

In vivo Anti-diabetic and Anti-hyperlipidemic Activities of ethyl Acetate/Methanol Fractions of *Feronia elephantum* Fruit in type 2 Diabetic Rats: Via α -amylase and PPAR- γ by using *in silico* Approach

Gangavaram Jyothi Reddy^{1,*}, Kesavan Bhaskar Reddy², Gopireddy Venkata Subba Reddy³

¹Department of Pharmaceutical Sciences, JNTUA, Ananthapuramu, Tirupati, Andhra Pradesh, INDIA.

²Sri Venkateswara College of Pharmacy, RVS Nagar, Chittoor, Andhra Pradesh, INDIA.

³Department of Chemistry, JNTUA College of Engineering, Pulivendula, Andhra Pradesh, INDIA.

ABSTRACT

Background: Obesity and overweight, together with physical inactivity, are projected to cause a huge proportion of the global diabetes burden. Natural products play a key role in the discovery of novel therapeutic agents. **Aim:** The present study aimed to elucidate *in silico* and *in vivo* anti-diabetic and anti-hyperlipidemic potential of Ethyl acetate (EFE) and ethyl acetate:methanol (EMFE) fractions of *F. elephantum* fruit in diabetic rats. **Methods:** Male Sprague-Dawley rats were induced type 2 diabetes by high fat diet (HFD) for a period of 2 weeks and then injected intraperitoneally with Streptozotocin (STZ, 35 mg/kg b.w). **Results:** Significant antihyperglycemia was displayed in rats treated with EFE and EMFE in OGTT in normal rats. Daily oral treatment with EFE and EMFE each at 100 and 200 mg/kg b.w for 5 weeks resulted in significant reduction in plasma glucose levels and HbA_{1c}, increased plasma insulin levels, normalised lipid profile and liver function parameters in HFD and STZ model. In addition, degenerative histopathological changes were found to be normalized. Molecular docking studies revealed strong binding efficacy of the active constituents of the extract viz., 22,23-dihydrostigmasterol, tocopherol and campesterol with α -amylase enzyme and PPAR- γ . **Conclusion:** These results suggest a promising anti-diabetic and anti-hyperlipidemic effect of *Feronia elephantum* fruit without apparent toxic effects.

Key words: Molecular Docking, *Feronia elephantum*, Anti-diabetic, Anti-hyperlipidemic, Streptozotocin, High Fat Diet.

INTRODUCTION

Obesity and overweight, together with physical inactivity, are projected to cause a huge proportion of the global diabetes burden.¹ DM is a serious challenge across the globe, as it affects around 463 million people in 2019.² Persistent weight loss has been reported to mend glycaemic control, avoiding the prerequisite of glucose-lowering medications, which exhibit some inadequacies and serious adverse effects as well. Natural products play a key role in the discovery of novel therapeutic agents as they are less toxic than synthetic ones and possess wide range of ingredients that are

believed to act on a variety of therapeutic targets. They have been used as an effective alternative in patients with chronic disorders like diabetes and allied complications.^{3,4} Therefore, to help mankind, it is an urge to develop effective natural therapeutic alternatives with established long-term safety in patients with coexisting diabetes and metabolic disorders.

Feronia elephantum Correa belongs to the family Rutaceae, also known as *Limonia acidissima*, *Feronia limonia* Swingle⁵ and is commonly called as elephant apple, wood apple. The fruit is used as a liver and cardiac

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Correspondence:

Mrs. G Jyothi Reddy

Academic Consultant, Department of Pharmacology, SVU College of Pharmaceutical Sciences, S V University, Tirupati-517502, Andhra Pradesh, INDIA.

Phone: +91 8309141659

E-mail: jyothi.reddy992@gmail.com



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tonic, as an astringent means of halting diarrhoea and dysentery.⁶ A GCMS study on methanol extract of the fruit pulp has reported the presence of furfural; 2-furan carboxaldehyde, 5-methyl; 2,5 furandione, 3-methyl; maltol; benzofuran, 2,3- dihydro; gamma tocoferol; campesterol; stigmasterol; Stigmasterol 22,23-dihydro; 2 propenenitrile, 3-(3,4-dimethoxy phenyl); cyclopropaneoctanol, 2-octyl.⁷ Though various ethanopharmacological properties of the plant have been reported, the plant has been underutilized due to limited scientific studies. Therefore, the current study aimed to investigate the *in silico* and *in vivo* anti-diabetic and anti-hyperlipidemic potential of ethyl acetate and ethyl acetate:methanol fractions of *F. elephantum* fruit in high fat diet and Streptozotocin induced type 2 diabetic model.

MATERIALS AND METHODS

Chemicals

STZ was purchased from M/S Sigma–Aldrich, St. Louis, MO, USA. Glibenclamide was received as a gift from M/S Cipla Limited, Mumbai, India. Insulin kit was procured from M/S Bhabha Atomic Research Center, Mumbai, India. All other biochemical studies were performed by using kits from M/S Span Diagnostics Ltd., Surat, India. All the chemicals used were of analytical grade.

Collection of plant material and preparation of fractions

Ripe fruits of *F. elephantum* were collected and processed as described earlier.⁸ The plant material was authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University (SVU) and voucher specimens (Voucher number:1328) were deposited at department of Botany, Sri Venkateswara University (SVU), Tirupati, India. *F. elephantum* fruits powder was extracted with methanol by soxhlation, filtered, concentrated using Rotavapor (Buchi R-200) and dried in incubator (37°C). It was fractionated using column chromatography on silica gel with n-hexane, ethyl acetate and methanol in their increasing order of polarity to yield 2 bioactive fractions viz., Ethyl acetate fraction of *F. elephantum* (EFE) and Ethyl acetate:methanol fraction 1:1 v/v of *F. elephantum* (EMFE), which were stored in refrigerator and used for further studies.

Experimental Animals

Sprague-Dawley rats weighing 180-200 g were procured from Sri Venkateswara Enterprises, Bangalore and were maintained as per standard guidelines.⁹ All the animal

studies conducted were approved by the Institutional Animal Ethics Committee (IAEC) of Sri Venkateshwara College of Pharmacy, Chittoor, India with reference No. 11-18/IAEC/CPCSEA/PO/SVCP/2017, dated 18.12.2017.

Determination of acute toxicity

Two groups of Sprague-Dawley rats ($n=6$) of either sex were used to assess acute toxicity of EFE and EMFE at 2000 mg/kg body weight (b.w) p.o according to OECD 423 guidelines.

Assessment of Hypoglycemic effect of EFE and ENFE using Oral Glucose Tolerance Test (OGTT)

OGTT was executed in fasted (6.00 AM to 2.00 PM) rats.¹⁰ They were allocated into five groups ($n=6$) according to the below treatment schedule;

Group I: Glibenclamide treated - 20 mg/kg p.o.

Group II: Ethyl acetate fraction of *F. elephantum* (EFE) 100 mg/kg b.w p.o.

Group III: Ethyl acetate fraction of *F. elephantum* (EFE) 200 mg/kg b.w p.o.

Group IV: Ethyl acetate:methanol fraction of *F. elephantum* (EMFE) 100 mg/kg p.o.

Group V: Ethyl acetate:methanol fraction of *F. elephantum* (EMFE) 200 mg/kg p.o.

30 min after the respective treatment, glucose 2 g/kg b.w was administered orally to all the groups of rats. Tail blood glucose levels (BGL) were measured using a glucometer (Accu-Chek® Active, Roche Diagnostic Corporation, Mannheim, Germany) at 0, 30, 60, 90 and 120 min of glucose administration.

Assessment of antihyperglycemic effect of fractions in HFD and STZ induced type 2 diabetic rats

Forty six male Sprague-Dawley rats (180–200g) were divided into two groups. First group consisted of normal control rats ($n=6$) and were provided with standard pellet diet (Amrut Rodent Diet-Hypro, Krishna Valley Agro LLP, Pune, India) *ad libitum*. Second group rats ($n=42$) were, induced type 2 diabetes by providing with high fat diet (HFD) for a period of 2 weeks and then fasted for 12-14 hr, injected intraperitoneally with Streptozotocin (STZ 35 mg/kg b.w) dissolved in freshly prepared 0.01 M citrate buffer, pH 4.5.¹¹ The composition and preparation of the HFD was as per Srinivasan *et al.*¹² Some animals may die quickly after STZ administration owing to substantial necrosis of islet β -cells and abrupt release of insulin which results in fatal hypoglycemia typically within 48 hr of STZ injection. To prevent this, 6hr after STZ administration,

the rats were provided with 10% sucrose solution for the next 24 hrs.¹³ After 72 hr, rats manifesting marked hyperglycaemia (fasting blood glucose >250 mg/dL) were used for the study according to below treatment schedule with glibenclamide 20 mg/kg b.w as standard reference.¹⁰ Four rats were omitted due to insufficient or excess hyperglycemia. Body weights of the rats was monitored regularly during the study.

Treatment Schedule

Group I: Normal Pellet Diet + 1 ml distilled water p.o

Group II: HFD + STZ induced rats + 1 ml distilled water p.o

Group III: HFD + STZ induced rats + Glibenclamide 20 mg/kg b.w p.o.

Group IV: HFD + STZ induced rats + EFE low dose (100 mg/kg b.w) p.o.

Group V: HFD + STZ induced rats + EFE high dose (200 mg/kg b.w) p.o.

Group VI: HFD + STZ induced rats + EMFE low dose (100 mg/kg b.w) p.o.

Group VII: HFD + STZ induced rats + EMFE high dose (200 mg/kg b.w) p.o.

Freshly prepared solutions dissolved in distilled water were administered daily at noon (2.00 PM) for 5 weeks. Fasting blood glucose (FBG) levels were estimated weekly on fasted animals on days 1, 7, 14, 21, 28 and 35 of the treatment. As rats are nocturnal feeders, fasting was initiated on the morning of blood sampling i.e, from 6.00 AM to 2.00 PM.

Bio-chemical Studies: At the end of the study (on 36th day), all the fasted animals were euthanized under light ether anesthesia by cervical decapitation and blood was collected via cardiac puncture in tubes with and without anticoagulant to study the biochemical parameters such as Serum insulin,¹⁴ glycosylated haemoglobin (HbA_{1c}),¹⁵ serum alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT),¹⁶ total Protein, total cholesterol (TC),¹⁷ triglycerides (TG)¹⁸ and High density lipoprotein cholesterol (HDL-c)¹⁹ using commercially available kits. Very low-density lipoprotein cholesterol (VLDL-c) and low-density lipoprotein cholesterol (LDL-c)²⁰

Histopathological studies: Liver, kidney and pancreas were washed immediately with saline and fixed in 10% phosphate buffered formalin, cut into 5 µm-thick sections and stained with hematoxylin and eosin (H and E) following standard protocols. The sections were examined in light microscope (Olympus BX53) for the incidence of histopathological alterations and photomicrographs were taken.

Statistical Analysis

All information was presented as mean ± SD ($n=6$). Results were analysed by Microsoft Excel Spread Sheet for Windows (2010) using one-way ANOVA followed by independent-sample *t* test. Values of *P* less than 0.05 were considered statistically significant.

In silico Analysis

Molecular docking studies were performed using Autodock Vina²¹ in PyRx platform in order to evaluate the binding possibilities of furfural; 2-furan carboxaldehyde, 5-methyl; 2,5 furandione, 3-methyl; maltol; benzofuran, 2,3- dihydro; gamma tocoferol; campesterol; stigmasterol; Stigmasterol 22,23-dihydro; 2 propenenitrile, 3-(3,4-dimethoxy phenyl); and cyclopropaneoctanol, 2-octyl against α -amylase and peroxisome proliferator-activated receptor gamma (PPAR- γ). The available crystal structures with PDB ids 4W93 and 2PRG respectively were obtained from Protein Data Bank. All water molecules and hetero atoms were removed and subjected to UCSF Chimera to add partial charges and polar hydrogen atoms. Grid was prepared around the ligand binding site of each target protein^{22,23} and docked with the compounds by using Genetic Lamarkian algorithm.

RESULTS

Acute Toxicity Study

No adverse effects, mortality and change in the behavioral or autonomic responses of all the rats were detected, even after acute oral administration of EFE and EMFE at 2000 mg/kg b.w. p.o. Hence, these fractions were safe and two doses 100 and 200 mg/kg b.w. were selected for further studies.

Effect of EFE and EMFE on OGTT in normal rats

Oral glucose loading (2 g/kg) to the rats produced significant change in BGL after 30 min in all the groups of rats, confirming the induction of hyperglycaemia. The rats treated with glibenclamide, EFE and EMFE at two doses (100 and 200 mg/kg) showed reduction in BGL from 60 min onwards and no overt hypoglycaemia was observed in all the treated groups as represented in Table 1.

Effect of long term treatment of EFE and EMFE on body weight in HFD and STZ induced diabetic rats

Increase in body weight (Day 14 ~50.0 g) was observed in rats provided with HFD during first two weeks. However, after administration of STZ and subsequent treatment with plant fractions, there was no significant

change in body weight among all groups as presented in Table 2.

Effect of long term treatment of EFE and EMFE on blood glucose levels in HFD and STZ induced diabetic rats

FBG level was measured in all groups of rats on days 1, 7, 14, 21, 28 and 35 of the treatment as shown in Table 3. Diabetic control rats had 2-5 folds increase in FBG levels throughout the study period than normal control rats ($P<0.001$). Daily oral treatment with EFE and EMFE (100 and 200 mg/kg) significantly reduced FBG level from day 7 when compared to diabetic control rats ($P<0.01$). Furthermore, at the end of the experiment, EFE and EMFE at 100 and 200 mg/kg b.w produced significant fall in FBG levels, as that of glibenclamide treated rats ($P<0.005$).

Effect of EFE and EMFE on plasma insulin, glycosylated haemoglobin (HbA_{1c}), Total protein and hepatic function markers

At the end of the treatment, diabetic control rats showed significantly ($P<0.0005$) low levels of plasma insulin than those of normal control rats. The plasma insulin levels of EFE and EMFE treated rats were significantly ($P<0.005$) enhanced compared to diabetic control rats. There was a significant increase in HbA_{1c} levels of diabetic control, which on treatment with EFE and EMFE decreased significantly. Total proteins in diabetic control rats were significantly lesser and on treatment with the EFE and EMFE there was a significant upsurge which was comparable to that of glibenclamide group (Table 4). Hepatic function markers such as ALP, AST and ALT showed significantly elevated activities in diabetic control rats, treatment with glibenclamide, EFE and EMFE (100 and 200 mg/kg b.w) for 35 days has normalised the activities of ALP, AST and ALT, in a dose dependent manner as given in Table 4.

Anti-hyperlipidemic effect of EFE and EMFE in HFD and STZ induced diabetic rats

TC, TG, LDL-c and VLDL-c levels were found to be increased significantly, while the HDL-c levels were significantly declined in diabetic control rats at $P<0.05$ (Table 4). Treatment with EFE and EMFE showed a significant reduction in the TC, TG, LDL-c and VLDL-c cholesterol levels and significant increase in HDL-c levels, which were comparable to that of glibenclamide treated group.

Effect of EFE and EMFE on histopathological analysis

Liver: The liver of the diabetic control rat revealed extensive hydropic degeneration and necrotic change with partially rounded, irregularly-edged vacuoles, hemorrhages in the sinusoidal spaces, congestion of central vein and mild fibrosis. These findings were found to be significantly decreased in the liver of glibenclamide, EFE 200 mg/kg and EMFE 200 mg/kg treated groups, while EFE and EMFE (100 mg/kg) treated rats shown nearly normal architecture with slight congestions in central vein and improvement from the degenerative changes (Figure 1).

Kidney: The histological view of diabetic control rat kidney presented severe necrosis with hydropic degeneration resulting in distracted bowmans capsule, hemorrhage and focal inflammatory cells were seen in the intertubular areas. In glibenclamide, EFE 200 mg/kg and EMFE 200 mg/kg treated rats the findings were detected to be significantly reduced, while EFE and EMFE (100 mg/kg) groups shown mild congestion (Figure 2).

Pancreas: The islets of Langerhans in diabetic control group were observed with hydropic degenerative and necrotic manifestations with disrupted cellular order. A significant improvement was observed with normalised degenerative, necrotic changes and less beta cell

Table 1: Effect of EFE and EMFE on OGTT in normal rats.

Groups	Blood glucose levels (mg/dL) at time intervals (min)				
	0	30	60	120	180
Glibenclamide 20 mg/kg	95.17±5.3	142.00±6.8	121.33±7.2	99.33±6.3	93.83±14.4
EFE 100 mg/kg	93.17±6.2	163.50±7.1	138.50±5.9	115.50±5.5	99.73±6.9
EFE 200 mg/kg	91.83±6.3	156.00±7.4	125.67±6.4	101.33±7.2	94.67±6.2
EMFE 100 mg/kg	90.50±5.7	160.50±5.7	128.50±6.3	109.83±5.8	101.17±8.7
EMFE 200 mg/kg	94.17±4.5	144.33±6.1	118.83±5.8	103.50±7.1	98.83±14.3

Values are given as Mean ± S.D. n=6

granulation in glibenclamide, EFE and EMFE treated rats at both doses (Figure 3).

In silico studies

We carried out docking studies using molecular targets of diabetes like α -amylase and PPAR- γ with various phytochemical compounds reported in methanolic extract of *F. elephantum* fruit. Of the compounds studied, three compounds viz., 22,23-dihydrostigmasterol, tocopherol and campesterol showed better binding against α -amylase with -9.4, -9.4 and -9.0 kcal/mol binding energies, when compared to standard anti-diabetic drug glibenclamide which showed -9.0 kcal/mol free binding energy against α -amylase active site respectively. As Figure 4a, the oxygen atom of side chain carboxyl group present in the Glu240 forms a hydrogen bond with the hydrogen atom of the hydroxyl group of 22,23-dihydrostigmasterol. Additionally, Ile235, Leu162, Tyr62 are involved in the formation of hydrophobic contacts with the ligand. Figure 4b shows the binding orientation and interaction of tocopherol with α -amylase. Here, the hydroxyl group of the ligand forms two hydrogen bonds with the main chain amino and carboxy groups Ile235. Whereas, Trp58, Trp59, Tyr62, Leu162, His201, Ile235 are involved in the hydrophobic environment to accommodate tocopherol. As shown in Figure 4c the main chain carboxy group and the side chain amino groups are involved in hydrogen bond formation with the hydroxyl group of campesterol, which is accommodated in the hydrophobic environment formed with Tyr62, Leu162, Ile235. Likewise, the 2D plot in the Figure 4d shows two

hydrogen bonds between Glu233 and glibenclamide. However, Trp59 is involved in π - π staking with the ligand in addition to the hydrophobic interactions formed by His201, Ile235, Leu165, Trp59, Tyr62.

On the other hand, glibenclamide showed -8.2 kcal/mol binding energy against PPAR- γ . Only two compounds viz., 22,23-dihydrostigmasterol and tocopherol showed better binding with -11.3 and -10.4 kcal/mol binding energies against PPAR- γ active site. The results showed that among the docked compounds dihydrostigmasterol and tocopherol showed more affinity compared to glibenclamide. Hydrophobic interactions play the key role to accommodate these compounds as shown in Figure 5 the major amino acids involved in the interaction are Leu228, Leu270, Gly284, Cys285, Phe287, Arg288, Ser289, Glu291, Ile326, Tyr327, Leu330, Leu333, Leu340, Ile341, Ser342, Glu343, Gly344, Phe363, Met364, Lys367, His449.

DISCUSSION

In the present study, we have established that EFE and EMFE ameliorates hyperglycemia, hypoinsulinemia, hyperlipidaemia and pathological changes in liver, pancreas and kidney in HFD and STZ induced type 2 diabetic rats in a dose-dependent manner. Further, OGTT in normal rats was performed to define the disturbance of glucose metabolism and to assess apparent insulin release and any possible hypoglycemic action of the plant.²⁴ A significant fall in BGL of EFE and EMFE treated rats infers better glucose utilization capacity, without any subsequent hypoglycemia and

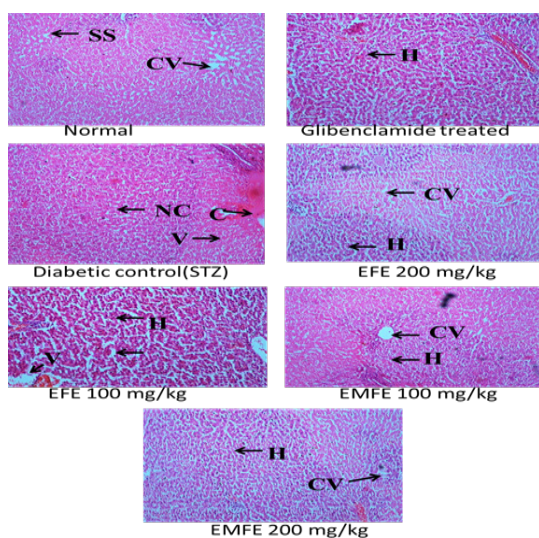


Figure 1: H and E stained histopathological sections of liver after 5 weeks of treatment. CV=Central vein, SS= Sinusoids space, H= Hepatocyte, C= Congestion, NC= Necrotic Change, V= Vacuolization.

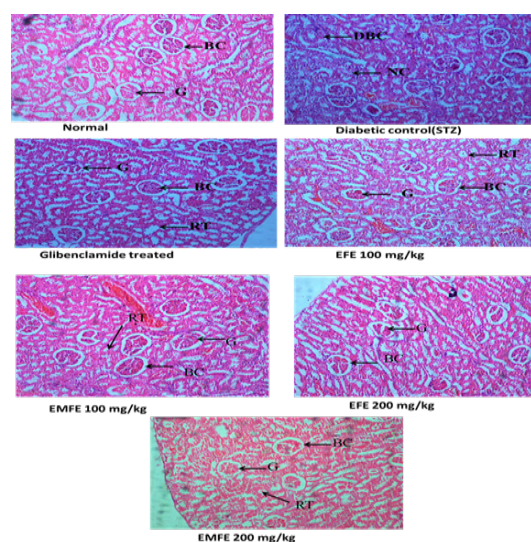


Figure 2: H and E stained kidney sections after 5 weeks of treatment; BC= Bowmans capsule, G= Glomerulus, DBC=Distractive Bowmans Capsule, RT= Renal tubule. NC= Necrotic changes.

beneficial effect of the plant in the avoidance of postprandial hyperglycaemia and related complications. This effect could be related to the inhibitory action on carbohydrate metabolizing enzymes like α -glucosidase and pancreatic α -amylase as reported previously in our *in vitro* study.⁸

HFD tends to increase body weight as evident from the the present results however no significant change was observed post-STZ administration in all groups. The possible intervention of STZ in lowering body weight should be considered. The present results are consistent with previous study where *F. elephantum* treatment in STZ

induced rats retained original body weight.²⁵ Shimoda *et al.* in a study suggested that the administration of 0.5% and 1% Green Coffee Extract (GCE) reduced visceral fat content and body weight. Another study exposed no body weight lowering effect after light roasted GCE administration for 8 weeks in high sucrose HFD and STZ induced model.²⁶

HFD and STZ diabetic model shares many similarities with human type 2 DM. HFD rats may be vulnerable to insulin resistance as the receptor cells are blocked by fat deposits and the diabetic action of a low dose of STZ is boosted. STZ enters the beta cells through GLUT 2 and causes damage to the beta cells by alkylation of DNA and also by generating free radicals, resulting in hyperglycaemia and steady diminution of insulin production.^{27,28} It has been reported that when fed with a HFD and injected with a low dose of STZ, rats show hyperglycemia, hypoinsulinemia, kidney, pancreas and liver damage.²⁹ EFE and EMFE administration ameliorated hyperglycemia, improved insulin secretion and glycemic control, decreased HbA_{1c} levels. FBG levels of EFE and EMFE groups were decreased notably on the 35th day. These results propose that EFE and EMFE may act positively in controlling FBG levels of diabetic rats. The possible mechanism for the antihyperglycaemic effect of EFE and EMFE could be due to protection of pancreatic β -cells from further atrophy or by potentiation of insulin secretion from residual β -cells or due to their insulin like action,³⁰ as evident from the increased plasma insulin levels in EFE and EMFE treated rats.

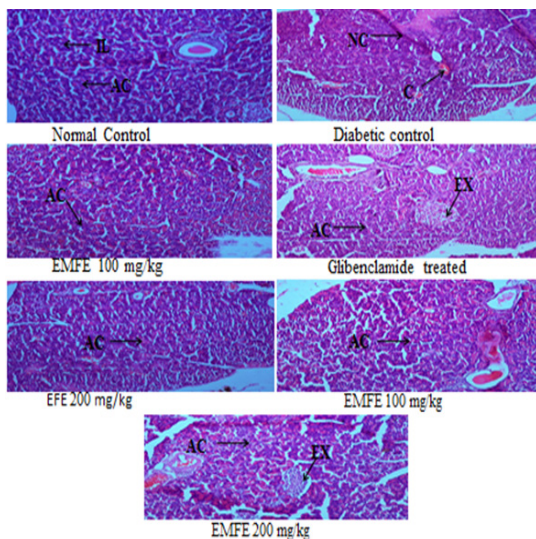


Figure 3: H and E stained sections of Pancreas after 5 weeks of treatment; EX= Exocrine, IL= Islets of Langerhans, AC= Acinar Cells, NC= Necrotic Change, V= Vacuolization.

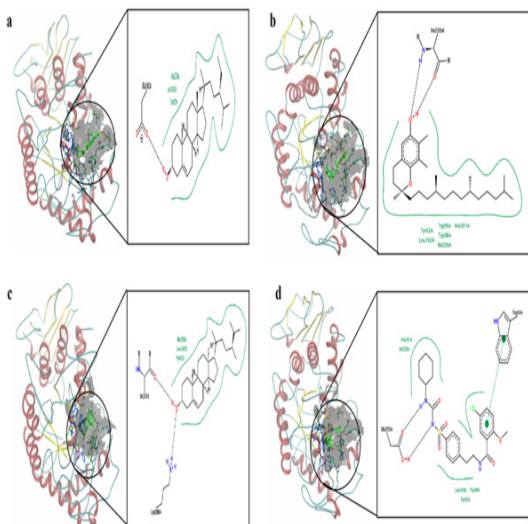


Figure 4: Schematic representation of binding mode and interaction of a) α -amylase-dihydrostigmasterol b) α -amylase-tocopherol c) α -amylase-campesterol and d) α -amylase-glibenclamide.

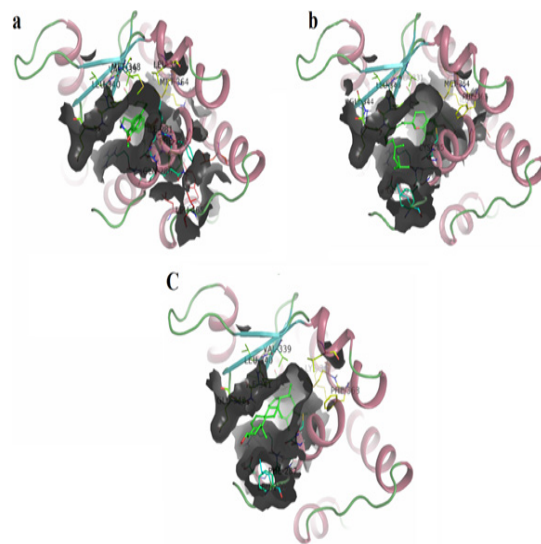


Figure 5: Schematic representation of binding mode and interaction of a) PPAR- γ -glibenclamide b) PPAR- γ -tocopherol c) PPAR- γ -dihydrostigmasterol.

Table 2: Effect of long term treatment of EFE and EMFE on body weight in HFD and STZ induced diabetic rats.

Groups	Day 0 (Baseline weight) (g)	Day 14 (After Induction) (g)	Day 28 (During Treatment) (g)	Day 42 (After Treatment) (g)
Normal control	198 ±4.5	212±4.6	229±4.0	248±3.8
Diabetic Control	193 ±4.8	245±5.1	254±9.3	263±8.9
Glibenclamide 20 mg/kg	190 ±5.1	242±4.2	254±6.9	252±5.9
EFE 100 mg/kg	195±4.6	245±4.9	252±8.2	249±5.2
EFE 200 mg/kg	194±5.3	240±5.5	247±9.5	239±6.1
EMFE 100 mg/kg	198±4.0	241±5.9	251±7.6	245±5.6
EMFE 200 mg/kg	200±3.9	247±5.7	249±8.2	238±6.2

Results are expressed in mean ± S.E.M, n=6.

Table 3: Effect of oral treatment of *F. elephantum* fractions for 5 weeks on Blood glucose levels in HFD and STZ induced diabetic rats.

Groups	Blood glucose levels (mg/dL)					
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
Normal control	77.7±2.5	81.3±1.9	88.0±1.8	89.5±2.4	81.5±1.9	79.0±2.4
Diabetic Control	315.7±6.2 [#]	340.8±9.2 [#]	341.7±8.5 [#]	367.2±9.7 [#]	388.7±8.5 [#]	398.3±8.4 [#]
Glibenclamide 20 mg/kg	319.0±1.4	190.7±8.4 [*]	163.0±6.5 [*]	149.3±6.3 ^{**}	136.5±4.4 ^{**}	121.2±5.8 ^{**}
EFE 100 mg/kg	314.8±8.2	242.2±8.3 [*]	191.3±8.7 [*]	166.3±9.7 [*]	154.5±6.8 [*]	145.5±6.1 ^{**}
EFE 200 mg/kg	321.0±7.1	227.5±8.1 [*]	187.3±9.7 [*]	158.3±7.1 [*]	144.3±6.5 ^{**}	138.5±6.1 ^{**}
EMFE 100 mg/kg	320.0±8.6	234.3±8.3 [*]	176.7±8.3 [*]	166.3±9.7 [*]	156.8±7.4 [*]	149.0±6.6 ^{**}
EMFE 200 mg/kg	317.0±7.5	208.0±9.3 [*]	166.2±8.2 [*]	148.0±9.9 ^{**}	147.7±7.1 ^{**}	126.7±4.7 ^{**}

Results are expressed in mean ± S.E.M, n=6; [#]P<0.001 diabetic control compared to normal control; ^{**}P<0.005 treated group compared to diabetic control; ^{*}P<0.01 treated group compared to diabetic control.

An increase in the plasma ALP, ALT, AST and total protein levels in diabetic control rats indicate that some of the detrimental effects of DM may be related to hepatic dysfunction resulting from hepatic necrosis. These deleterious effects in diabetic rats were corrected after daily oral administration EFE and EMFE at the doses of 100 and 200 mg/kg which is evident from the decreased plasma levels of ALP, ALT, AST and total protein and also from the histology of liver (Figure 1). The results of the present study are in agreement with that of the plants having good hepatoprotective property.^{31,32}

Hyperglycaemia also hinders the lipid metabolism resulting in hyperlipidemia, which is the key factor involved in the evolution of secondary complications of diabetes like atherosclerosis and coronary heart disease.³³ In the present study, the rise in blood glucose was complemented by marked elevations in TC, TG, LDL-c, VLDL-c and significant reduction in HDL-c levels in HFD and STZ induced diabetic control rats. EFE and EMFE supplementation corrected the serum lipid profile which may be related to their

effect on carbohydrate metabolism or modification of hepatic enzymes. Both fractions thus have potential antihyperglycaemic and antihyperlipidemic properties and could be used to prevent the development of cardiac diseases and atherosclerosis. These results are in agreement with previous published data showing reversal of diabetic mice by formation of new islets from ductal cells.³⁴ This observation was further supported by histology of pancreas of EFE and EMFE treated rats (Figure 3).

In this study, histopathological results of diabetic rats were in agreement with previous reports.³⁵ In the present study, EFE and EMFE treated rats exhibited normalisation of the necrotic changes in liver, kidney and pancreas, which suggest that administration of EFE and EMFE may perhaps greatly ameliorate degenerative and necrotic destruction by repairing the cell damage associated with development of type 2 DM.

Retardation of starch digestion by inhibition of α -amylase enzyme would play a key role in the control of diabetes.³⁶ In the present study, using computational

Table 4: Effect of oral treatment of *F. elephantum* fractions for 5 weeks on biochemical parameters in HFD and STZ induced diabetic rats.

Groups	Plasma Insulin (μ IU/mL)	HbA1c (%)	Total Protein (g/dL)	ALP (mg/dL)	AST (IU/L)	ALT (IU/L)	TC (mg/dL)	TG (mg/dL)	HDL-c (mg/dL)	VLDL-c (mg/dL)	LDL-c (mg/dL)
Normal Control	17.52 \pm 0.3	4.92 \pm 0.19	4.57 \pm 0.4	184.50 \pm 5.9	81.17 \pm 6.8	22.50 \pm 2.6	92.83 \pm 3.12	65.33 \pm 3.72	42.41 \pm 1.68	13.06 \pm 0.74	37.42 \pm 1.95
Diabetic Control	5.37 \pm 0.4 [#]	8.78 \pm 0.28 [#]	8.28 \pm 0.3 [#]	495.50 \pm 7.6 [#]	157.50 \pm 7.05 [#]	46.67 \pm 2.2 [#]	215.33 \pm 6.97 [#]	161.83 \pm 5.45 [#]	26.75 \pm 1.54 [#]	32.36 \pm 1.09 [#]	161.62 \pm 4.83 [#]
Glibenclamide 20 mg/kg	15.13 \pm 0.6 ^{**}	6.67 \pm 0.37	5.25 \pm 0.3 [*]	261.33 \pm 9.1 [*]	92.17 \pm 6.5 [*]	22.50 \pm 1.9 [*]	105.00 \pm 3.89 [*]	83.16 \pm 3.43 [*]	36.91 \pm 1.42 [*]	16.63 \pm 0.68 [*]	52.09 \pm 2.01 [*]
EFE 100 mg/kg	10.55 \pm 0.4 [*]	7.52 \pm 0.25 [*]	6.41 \pm 0.3 [*]	320.83 \pm 10.3 [*]	126.04 \pm 5.1 [*]	34.67 \pm 4.3 [*]	137.50 \pm 4.27 [*]	91.16 \pm 3.92 [*]	35.91 \pm 1.35 [*]	18.23 \pm 0.78 [*]	83.67 \pm 2.67 [*]
EFE 200 mg/kg	13.30 \pm 0.5 ^{**}	6.50 \pm 0.3 [*]	6.04 \pm 0.1 [*]	295.67 \pm 9.0 [*]	101.17 \pm 7.2 [*]	28.83 \pm 2.3 [*]	108.33 \pm 5.60 [*]	80.66 \pm 4.32 [*]	37.00 \pm 1.41 [*]	16.13 \pm 0.86 [*]	55.33 \pm 2.19 [*]
EMFE 100 mg/kg	11.05 \pm 0.5 [*]	6.95 \pm 0.36 [*]	6.32 \pm 0.3 [*]	321.17 \pm 8.5 [*]	128.01 \pm 5.1 [*]	31.01 \pm 2.8 [*]	121.83 \pm 3.54 [*]	99.00 \pm 3.89 [*]	34.16 \pm 1.36 [*]	19.80 \pm 0.77 [*]	67.58 \pm 2.40 [*]
EMFE 200 mg/kg	14.13 \pm 0.6 ^{**}	6.42 \pm 0.52 [*]	5.68 \pm 0.4 [*]	278.02 \pm 6.8 [*]	114.33 \pm 5.4 [*]	27.33 \pm 2.2 [*]	107.83 \pm 4.16 [*]	91.01 \pm 3.74 [*]	36.75 \pm 1.17 [*]	18.20 \pm 0.74 [*]	52.25 \pm 2.76 [*]

Results are expressed in mean \pm S.E.M, n=6; [#]P<0.0005 diabetic control compared to normal group; ^{**}P<0.0005 treated group compared to diabetic control; ^{*}P<0.005 treated group compared to diabetic control. HbA_{1c}: glycosylated haemoglobin, ALP: Alkaline phosphatase, ALT: Alanine transaminase, AST: Aspartate transaminase, TG: Triglyceride; TC: Total cholesterol; HDL-c: High density lipoprotein cholesterol; LDL-c: Low density lipoprotein cholesterol; VLDL-c: Very low density lipoprotein cholesterol.

molecular docking software, out of the compounds reported in methanol extract of *F. elephantum* fruit, three compounds viz., 22,23-dihydrostigmasterol, tocopherol and campesterol possess good binding efficacy against pancreatic α -amylase. Moreover, two compounds viz., 22,23-dihydrostigmasterol and tocopherol showed better binding against the target PPAR- γ than standard reference glibenclamide. PPAR- γ receptor has been found to be an important drug target for regulating fatty acid storage, marked improvement in insulin sensitivity and glucose metabolism in diabetic patients.³⁷ The present *in silico* results suggests that the possible antihyperglycemic mechanism may be due to inhibition of α -amylase enzyme or increase in insulin-stimulated glucose uptake through action on PPAR- γ receptor. The results are in accordance with previous reports where they identified potential PPAR- γ partial agonist molecules from plants by virtual screening procedures.³⁸ The present biochemical, histopathological outcomes and *in silico* results, substantiate the protective role of *F. elephantum* fruit pulp in overcoming type 2 DM, hyperlipidemia and associated complications. These effects of EFE and EMFE may be attributed to 22,23-dihydrostigmasterol, tocopherol and campesterol. The presence of these compounds was confirmed by GC-MS analysis of EFE and EMFE.^{7,39}

CONCLUSION

The current study confirms that daily oral administration of EFE and EMFE of *F. elephantum* fruit pulp displayed pronounced anti-hyperglycaemic and antihyperlipidemic effects in HFD and STZ induced type 2 DM rats. Moreover, the active constituents of *F. elephantum* fruit exhibited good binding efficacy against α -amylase and PPAR- γ in molecular docking studies. Therefore, our study justify the traditional claim of the plant, however, the exact mechanism of action has to be elucidated so as to develop it as an excellent candidate for future pharmacological interventions.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ABBREVIATIONS

EFE: Ethyl acetate fraction of *F. elephantum*; **EMFE:** Ethyl acetate:methanol fraction of *F. elephantum*; **IR:** Insulin Resistance; **DM:** Diabetes mellitus; **FBG:** Fasting Blood Glucose; **BGL:** Blood Glucose Levels; **H&E:** hematoxylin and eosin; **IAEC:** Institutional Animal Ethics Committee; **OGTT:** Oral Glucose Tolerance Test; **HbA_{1c}:** Glycosylated haemoglobin; **ALP:** Alkaline phosphatase; **AST:** Aspartate transaminase; **ALT:** Alanine transaminase; **TC:** Total Cholesterol; **TG:** Triglycerides; **HDL-c:** High-density lipoprotein cholesterol; **VLDL-c:** Very low-density lipoprotein cholesterol; **LDL-c:** Low-density lipoprotein cholesterol.

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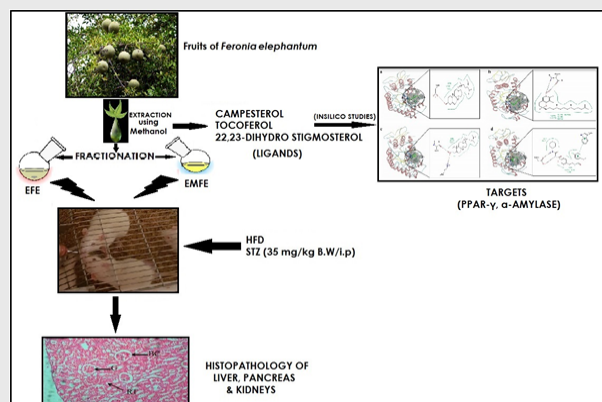
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PICTORIAL ABSTRACT



About Authors



Gangavaram Jyothi Reddy: Department of Pharmaceutical Sciences, JNTUA, Ananthapuramu, Tirupati, Andhra Pradesh, INDIA.



Kesavan Bhaskar Reddy: Sri Venkateswara College of Pharmacy, RVS Nagar, Chittoor, Andhra Pradesh, INDIA.



Gopireddy Venkata Subba Reddy: Department of Chemistry, JNTUA College of Engineering, Pulivendula, Andhra Pradesh, INDIA.

SUMMARY

- High fat diet and STZ (35 mg/kg) induced diabetic rat model used to evaluate the anti-diabetic and antihyperlipidemic activity of Ethyl acetate fraction of *F. elephantum* (EFE) and Ethyl acetate:methanol fraction (EMFE) of *F. elephantum* fruits (100 and 200 mg/kg b.w) for 5 weeks with Glibenclamide as standard drug.
- EFE and EMFE considerably decreased elevated plasma glucose levels and HbA_{1c}, amplified plasma insulin levels, stabilized lipid profile and liver function parameters. Moreover, deteriorating histological changes seen in liver, pancreas and kidney of the diabetic rats were found to be controlled and were normalised in EFE and EMFE treated rats as comparable to that of glibenclamide treated rats.
- Furthermore, molecular docking studies revealed strong binding efficacy of the active constituents of the extract with α -amylase enzyme and PPAR- γ .
- EFE and EMFE possess promising antihyperglycemic and antidyslipidemic potential without apparent toxic effects and are good candidates for future research.

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