control and treatment groups by using one-way ANOVA followed by Dunnette's multiple comparison test. NC: Normal Control Group; DC: Disease Control Group, diabetic rats maintained on regular rat food and drinking water ad libitum (STZ 55 mg/kg); PC: Positive control group, Diabetic rats were treated with standard drug Metformin (150 mg/kg); FGE200 (Diabetic rats + feronia gum extract 200 mg/kg) p.o.; FGE300 (Diabetic rats + feronia gum extract 300 mg/kg) p.o.; FGE400 (Diabetic rats + feronia gum extract 400 mg/kg) p.o.

4. DISCUSSION

Present investigation was carried out to check the antidiabetic and antihyperlipidemic activities of Feronia elephantum gum in Streptozotocin induced diabetic rats. Blood glucose levels were significantly reduced after administration of Feronia elephantum gum extract in dose dependent manner showing antihyperglycemic effect of the gum extract. In vitro study showed significant inhibition of alpha amylase and alpha glucosidase enzymes by Feronia gum extract. A significant reduction was observed in all biochemical parameters.

After administration of FGE 200, FGE 300 and FGE 400 for 20 days, levels of blood sugar were significantly decreased in diabetic rats in dose dependent manner. A significant decline was noted on days 5, 10, 15, and 20, indicating that gum of the plant possesses antidiabetic properties. Inspite of its application in traditional medicine, research on a scientific basis to investigate its antihyperglycemic properties has not been done previously. No any investigations regarding phytochemical study of gum has been carried out yet, but bark of the plant Feronia elephantum has shown the presence of tannins, glycosides, flavonoids, steroids, phenols, coumarins, alkaloids and triterpenoids.

Gums and gum resins are obtained from wood and bark produced by duct structure[19]. The duct has close morphological similarity with resin producing ducts. Plant gums are specially carbohydrates complexes that may contain proteins resins and other components[20]. The plant gums unlike other parts, produces less amount of few secondary metabolites in reckonable amount[21]. We have tried to separate some of fatty material from the feronia gum and assessed the pharmacological activity. The conjugation chemistry technique was used using polyethylene glycol to conjugate the phytochemical containing –COOH and –NH2 group[22]. Polyethylene glycol was converted to PEG-COOH using succinic anhydride and

then allow to react with NHS (n- hydroxyl succinimide) to synthesize PEG-NH2 complex which is reactive with the compound containing terminal COOH in the gum solution. The reaction was carried out using DCC coupling chemistry. Conjugation of plant compound containing COOH terminal was synthesized in the final reaction. The presence of Nitrogen and oxygen was identified in elemental analysis (Figure 1). The conjugated compound was studied by NMR spectroscopy to find out the compounds conjugated with PEG complex. The standard pattern of NMR signals of PEG 2000 was observed with additional peaks for the compound (Figure 2). The proton NMR spectra of the complex showed characteristics signal of conjugated compounds having different group attached. It is typical spectra of mixture of unsaturated fatty acids that could identify amount of maximum unsaturation in the compound. The saturated chain of UFA containing methyl and methylene CH2 overlapping at about 1.3 ppm. Oleic acid double bound at carbons 9 is coupled to a single olefinic hydrogen overlapped with a signal at about 4.8 ppm. The two double bonds of Linoleic acid at carbon 6 and 9 at 1.9 ppm. The peaks at 1.6 to 1.9 suggest presence of mixture of these unsaturated fatty acids including traces of linolenic acid in the plant gum. The shielding of these protons are due to conjugation with PEG 2000. The plant gum unsaturated fatty acid mixture was identified in the study which gave an idea of presence of oleic acid, linoleic acid and also linolenic acid. Unsaturated fatty acids are also reported by Vinod et al[23] from plant exudates. Only few reports show presence of secondary metabolites including sterols, aglycons, and fatty acids in plant gums and its pharmacological activity[24, 25]. The guggul gum was identified that used in management of lipid profile in animals.

The probable hypoglycemic mode of action of the plant was probably through amplification of beta cells insulin secretion or because of increased peripheral blood glucose. Glucose molecules interacts with hemoglobin resulting in formation of glycosylated hemoglobin, and as there is increase in glycosylated hemoglobin there will be increase in blood glucose level[26]. Hence glycosylated hemoglobin is having important role for evaluation of diabetes status. Formation of HbA1c in RBC's is a continuous, stable and permanent process which shows definite blood glucose level and is not affected by diet or exercise[27]. Therefore, HbA1c level is found to be most accepted and trusted parameter in prevention and cure of diabetes[28]. In the present investigation, significant decrease in HbA1c in FGE 400 rats was observed after administration of gum extract for 20 days[29]. This may be due to increased secretion of insulin which lead to decreased blood glucose level; decreased HbA1c level is directly proportional to decreased blood glucose level[30].

Previous study reveals that altered lipid profile leads to cardiac and diabetic issues.

Present study DC group showed increased levels of TC, TG and LDL whereas decreased levels of HDL was observed. Administration of FGE 400 showed significant decreased levels of TC, TG and LDL while increased level of HDL was found. This was a very important point during investigational period.

Present investigation revealed significant increased urine volume level in DC, which reveals renal failure. Increased urine volume in DC may be due to increased protein catabolism or amino acid deamination for gluconeogenesis[31]. Treatment with FGE 400 significantly decreased the urine volume level which might be due to its antidiabetic property.

Liver enzyme markers are indicative for liver functioning. For carbohydrate metabolism, liver shows major role. Increased blood glucose level can cause severe destruction of liver. DC group showed

increased SGOT, SGPT and ALP levels which can cause serious damage to liver[32]. Treatment with FGE 400 revealed significantly high levels of SGOT, SGPT and ALP which can recover liver tissues. In current investigation, glucose metabolic enzyme assessment was carried out. DC group showed decreased level of glycolytic enzymes and increased level of gluconeogenic enzymes which was retained to normal level by treatment with FGE 400.

Present study shows curative effect of FGE 400 for increased level of antioxidant enzymes SOD, CAT, GST and GPx which lead to increased antioxidant activity.

Histopathological studies of pancreas revealed normal arrangement of islets and beta cells for normal control group. Disease control group showed astonishing draining of islets and beta cells with dispersed pancreas. Metformin treated group showed general features of pancreas with large development of islets and beta cells. FGE 200 showed minute growth of beta cells while islets were not seen. FGE 300 showed minute evolution of beta cells with islets. FGE 400 showed broad ducts with acinus cells and normal growth of islets with beta cells. It shows maximum protection of pancreas by FGE 400.

Histopathological observations of liver tissues revealed normal aspects of liver with central vein and hexagonal lobules were observed in normal control group. Disease control group revealed awful destruction of liver tissue with high swelling. Treatment with metformin revealed normal structure of hepatic cells with normal liver tissues. FGE200 showed very less cure of hepatocyte inflammation. FGE300 showed less protection for hepatic swelling. FGE400 revealed recovery of hepatocytes with central vein and renewed liver tissues. Recovery of liver tissues by FGE 400 was found to be in significant amount.

IRS2 plays key role in liver by controlling insulin secreation[33]. PPARα is having prime function in lipid metabolism and decreased level leads to hepatic stress[34]. GRIA2 plays an important role for insulin secretion. DC group showed decreased levels of IRS2, PPARα and GRIA2 genes which was significantly increased by treatment with FGE 400 group.

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