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STUDY OF EFFECT OF *STERCULIA FOETIDA* LINN.(STERCULIACEAE) SEEDS ON INSULIN RESISTANCE IN TYPE II DIABETES MELLITUS IN RATS

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INTRODUCTION

Diabetes prevalence is increasing at an accelerating rate approaching epidemic levels. Type II Diabetes is heterogeneous, progressive disorder initially characterized by glucose intolerance and compensatory hyperinsulinaemia which in later stages progress to insulin resistance and impaired beta cell function. Type II Diabetes which accounts for 90-95% of diabetic cases is largely caused by social and Lifestyle that can be readily controlled. Obesity is one of the major factors which contribute in development of Diabetes Mellitus.[1] Obesity leads to an increase in the adipose tissue mass. This in turn triggers insulin Resistance in fat, skeletal muscle and liver leading to Type II Diabetes Mellitus. Current research is focused on the development of newer drug leads from phytoconstituents of medicinal plants which have been used in traditional practices, so as to get more potential and effective agents with lesser side effects than existing agents.[3]

Sterculia Foetida L. is a tropical plant belonging to the Sterculiaceae family which is also called as 'Java-Olive', 'Bastard poon tree', 'Hazel sterculia', 'Skunk tree', 'Poon tree' and 'Sam-rong' in Thai. In India it is known as 'Janglibadam' (Hindi, Bengali), 'Gorapu-badam' (Tamil).[4] Leaves of this plant are used as herbal medicine as aperients, diuretic, anti epileptic and as insect repellent. Decoction of wood boiled with seed oil is said to be employed in rheumatism.[5] A gum that resembles 'gum tragacanth' is obtained from the trunk and branches and is used for bookbinding and similar purposes. In Ghana, seeds are taken as a purgative.[6] Oil from the seed is extracted on a local scale to be used in medicine internally in itches and other skin diseases and is applied externally as a paste. It is used locally for illuminating purpose. Seed oil also exhibits activities like antifungal insecticide, antibiotic, and antiviral, hormonal, carcinogenic or anti tumour.[7]

The qualitative photochemical analysis of different parts extract of S*terculia foetida L.* showed the presence of alkaloids, glycosides, flavonoids, Phenolic compounds, saponins, steroids and tannins.[8] Previous studies have proved that the chemical Constituents such as flavonoids, alkaloids and saponins are promising agents for treatment of diabetes and its complications.[9]

Different components of *Sterculia foetida L.* especially leaves have been reported to possess antihyperglycemic activity which is attributed to presence of phytoconstituents such as alkaloids, glycosides, flavonoids, Phenolic compounds, saponins. However, till date no studies have so far been reported for Insulin Resistance in Type II Diabetes In the absence of any scientific evidence, we have attempted the present study for exploring effect of *Sterculia Foetida* seeds on Insulin Resistance in Type II Diabetes Mellitus in Rats and to focus on its possible mode of action.

MATERIALS AND METHODS

Collection and authentication of plant material

The Seeds *sterculia foetida* Linn. Were collected, identified and authenticated from the Savatribai phule, Pune University Pune in the month of October 2015. The Seeds were dried and converted to a coarse powder by using dry grinder.

Preparation of Extract

Seed contain oily material. To remove excess oil from seeds they are subjected to Soxhelt Extraction by using petroleum ether as a solvent.

Preparation of Hydroalcholic Extract

The crude Hydroalcholic extract was prepared by cold maceration technique using 1000 ml of solvents methanol: water (70:30) for 72 hours at room temperature. The yield of Hydroalcholic extract was 32.30%w/w.

Preparation of Fraction

This crude extract was partitioned into ethyl acetate soluble and ethyl acetate insoluble fraction using 500ml of ethyl acetate solvent. The solvent was filtered using absorbent cotton wool and filter paper. The process was repeated several times to ensure maximum yield of ethyl acetate soluble compounds form crude extract. Filtrates

were collected and evaporated on Rota evaporator at 40 ˚C to obtain a yield of 14.16% w/w. The obtained ethyl acetate fraction (EASF) was preserved in refrigerator till further use.

Experimental animals

Wister rats of either sex (180-220 g) were procured from National Institute of Bioscience, Chaturshrungi, Pune. Animals were housed in standard polypropylene cages (32.5×21×14 cm) lined with raw husk (renewed after 48h). The animal house was maintained on 12h light/dark cycle at approximately 22±20C, relative humidity 60–70% and the animals were provided with standard laboratory diet (Nutrivet life sciences, Pune) and water *ad libitum*. The animals were randomly assigned to different groups and a minimum period of 7 days was allowed for adaptation on each experiment. The animals described as fasting were deprived of food for 24 h before experimentation.

Chemicals

Streptozotocin (STZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Preliminary photochemical study

The EASF was screened for the presence of various phytoconstituents like alkaloids, glycosides, flavonoids, tannins, carbohydrates, amino acids and proteins.[10]

Determination of total flavonoids content

Total flavonoids content was measured by means of an aluminium chloride assay. with slight modification.[11] An aliquot (1 ml) of the fraction (1 mg/ml) or standard solutions of quercetin (10-50 µg/ml) was added to a 10 ml volumetric flask containing 4 ml of 50% solution of methanol. To the flask, 0.3 ml of 5% NaNO2 was added. The 0.3 ml of 10% AlCl3 was added after 5 min. At the sixth minute, 2 ml of NaOH (1 M) solution was added; the total volume was made upto 10 ml with distilled water. The solution was well mixed and absorbance was measured against reagent blank at 510 nm. The total flavonoids content (mg/g) was determined from the calibration curve and expressed as mg quercetin equivalents. All the determinations were carried out in triplicate, and the mean values were calculated.

Thin layer chromatography (TLC)

EASF were subjected to thin layer chromatography using precoated silica plates of 250 µm thickness (TLC Silica gel 60 F_{254} Merck, Germany). The spots were developed in eight different solvent systems as follows:

A. Ethyl acetate: formic acid: glacial acetic acid: water (100 : 11 : 11 : 2.6)
B. Chloroform: ethyl acetate: formic acid (7.5 : 6.0 : 0.5)
C. Tolune:Ethyl acetate:Ethnaol (1:1:1)
D. Tolune:Ethyl acetate:Methanol (8:1:1)

The spots were visualized by using iodine chamber
R_f value was calculated using following equation
R_f = Distance travelled by the sample/Distance travelled by the solvent

High performance thin layer chromatography

HPTLC chromatography of EASF was performed on 20×10 cm aluminum Lichrosphere HPTLC plates precoated with 200 µm layers of silica gel 60F254 (E. Merck, Germany). EASF (10 µl) and standard biomarkers quercetin (600 ng/band) was applied as bands 6 mm wide and 10 mm apart by means of Camag Linomat V sample applicator (Muttenz, Switzerland) equipped with a 100-µl syringe.[12] The constant application rate was 160 nl s^{-1}. The Linear ascending development with mobile phase toluene: ethyl acetate: formic acid (5:4:1, v/v/v) was performed in a 20 cm×10 cm twin-trough glass chamber (Camag) previously saturated with mobile phase for 15 min at room temperature (25±2^o C) and relative humidity 60%±5%. The development was done using 20 ml of mobile phase. HPTLC analysis was performed at 254 nm in reflectance mode with a Camag TLC scanner III operated by WinCATS software (Version 1.2.0). The slit dimensions were 5 mm×0.45 mm and the scanning speed of 20 mm $s^{-.1}$

Acute toxicity study

Acute toxicity study was performed according to OECD guidelines no. 423.[13] Wistar rats selected by random sampling technique were employed in this study. The animals were fasted overnight with free access to water. Ethyl acetate fraction was administered orally to different groups at increasing dose levels of 50, 100, 300, 2000 and 5000 mg/kg body weight. After dosing, the animals were observed for 2 hours and then intermittently for further 4 hours for changes in behavioural (alertness, restlessness, irritability, fearfulness), neurological (spontaneous activity, convulsion, gait, bleeding, touch and pain response), autonomic (defecation) profiles and finally recording mortality up to 24 hours till 14 days.

Experimental induction of diabetes

Wister rats (150-180 g) of either sex were procured from National institute of Bioscience, Chaturshrungi, and Pune. T2DM rats prepared as described previously. In brief, the rats were fed with high fat diet containing 58% fat, 25% protein and 17% carbohydrates for 4 weeks. After, administration of High fat diet for 4 Weekes, inject 35mg/kg STZ to HFD animals (14). Streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5) Insulin resistance was induced in overnight fasted Wistar strain albino rats by a single intraperitoneal injection of 35 mg/kg streptozotocin. Since STZ is capable of producing fatal hypoglycaemia as a result of massive insulin release, rats were treated with 5% glucose solution for 24 h. Normal control rats received an equivalent volume of citrophosphate buffer. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h

after administration. Rats with fasting blood sugar levels around 160 to 300 mg/dl were selected for the study.

Experimental groups

Total of 36 rats (6 normal; 30 diabetic) were divided into seven different groups consisting of six animals in each group as follows.

Group 1: Normal control rats administered cold citrophosphate buffer (pH 4.3).

Group 2: Diabetic control rats i.e. rats treated with HFD-STZ (35mg/kg, i.p) Group 3: Diabetic rats treated with 100 mg/kg of EASF.

Group 4: Diabetic rats treated with 200 mg/kg of EASF.

Group 5: Diabetic rats treated with 400 mg/kg of EASF.

Group 6: Diabetic rats treated with 3 mg/kg Sitagliptine (standard oral hypoglycaemic drug)

The ethyl acetate fraction and Sitagliptine were dissolved in water using sonicator and administered orally for 28 days once daily to the respective groups.

Fasting blood glucose

Fasting blood glucose was determined at 0, 7, 14, 21 and 28 day of study period using glucometer (Accu check, Germany). Blood samples were collected by snipping the tail with the help of sharp razor.

Determination of body weight, food and fluid intake

During the study period of 28 days the rats were weighed daily using electronic balance and their body weights were recorded. Daily food and fluid intake were also recorded during 28 days study period.[14]

Glycosylated haemoglobin and Plasma Insulin

Glycosylated haemoglobin (HbA1c %) was determined in EDTA-blood samples obtained at the end of the 28th day study. Insulin concentrations and Glycosylated haemoglobin results were obtained from Dr.Lal Pathology Laboratory Pune.[14]

Oral glucose tolerance Test

Oral glucose tolerance test was performed in overnight fasted (18 h) diabetic rats at the end of 28th day of study.[15] Glucose (3g/kg) was fed 30 min after the administration of extracts or standard drug. Blood glucose was determined at different time interval at 0, 30, 60, 90 and 120 min of glucose administration.

Evaluation of Liver Function Parameters

Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphates (ALP) were analysed by using commercial kits (Crest Biosystems, Goa, India).[16,17]

Determination of Plasma Leptin and TNF-α

Plasma leptin were measured using ELISA Kit. Plasma TNF was measured by using ELISA kit. (Dr.Lal pathology Lab. Pune).[18]

Histopathological studies

At the end of study duration all the animals were sacrificed and the pancreas and Heart were isolated for Histopathological estimation.[19,20]

Statistical analysis of data

All the data are presented as mean ± SEM of measurements made on six animals in each group. Statistical analysis was performed with one-way analysis of variance (ANOVA) followed by Dunnet's multiple test for comparison using Graph Pad Instat (version-3) software. A value of $p<0.05$ was considered to be statistically significant compared with the respective control.

RESULTS

Preliminary Phytochemical Analysis

The percentage yield of hydro alcoholic extract of *sterculia foetida* seeds was found to be 32.30%w/w while the percentage yield of Ethyl acetate fraction of *sterculia foetida* seeds(EASF) 14.15%W/W respectively.

Qualitative photochemical screening

The qualitative photochemical analysis of these extracts showed the presence of active constituents such as alkaloids, flavonoids, Phenolic compound.

Thin Layer Chromatography

TLC analysis confirmed the presence of flavonoids (0.82 RF value) by comparison with RF values of standards quercetin.

Fig. 1: Spots observed in thin layer chromatography of ethyl acetate fraction of *Stercuila foetida* seeds Linn.

HPTLC

HPTLC fingerprinting of Quercetin and EASF were shown in the fig 2 and 3. Quercetin and EASF were resolved at Rf values 0.43 and 0.42 respectively. HPTLC fingerprinting of EASF clearly reveals that EASF contains Quercetin.

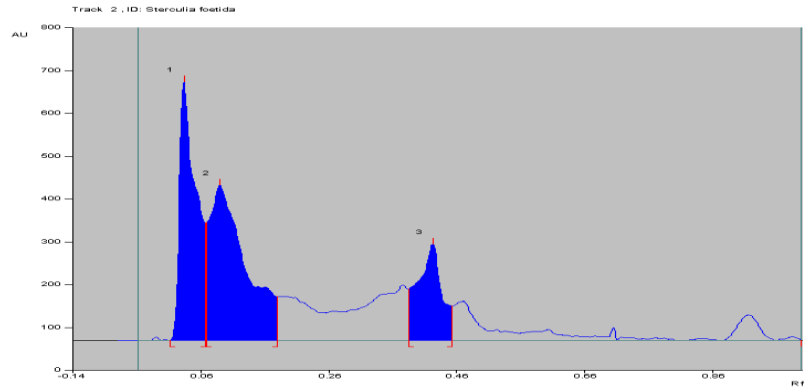


Fig. 2: HPTLC fingerprinting of Quercetin.

R_f values of spots observed in HPTLC fingerprinting

Sr. No.	Peak No.	Concentration	Wavelength	Rf Value	Area
1.	3	3,00,000ng/spot	254nm	0.42	6877.5AU

Mobile Phase – Toluene : Ethyl acetate : Ethanol (8 : 1 : 1)
Standard Preparation – 100 mg of sample dissolved in 1ml ethyl acetate(50000ppm)

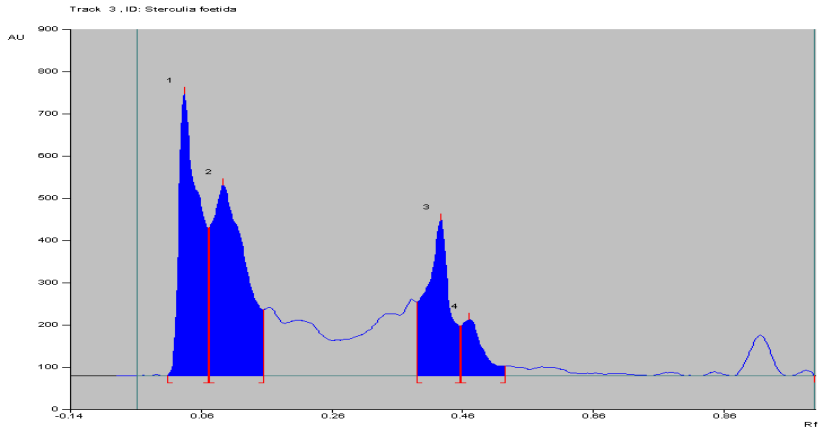


Fig. 3: HPTLC fingerprinting of Ethyl acetate fraction of *Sterculia foetida Linn.*

R_f values of spots observed in HPTLC fingerprinting

Sr. No.	Peak No.	Concentration	Wavelength	Rf Value	Area
1.	3	5,00,000ng/spot	254nm	0.42	10252.6AU

Mobile Phase – Toluene : Ethyl acetate : Ethanol (8 : 1 : 1)
Standard Preparation – 100 mg of sample dissolved in 1ml ethyl acetate(50000ppm)

Acute toxicity study

Acute toxicity study revealed the non-toxic nature of EASF. There was no lethality or any toxic reaction in animals at a single large dose of 5000 mg/kg. No mortality was recorded within the 14 days of observation.

Fasting blood glucose

Treatment with EASF for 4 weeks exhibited a significant ($p<0.01$) decrease in fasting blood glucose in STZ diabetic rats as compared to diabetic control (Fig.2).

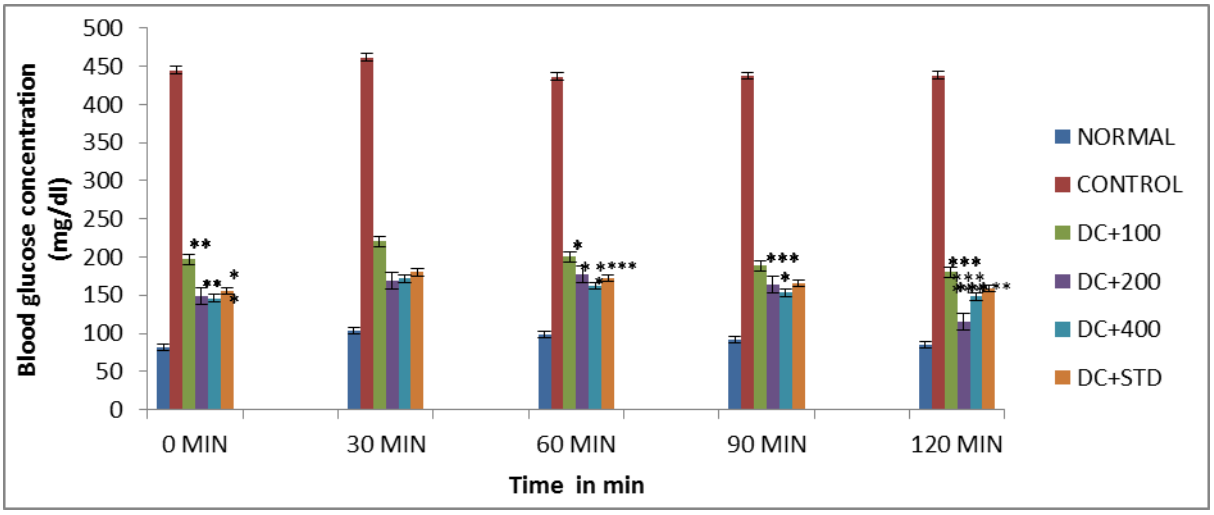


Fig. 4: Effect of administration of EASF on Fasting Blood Glucose in High fat diet plus low dose STZ induced Type II Diabetic Rats.

NC: Normal control; DC: Diabetic control; EASF: Ethyl acetate fraction of Sterculia foetida SG: Sitagliptine Data are expressed as mean ± S.E.M. (n=6). #: p<0.05, ##: p<0.01 as compared to NC; *: p<0.05, **: p<0.01 as compared to DC. Data analyzed by one way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.

Oral glucose tolerance Test (OGTT) in STZ+HFD Rats

The effect of EASF on oral Glucose tolerance test (OGTT) is shown in fig.6.7. Blood glucose of normal and diabetic rats was increased significantly (p<0.01%) at 30 min after glucose administration. EASF at all dose level significantly ([p<0.01%) reduced the increase in blood glucose level in glucose loaded rats compared with diabetic control rats which showed elevation in blood glucose throughout the total measurement period (120min).

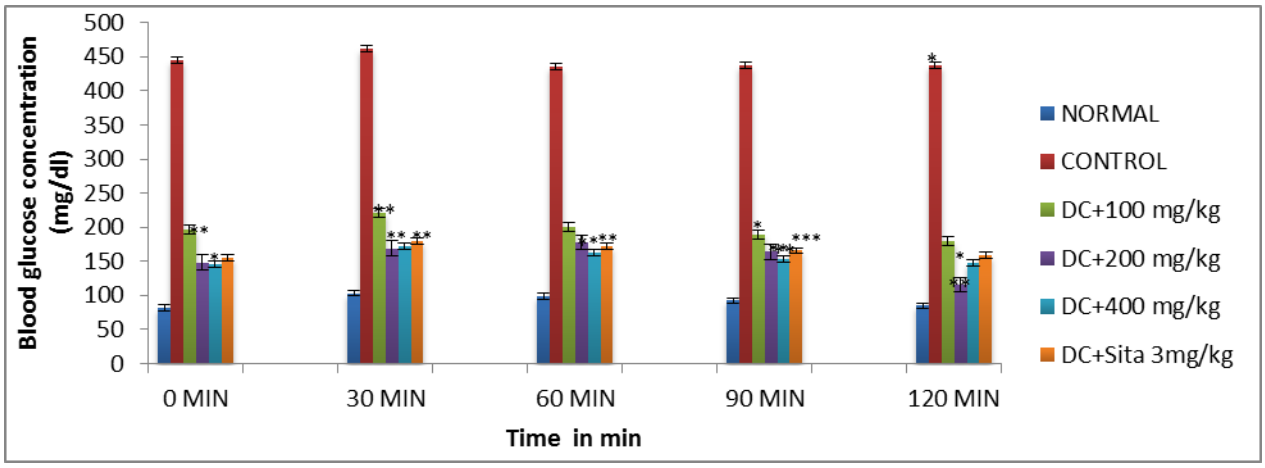


Fig 5: Effect of administration of EASF on Oral glucose tolerance Test in High fat diet plus low dose STZ induced Type II diabetic Rats.

NC: Normal control; DC: Diabetic control; EASF: Ethyl acetate fraction of Sterculia foetida SG: Sitagliptine Data are expressed as mean ± S.E.M. (n=6). #: p<0.05, ##: p<0.01 as compared to NC; *: p<0.05, **: p<0.01 as compared to DC. Data analyzed by one way Analysis of Variance (ANOVA) followed by Dunnet's multiple test for comparison.

Lipid Profile

The protective effects of EASF on lipid profile are shown in above figure. There are significant elevation in the levels of T-CH, TG, LDL-CH and VLDL-CH and significant decrease in serum HDL-CH when compared with normal group. Administration of EASF for 28 days showed significant reduction in elevated level of serum lipid.

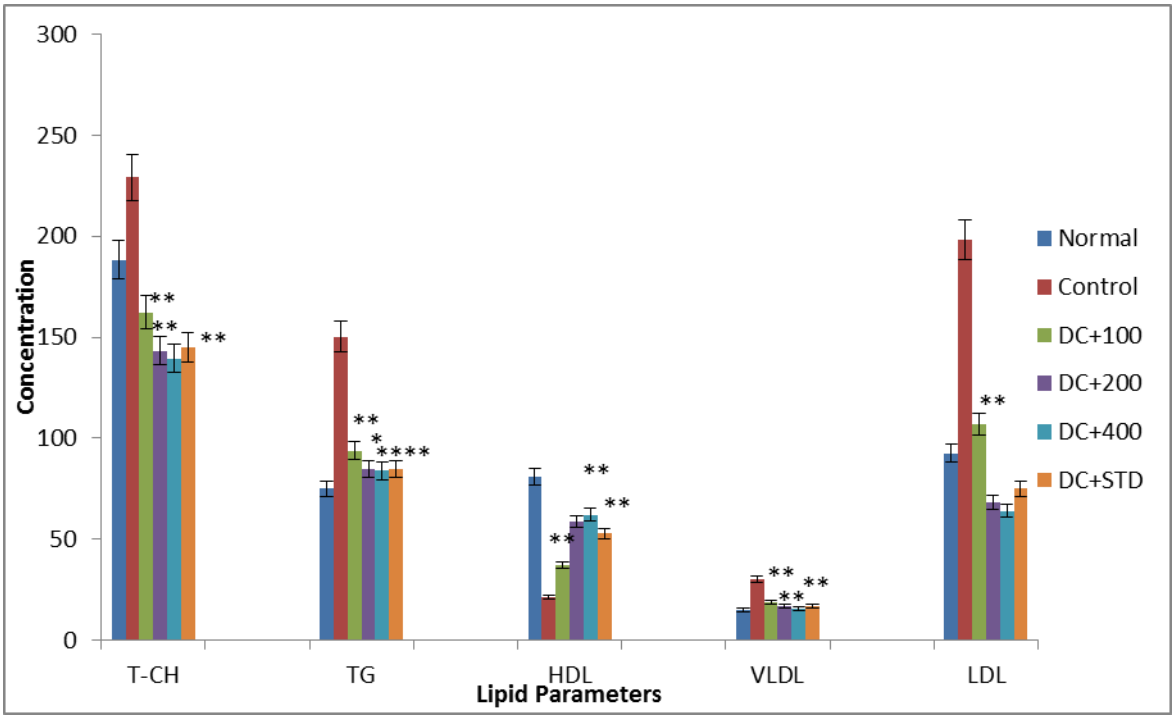


Fig. 6: Effect of administration of EASF on Lipid Profile of High fat diet plus low dose STZ induced type II diabetic rats.

NC: Normal control; DC: Diabetic control; EASF: Ethyl acetate fraction of Sterculia foetida SG: Sitagliptine Data are expressed as mean ± S.E.M. (n=6). #: p<0.05, ##: p<0.01 as compared to NC; *: p<0.05, **: p<0.01 as compared to DC. Data analyzed by one way Analysis of Variance (ANOVA) followed by Dunnet's multiple test for comparison.

Liver Enzyme

The effect of EASF on the hepatic function markers such as SGOT, SGPT and ALP indifferent experimental groups of rats is shown in fig.6.12. In comparison with normal control group, the level of SGOT, SGPT and ALP were increased significantly in diabetic untreated groups and after treatment with EASF for 28 days the level of SGOT, SGPT and ALP were significantly decreased to normal value as compared to diabetic rats.

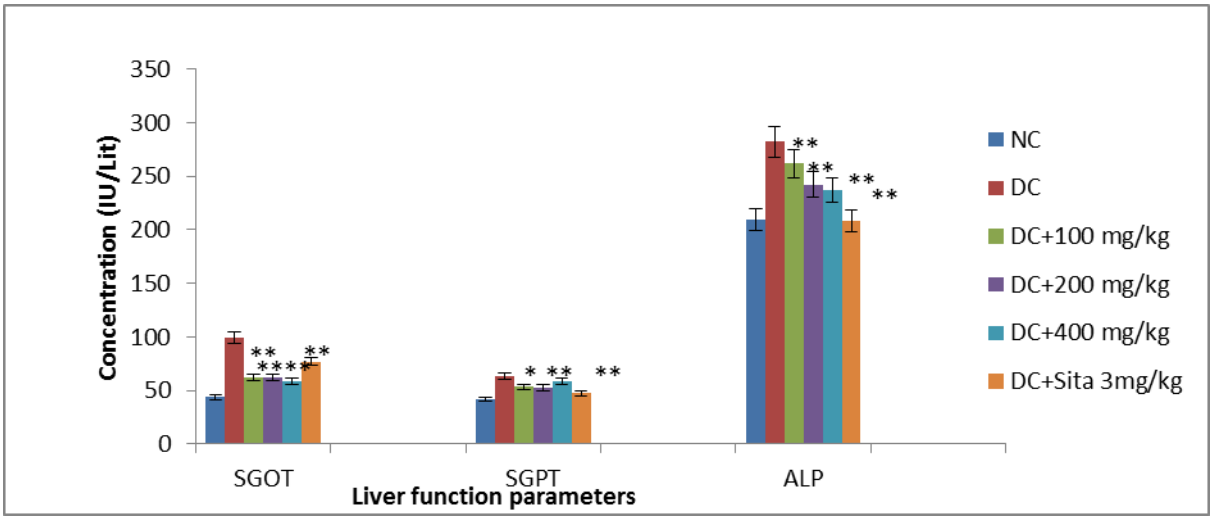


Fig. 7: Effect of oral administration of EASF on serum SGOT, SGPT and ALP High Fat diet plus Low Dose STZ Induced type II Diabetes in Rats.

NC: Normal control; DC: Diabetic control; EASF: Ethyl acetate fraction of Sterculia foetida SG: Sitagliptine Data are expressed as mean ± S.E.M. (n=6). #: p<0.05, ##: p<0.01 as compared to NC; *: p<0.05, **: p<0.01 as compared to DC. Data analyzed by one way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.

Biochemical Parameters

Table 1: Effect of administration of EASF on various biochemical parameters parameters in High fat diet plus low dose STZ induced type II diabetic rats.

Experimental groups	HbA_{1c} (%)	Plasma Insulin	Plasma Leptin	PlasmaTNF(pg/ml)
NC	5.55±0.34	14.54±1.09	4.6 0.±006	38.2±0.7
DC	8.22±0.36	2.66±0.23	6.7 0±.009	42.3±1.02
DC+EASF(100mg/kg)	5.65±0.15	2.57±0.19 **	3.51 0.±010 **	27.4±1.37 **
DC+EASF(200 mg/kg)	5.760±0.18	3.08±0.19 *	3.94 0.±011**	26.4±0.50 *
DC+EASF (400mg/kg)	5.90±0.39	2.95±0.29 **	3.46 0.±008**	21.5±1.29*
DC+STD (3mg/kg)	4.91±0.26	7.49±0.51**	5.24 0.±011**	31±1.16 **

NC: Normal control; DC: Diabetic control; *EASF*: Ethyl acetate fraction of *sterculia foetida* seeds n=5, Values are mean ± S.E.M., #p<0.05, ##p<0.01 as compared to normal control group *p<0.05, **p<0.01 as compared to diabetic control group Data analyzed by one way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Test for comparison.

Biochemical Parameters

The Table 1 deplicate effect of administration of different doses of EASF on Various Biochemical Parameters like Plasma Insulin, Plasma TNF and Plasma Insulin. High fat diet control group showed an increase in Plasma insulin level. Administration of EASF maintain Plasma insulin significantly (p<0.01%) in EASF treated high fat diet rats as compared to high fat diet control rats. STZ-HFD rats showed elevation in plasma TNF and leptin level. Treatment with EASF for 28 days showed significant reduction in elevated level of TNF and leptin.

Body Weight, Food Intake and Water Intake

Table 2 deplicate effect of administration of different doses of EASF on Body weight, food intake and water intake.

Table 2: Effect of EASF on body weight, food and water intake in STZ diabetic rats.

Experimental Group	Body Weight		Water Intake		Food Intake	
	Initial	Final	Initial	Final	Initial	Final
Normal Control(NC)	162.60±5.63	188.60±5.30	13±0.45	11.96±0.57	12.5±0.77	17.55±1.047
Diabetic Control(DC)	264.60±12.61	230.40±10.11	58.38±4.56**	58±4.60**	23±1.68**	27.45±1.24**
DC+EASF(100mg)	228±6.04	238.60±8.61	45.50±3.52	39.65±1.73*	23.46±0.45	22.43±0.90*
DC+EASF(200mg)	209.60±18.23	204±20.94	52.68±4.94	38.35±1.56**	25.62±1.21	21.96±0.83**
DC+EASF (400mg)	262±9.30	250.40±7.41	54.15±2.29	39.20±2.34**	25.42±2.35	21.32±1.26**
DC+STD(3mg/kg)	168±11.44**	147.80±8.59**	55±2.63	35.6±3.86**	20.52±0.76	19.34±0.97**

*NC: Normal control; DC: Diabetic control; EASF: Ethyl acetate fraction of Sterculia foetida SG: Sitagliptine Data are expressed as mean ± S.E.M. (n=6). #: p<0.05, ##: p<0.01 as compared to NC; *: p<0.05, **: p<0.01 as compared to DC. Data analyzed by one way Analysis of Variance (ANOVA) followed by Dunnet's multiple test for comparison.*

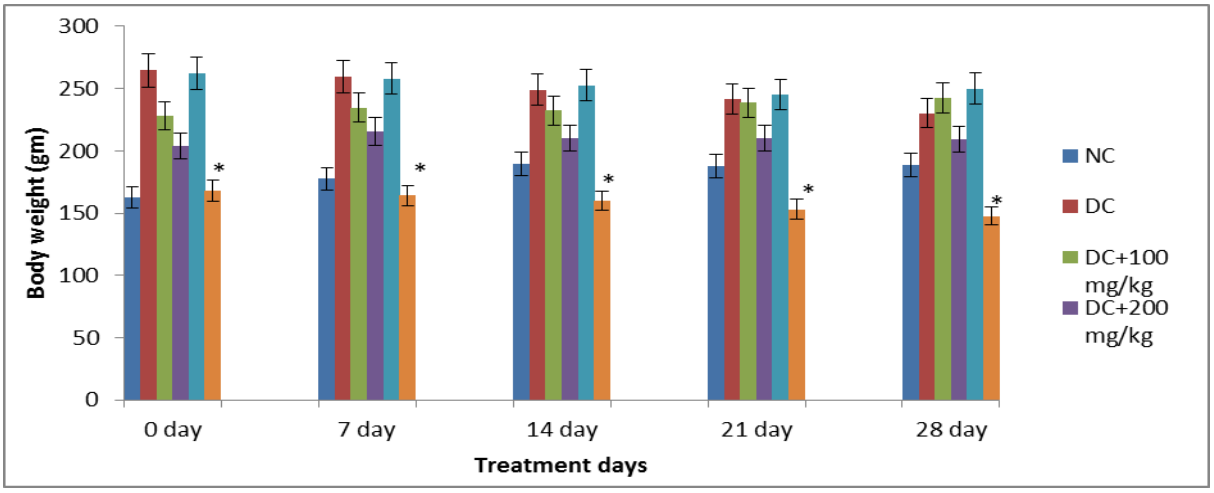


Fig. 8: Effect of administration of EASF on Body Weight of High fat diet plus low dose STZ induced type II diabetic rats.

NC: Normal control; DC: Diabetic control; *EASF*: Ethyl acetate fraction of *sterculia foetida* seeds n=5, Values are mean ± S.E.M., #p<0.05, ##p<0.01 as compared to normal control group *p<0.05, **p<0.01 as compared to diabetic control group Data analyzed by one way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Test for comparison.

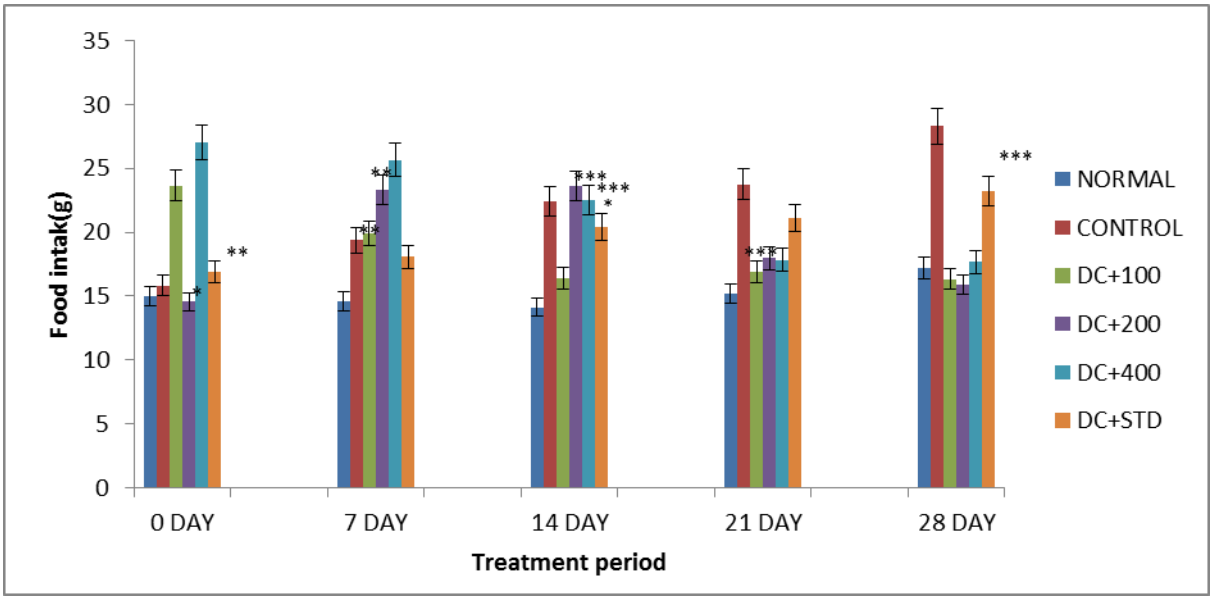


Fig. 9: Effect of administration of EASF on food intake of High fat diet plus low dose STZ induced type II diabetic rats.

NC: Normal control; DC: Diabetic control; *EASF*: Ethyl acetate fraction of *sterculia foetida* seeds n=5, Values are mean ± S.E.M., #p<0.05, ##p<0.01 as compared to normal control group *p<0.05, **p<0.01 as compared to diabetic control group Data analyzed by one way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Test for comparison.

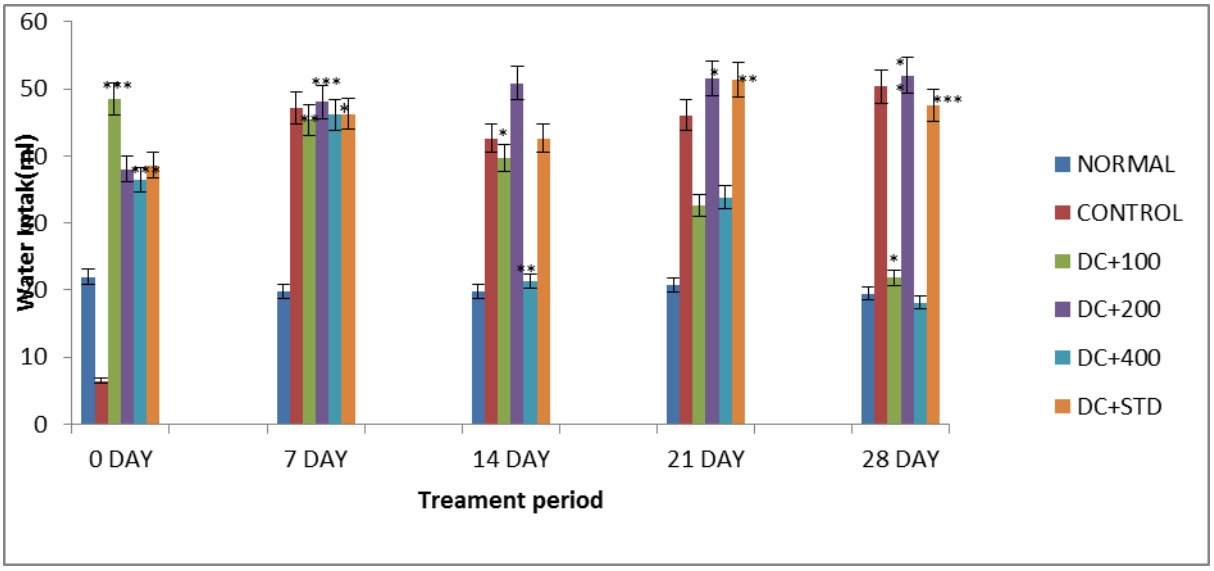


Fig. 10: Effect of administration of EASF on Water intake of High fat diet plus low dose STZ induced type II diabetic rats.

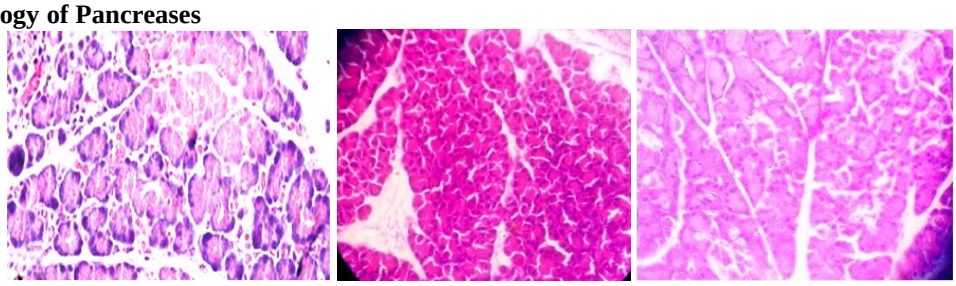
NC: Normal control; DC: Diabetic control; *EASF*: Ethyl acetate fraction of *sterculia foetida* seeds n=5, Values are mean ± S.E.M., #p<0.05, ##p<0.01 as compared to normal control group *p<0.05, **p<0.01 as compared to diabetic control group Data analyzed by one way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Test for comparison.

Body weight, food intake and water intake

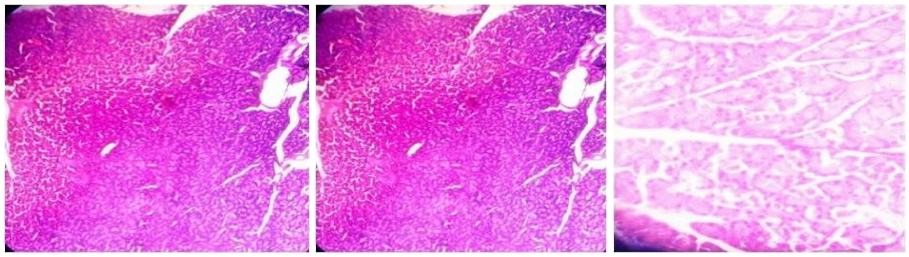
Body weight of streptozotocin induced diabetic control rats was found to be significantly (p<0.05) less compared to normal control rats. After 4 weeks of treatment with HACA body weight significantly (p<0.05) increased compared to diabetic control groups. Food intake was significantly high in diabetic control rats as compared to normal control. At the end of 28 days of treatment food intake of treated groups significantly (p<0.01) decreased as compared to diabetic control. Significant decrease (p<0.01) in water intake was observed in treated groups as compared to diabetic control at the end of study period.

Histopathology study

1. Histopathology of Pancreases



Normal **Diabetic Control DC+STD** **Sita3mg/kg**



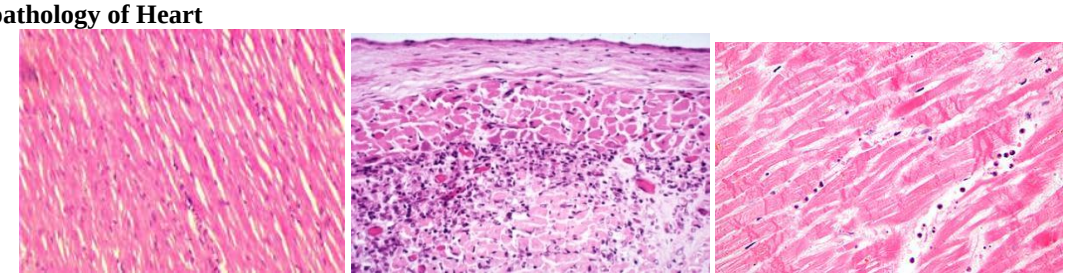
DC+100mg/kg **DC+200mg/kg** **DC+400 mg/kg**

Pancreas sections were stained with haematoxylin-eosin and observed under 40X magnification of digital microscope. (A) Normal control with typical histological structure of rat pancreas. (B) Diabetic control group showing necrosis of pancreatic islet cells, dilation of blood vessel. (C) DC + Sitagliptin (3 mg/kg). (D) DC+EASF (100 mg/kg). (E) DC+ EASF (200 mg/kg). (F) DC+EASF (400mg/kg). All EASFtreated group shows significant improvement in pancreas histopathology as compared to DC.

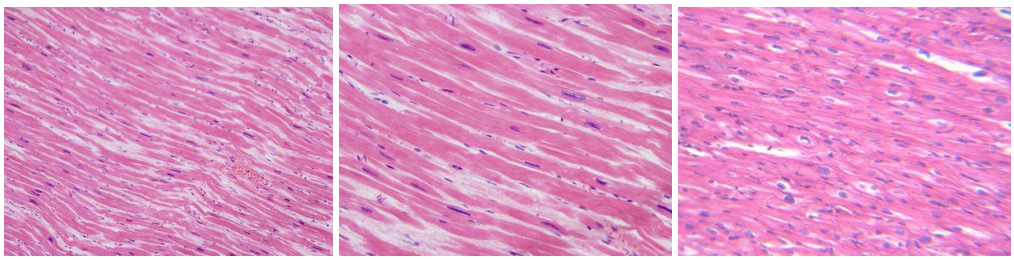
Fig 6: Effect of administration of EASF on Histopathology of Pancreas of High fat diet plus low dose STZ induced type II diabetic Rats.

The Histopathological changes of pancreas of diabetic rat showed severe necrotic changes of pancreatic islets, nuclear changes with dilatation and congestion of blood vessels. Treatment with EASF shows slightly restoration of architecture of pancreas that was earlier affected with HFD+STZ.

2. Histopathology of Heart



Normal **Diabetic Control** **DC+STD**



DC+100mg/kg **DC+200mg/kg** **DC+400mg/kg**

Fig. 7: Effect of administration of EASF on Histopathology of Heart of High fat diet plus low dose STZ induced type II diabetic Rats.

The Histopathological studies revealed that administration of STZ+HFD for week Caused morphological abnormalities such as moderate diffuse myocardial degeneration and necrosis with inflammatory infiltration in heart muscles and cystic dilation of tubules, atrophy of the tubular epithelium and decreased number of glomeruli with mild degree increase in glomerular filtration space in the kidney. These abnormalities were partially prevented by treatment of different doses of EASF.

DISUSSION

Insulin resistance is a condition in which defects in the action of insulin are such that normal levels of insulin do not trigger the signal for glucose absorption the resulting in hyperinsulinaemia. Insulin resistance is an early key defect associated with Type 2 Diabetes and other metabolic disorder such as Obesity and hypertension.[24] Before, the advent of insulin and other synthesis medicine for diabetes, the herbal medication was the mainstay in antidiabetic therapies. Now days, insulin and other synthesis medicine take the place of herbal medication gradually in foreign countries due to their remarkable therapeutic effects.[25] However, the complications in macro vascular retinal and neuropathic functions are still associated in the patients. Insulin resistance is another serious problem, which are a common consequence of overweight and a cause of impaired glucose tolerance in Type2 Diabetes.

Treatment of Diabetes with insulin and oral drugs failed to prevent Diabetic complications in many patients, therefore the use of alternative medicine, especially the consumption of botanical has been increasing, mostly because of the less frequent side effects and multiple effects when compared with modern medicine.[26] In this study the effect of *Sterculia Foetida L*.seeds on Insulin Resistance in Type II Diabetes is evaluated by Using High Fat Diet plus Low dose STZ in Wistar rats. The present results suggest that EASF exhibit significant antihyperglycemic, hypolipidemic and Insulin Resistance effects in STZ+HFD induced Type II Diabetes in rats. The administration of High fat diet for a period of four weeks followed by single injection of STZ (35 mg/kg) developed Insulin resistance with T2D rats. High Fat diet for four weeks and administration of single low dose of STZ is a well known model for induction of Type 2 Diabetes with insulin resistance.[27]

The feeding of high fat diet caused insulin resistance in rats and was characterised by increased body weight (Obesity), mild hyperglycaemia, hypertriglyceridaemia, hypercholesterolemia and compensatory hyperinsulinaemia. HFD-fed rats which are already hyperglycaemic due to insulin resistance, become more susceptible to the dibetogenic effect of STZ.[28] In other words, beta cells under the strain of compensatory hyperinsulinaemia could be easily damaged by lower dose of STZ.

These insulin-resistant HFD-STZ rats also showed abnormalities in lipid metabolism as evidenced from increased TG, TC, VLDL-CH, and LDL-CH levels, as in human type 2 Diabetes. The onset of hypertriglyceridaemia in these HFD+STZ rats may be due to increased absorption and formation of triglycerides in the form of chylomicrons following exogenous production of TG-enriched hepatic VLDL and decreased TG uptake in peripheral tissue. Hypercholesterolemia may be attributed to increased dietary cholesterol absorption from the small intestine following the intake of HFD in a diabetic condition. Polyphagia and polydipsia with concomitant reduction in body weight are major symptoms of diabetes mellitus which were also evidently observed in the diabetic group of our experiment.[29] In our study, although treatment with the either dosages of EASF did not affect the food intake, the dose-dependently lower fluid intake and relatively better body weight gain in EASF treated groups compared with DC group suggest its possible antidiabetic effect at least for T2D.

OGTT was performed on 28th day of experiment. Glucose loaded diabetic rats treated with EASF have shown significant antihyperglycemic effects at 60 and 120 mins, when compared with glucose loaded diabetic control rats. Impaired glucose tolerance reflects hepatic gluconeogenesis and reduced uptake of glucose from blood into skeletal muscle and adipose tissue following a meal Impaired glucose tolerance serves as a marker for the state of insulin resistance and predicts both large and small-vessel vascular complication.[32] EASF increases the glucose tolerance of STZ-HFD Induced Type 2 diabetic rats compared to diabetic control rat. OGTT results indicated that the control of post-prandial glucose level shown by EASF may be medicated by regulation of glucose uptake from the intestinal lumen, through the inhibition of carbohydrate digestion or absorption.

Glycosylated haemoglobin (HbA1c) has been the key measure of glycaemic control in diabetic patients.[33] HbA1c is an indicator of irreversible condensation of glucose with the N-terminal residue of the B-chain of haemoglobin A. In uncontrolled or poorly controlled diabetic animals increased glycosylation of number of proteins has been reported.[34] The concentration of HbA1c in blood is directly proportional to the mean concentration of glucose in the previous 6 to 8 weeks. In the present study, the concentration of HbA1c in Sitagliptine and EASF treated rats was found significantly reduced as compared to diabetic control rats as compared to diabetic control rats.

The EASF plays a key role in controlling coronary heart disease by exerting its effect on altered lipid profile in diabetic rats. In diabetic state, there is inactivation of the lipoprotein lipase by which free fatty acids are converted into phospholipids and cholesterol, which are finally discharged into blood, causing an elevation of serum phospholipids level. HDL helps to scavenge cholesterol from extra hepatic tissues.[35] Decreased HDL can contribute to the increased LDL cholesterol levels as there is a reciprocal relation between the concentration of LDL and HDL. In our study, markedly increased levels of TG, T-CH, VLDL-CH, LDL-CH and decreased level of HDL-CH in STZ diabetic rats contributed to the pathogenesis of diabetic rats as reported in previous studies.[36] This altered levels of TG, T-CH, VLDL-CH, LDL-CH and HDL-CH was reversed towards the normal control level by administration of EASF during treatment period. This implies that EASF may activate lipoprotein

lipase which would be helpful to reduce the incidence of lipid born complications such as renal damage.

The elevation of biomarker enzymes such as SGOT, SGPT and ALP indicated hepatic injury.[37] The diabetic complication such as gluconeogenesis and ketogenesis may be due to increase transaminase activity. It may be due to increased STZ mediated liver damage which may cause leaking of enzymes into blood.[38] Aspartic transaminase and almandine transaminase were used as markers to assess the extent t of liver damage in STZ induced insulin resistance in rats. The elevated enzymatic activity of SGOT with only moderately increased activity of SGPT suggest cardiac damage while elevated activity of SGPT with only moderate increases in SGOT activity suggest liver damage.[39] In present study SGPT, SGOT ALP level significantly decreased in extract traded group in comparison to STZ-HFD control.

Leptin is a protein hormone made up of 167 amino acids and released by adipocyte. It is considered to have a role in regulation of body Weight. And energy metabolism.[40] It reduces insulin release from human pancreatic B-cells and inhibits insulin biosynthesis by decreasing propane insulin mRNA expression in B-cells.) It plays a role in chronic inflammation and autoimmunity.[41] Serum leptin concentration is increased in obese subject. It is higher in subject with Type-2 DM. High Leptin level significantly markers of insulin resistance. HFD-STZ induced Diabetic Rats Showed Elevated Level of Leptin as Compared to Other Group. Adminsitaration of EASF Show Significant Decreased in Level of Leptin.

Tumor necrosis factor (TNF a) is a cytokine initially described as an endotoxin included factor TNF-α was the first cytokine to be implicated in the pathogenesis of obesity and insulin résistance.[42] Several potential mechanism for TNF as metabolic effects have been described including the activation of serine kinase such as JNK and B38 nitrogen activated protein kinase (MAPR) that increase serine phosphyration of ZRS-1 and IRS-2 making them poor substractly for insulin receptor activating kinase and increasing their level which lead to development of insulin resistance.[43,44] Plasma TNF of the HFD fed rats are significantly elevated compared to the control rats. Moreover this elevation is associated with another elevation of plasma level of triglycerides and free fatty acid. Theses observation seemed to support a strong link between TNF and insulin resistance that often is related to obesity.[45,46] EASF through effects action decreased plasma TNF level resulting in inhibition of excessive lipolysis and lipid turnover from the adipose tissue.

Histopathological examination of pancreas sections of High Fat Diet plus low dose STZ induced Type 2 Diabetic rats showed alterations such as islets shrinkage (atrophy), irregular islets, cellular swelling, ß-cell vacillation and apoptosis, and the presence of necrotic cells.[47,48] Treatment with EASF significantly suppressed further damage to endocrine cells, evidenced by the decreased number of necrotic cells. This effect by EASF is of important significance because cell necrosis is an irreversible process, whereas cell degeneration is reversible with the help of a good glycaemic-control agent to enable it to function normally again.[49,50]

In conclusion, the results of present study shows that EASF possess antihyperglycemic as well as Insulin Resistance activity along with hypolipidemic effect which is due to its active principles such as flavonoids. The findings of the current study showed that EASF have a hypoglycaemic effect in High fat diet plus low dose STZ induced Type 2 diabetic rats. In addition, they were highly effective in managing the complications of diabetes mellitus such as Hyperlipidemia, weight loss. The antidiabetic effects EASF may be mediated through an increase in insulin secretion, the inhibition of gluconeogenesis and glycogenolysis and/or protection of pancreatic β-cells from streptozotocin and glucose-induced oxidative stress. This summed effect seems to have a promising value for the development of a potent phytomedicine for diabetes. However, further studies are required for clarification of detailed mechanism of action *Sterculia Foetida* Linn Seeds which will provide new therapeutic targets for treatment of diabetes.

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