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ANTIDIABETIC AND ANTIHYPERLIPIDAEMIC ACTIVITIES OF *ENSETE SUPERBUM* FRACTIONS ON HIGH FAT FED WITH LOW DOSE STREPTOZOTOCIN INDUCED TYPE-2 DIABETES IN RATS

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ABSTRACT

***Objective:** To investigate in vitro and in vivo anti diabetic activity of Ensete superbum (E. superbum) fractions on high fat diet (HFD) with low dose Streptozotocin (STZ) induced type -2 diabetes mellitus in rats. **Methods:** Ensete superbum were successively extracted and then fractionated by Ethyl acetate and n-butanol. The in vitro antidiabetic activity of α-amylase and α- glucosidase was used to evaluate the potential activity of the fractions. The in vivo antidiabetic activity is evaluated against HFD/STZ induced type- 2 diabetic in rats at dose of 100mg/kg, 200mg/kg and 400mg/kg by mouth for 28 days. **Results:** Ethyl acetate fraction of Ensete superbum (EAFES) showed the highest antidiabetic activity and IC_{50} value of α-amylase inhibition (117.2±1.43) and α- glucosidase inhibition (105.80±1.65) than other fraction. In HFD/STZ (40mg/kg) diabetic rats the EAFES showed dose dependent significant hypoglycaemic property from body weight, blood glucose, serum lipids, serum cholesterol, serum triglycerides, urea, creatinine, hepatic enzymes and liver glycogen levels. The EAFES significantly ($P < 0.01$) increase the level of serum insulin. Histologically, focal necrosis was observed in diabetic rat pancreas; however, was less obvious in treated groups. **Conclusion:** The EAFES is a potent hypoglycaemic agent and beneficial in reducing the elevated blood glucose level, improve the lipid profile and insulin level and histopathological changes in pancreas of HFD/STZ-induced non-genetic rat model of type- 2 diabetes mellitus.*

***Keywords –** Antidiabetic, Ensete superbum, Streptozotocin, Type-2 diabetes, Pancreas.*

1. INTRODUCTION

Diabetes mellitus is a chronic disease caused by inherited and acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced. Such as deficiency results in increased concentrations of glucose in the blood, which is turn damage to various macro and micro vascular complication leading to damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels[1]. Estimated 366 million people worldwide (8.3 % of adults) had diabetes in 2011 and was predicted to go up to 552 million people (one adult in 10) by the year 2030[2].

Glucose homeostasis, especially post prandial increase in blood glucose levels, is facilitated by enteric enzymes namely α-amylase and α-glucosidase attached to the brush border of the intestinal cells[3]. Alpha-amylase (α-1,4 glucan-4- glucanohydrolase) at first converts starch to oligosaccharides by hydrolyzing α-1,4-glucan bonds. Thus, the first reaction in digestion of carbohydrates is initiated by α-amylase by forming oligosaccharides. Unabsorbed carbohydrates (disaccharides and oligosaccharides) will then get bound to α-glucosidase enzymes in the brush border of small intestine[4]. Inhibition of these carbohydrate digesting enzymes prolong overall carbohydrate digestion time by delaying its breakdown, causing a decrease in the rate of glucose absorption and consequently blunting the post prandial rise in plasma glucose[5].

Streptozotocin (STZ, 2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is used to induce both type 1 and type 2 diabetes mellitus in rats. High fat diet with STZ injected intraperitoneally at a dose of 40 mg/kg b.w. in rats produced type 2 diabetes mellitus showing sustained hyperglycemia[6].

Herbal medicine are prepared from various plant part are contain many bioactive compounds are used primarily for treating. Due to the demand in the field of herbal medicines, it has become necessary of systematic knowledge about herbal drugs. The safety of herbals, it is better not to bury our phytotherapeutic heads in the sand like frightened ostrich in the hope that herbal health problems will dissolve by themselves. Thus we should accept that herbal medicines entail certain health risks and to look out actively for safety problems associated with herbal medicines several considerations[7]. Consistency in composition and biological activity are essential requirements for the safe and effective use of therapeutic agents. Quality is the critical determinant of safety and efficacy of botanical medicines[8].

Ensete superbum (Roxb) Cheesman., belongs to the family Musaceae, which is being widely used as Folk and ayurvedic medicine. It is widely distributed throughout Western Ghats region of India [9]. The plant has more important medicinal uses. Many of the members of the Musaceae family are used as medication for dog bite[10]. Medicinally *Ensete superbum* is used as potent anti-fertility agent [11]**.** The plant was reported to possess anti-oxidant. In this study, the fraction of *Ensete superbum* were used to perform inhibitory studies on enzymes α-amylase, α- glucosidase and hypoglycaemic effects of ethyl acetate fraction of *Ensete superbum* (EAFES) on high fat fed with low dose Streptozotocin induced diabetes in rats. This evaluation is required to establish potential hypoglycaemic effects in type 2 diabetes of this valuable herbal preparation.

2. MATERIALS AND METHODS

2.1 Drugs and chemicals

Glucose assay, cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), triglyceride assay kits (Agappe Diagnostics, Kerala, India). Streptozotocin, α-amylase (HiMedia, Mumbai, India) and potato starch, maltose, (Merck Ltd, Mumbai, India), and α- glucosidase (Sisco lab Ltd, Mumbai, India), Tween-80 (SD fine chemicals, Mumbai, India) were purchased for the study. Glibenclamide (Aventis Pharma, Mumbai, India) and Acarbose (Orchid Pharma Ltd., Chennai, India) were obtained as gift sample. All the other chemicals used in the study were of analytical grade and were obtained commercially.

2.2 Instrument used

Shimadzu 1700 UV/Vis Spectrophotometer, Maxlyzer ultra semi auto analyzer, Systronics MKVI digital pH meter and SD check glucometer.

2.3 Collection of plants

Ensete superbum seeds were collected from Kottakal, Malappuram Dt, Kerala, India during the month of June. It was identified and authenticated by Dr. R. Prabakaran, head of the department of botany, Vivekananda College of arts and science for women, Tiruchengode, Tamilnadu, India, and the voucher specimen was deposited at the same institute for future reference.

2.4 Preparation of fraction

Five hundred grams of shade-dried powdered seeds of *Ensete superbum* were extracted using Soxhlet, successively with petroleum ether, chloroform and ethanol and for 72 h each. The ethanolic extract was prepared and dried separately. The solvents were evaporated in vacuum to obtain residues of the extract. The ethanol extract was done using its solubility profile[11]. 30 g of dried ethanol extract was taken in a stoppered flask, containing 250 ml of water and shaken mechanically for 1-2 h in a flask shaker. The ethanol extract was not completely soluble in water. The water insoluble portion of ethanol extract was separated using filtration and further fractionated with ethyl acetate and n-butanol using the same procedure. The supernatants obtained from the above fraction were concentrated and evaporated to dryness and their per cent yield was determined. The ethyl acetate fraction *of E. superbum* (EAFES) and n-butanol fractions of *E. superbum* (NBFES) were used to in-vitro antidiabetic activity. Since the activity was found in the ethyl acetate fraction and it was suspended in 1% Tween 80 freshly was prepared just before administration to the rats.

2.5 *In vitro* antidiabetic studies

In vitro antidiabetic potential was studied by performing of the ethyl acetate and n-butanol fractions of *Ensete superbum* using carbohydrate digesting enzymes: α-amylase and α-glucosidase.

2.6 *In vitro* inhibition of α-amylase

The different concentrations (5–1000 µg/ml) of ethyl acetate and n-butanol fractions of *Ensete superbum* /standard *acarbose* were prepared in dimethyl sulfoxide from 1 mg/ml stock solution and 500 µl of test/standard was added to 500 µl of α-amylase (0.5 mg/ ml) and was incubated for 10 min at room temperature. Then added 500 µl of 1% starch solution and incubated for another 10 min. After that 1 ml of the 3, 5-dinitrosalicylicacid as a colouring reagent was added to the reaction mixture and heated in a boiling water bath for 5 min. After cooling, it was diluted with 10ml of distilled water. The absorbance was then measured at 540 nm against reagent blank. The α-amylase inhibition was expressed as percentage of inhibition and the IC_{50} values determined by linear regression plots with varying concentration of fraction against percentage inhibition [12]. The percentage inhibition was calculated employing the following formula.

% inhibition = [(Abs of control - Abs of test)/ Abs of control] X 100

2.7 *In vitro* inhibition of α-glucosidase

From 1 mg/ml stock solution different concentrations (5–1000 µg/ml) of ethyl acetate and n-butanol fractions of *Ensete superbum* / standard *acarbose* in 5 % dimethyl sulfoxide. Five hundred micro liters of test/standard was added to 500 µl of α-glucosidase (1U/ml) and was incubated for 5 min at room temperature. Then added 500 µl (37 mM) maltose solutions and incubated for 30 min. After that 1 ml of the glucose kit reagent was added to the reaction mixture and kept aside for 15 min. 1 ml of Tris buffer was then added to the mixture. The absorbance was then measured at 505 nm against reagent blank. The α-glucosidase inhibition was expressed as percentage of inhibition and the assays were carried out in triplicate. The IC_{50} values were determined by linear regression plots with varying concentration of plant fraction against percentage inhibition[13]. The percentage inhibition was calculated employing the above formula.

2.8 *In vivo* antidiabetic studies

2.8.1 Selection of dose

LD_{50} was determined as per OECD guidelines for fixing the dose for biological evaluation. The LD_{50} of the ethyl acetate fractions of *Ensete superbum* (EAFES) falls under category 4 values with no death and no signs of acute toxicity at doses of 2000 mg/kg. The biological evaluation of the fraction was carried out at dose levels of 200 and 400 mg/kg body weight.

2.8.2 Animals

Throughout the experiment, experimental rats were processed in accordance with the instruction given by our institutional ethics committee for the purpose of control and supervision on experiments on animals (CPCSEA) [14]. Healthy Wister rats (150-200g) were used for the study. Animals were kept in standard polypropylene cage and maintained under standard laboratory conditions of temperature (24±1ºC), 12 hours dark like cycles, standard diet and water ad libitum. The study protocol was approved by the institutional ethical committee (JKKMMRFCP/IAEC/2014/001) and all the procedure were performed in accordance with the recommendations for the proper care and use of laboratory animals.

2.8.3 Oral glucose tolerance test (OGTT)

Rats were divided into five groups of 6 animals each. The group I received normal saline of 1% of Tween 80, group II was administered Glibenclamide (10 mg/kg, p.o.) and remaining groups received EAFES at 100mg/kg, 200mg/kg and 400 mg/kg, p.o. All groups received glucose solution (2g/kg) 30 minutes after the administration of tested drug. Blood sample were withdrawn from tip of tail and blood glucose levels were estimated at 0, 30, 60, 90 and 120 minutes using one touch glucometer (SD check, India).

2.8.4 Preparation of High fat diet (HFD)

High fat diet (HFD) was prepared as per Gandhi et al [15] and consisted of 73% of normal diet, 25% of coconut oil and 2% of dietary cholesterol, all of commercial grade.

2.8.5 Development of HFD and low dose of Streptozotocin (STZ) treated type 2 diabetic rats

The animals were fed with high fat diet once a day for two weeks followed by type 2 diabetes mellitus was induced in overnight fasted rats administering a single dose of freshly prepared solution of Streptozotocin (40 mg/kg. b.w. i.p) in 0.1 Mol/L of cold citrate buffer (pH 4.5). The STZ treated animals were allowed to drink 5% glucose solution overnight to drug induced hypoglycemia. After 7 days of injection of STZ rats with moderate diabetes having persistent glycosuria and hyperglycemia (blood glucose >250 mg/dl) were used for further experimentation[16].

2.8.6 Experimental design

The rats were divided into six groups of six rats (n=6) each. Group I and Group II served as normal control rats and High fat normal control rats were given 1ml of 1% Tween 80. Group III served as Diabetic control were given 1ml of 1% Tween 80. Group IV served as standard and was treated with 1% Tween 80 containing *Glibenclamide* (10mg/kg). Group V and Group VI were treated with dose of (200mg/kg and 400mg/kg) EAFES in 1% of Tween 80. The treatments were continued daily for 28 days.

Blood samples were collected from tip of rat tail and blood glucose levels were estimated on 0^{th}, 7^{th}, 14^{th},21^{st} and 28^{th} days of treatment using One touch glucometer (SD check, India). Body weight was measured initially and during treatment period. On 29th day blood was collected by retro-orbital puncture from the inner canthus of the eye under mild ether anaesthesia using capillary tubes in fresh vials and serum separated. Serum parameters like triglycerides, total cholesterol, urea, creatinine, insulin, low-density lipoproteins (LDL), high density lipoproteins (HDL), alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) were determined using standard kits obtained from Agappe Diagnostics, Cochin, India using a semi autoanalyser (Maxlyzer ultra). Immediately after the blood collection, the animals were sacrificed under anaesthesia, Hemi diaphragm, pancreas and liver tissues were dissected out for further studies.

2.8.7 Estimation of glycogen and glucose uptake by Hemidiaphragm and liver glycogenolysis

The liver tissue was separated in two portions, one for the glycogen estimation and another for liver glycogenolysis. The liver tissue was homogenized in 5 % w/v trichloroacetic acid and its glycogen content was determined by the method [17]. The hemidiaphragms and livers tissues were carefully excised and placed immediately in ice cooled perfusion solution with the following composition: NaCl (0.687%), KCl (0.04%), $MgSO_4$ (0.014%), $CaCl_2$ (0.028%), $NaHPO_4$ (0.014%) and $NaHCO_3$ (0.21%). Glucose was added to another batch of the

perfusate at a concentration of 400 mg%. This perfusate was used to study the glucose uptake/ transfer processes. The hemidiaphragms were incubated at 37°C for 1.5 h with appropriate aeration to enable stirring and also to provide oxygen to the tissue. At the end of the incubation period glucose concentration in the perfusate was assayed. The hemidiaphragms were removed, rinsed in water and dried in an oven at 55–60°C for 4–5 h or till a constant weight was obtained. The glucose uptake during the incubation period was calculated in terms of mg per100 mg dry weight of hemidiaphragm. Similarly, liver slices were incubated in the glucose enriched perfusate. The glucose concentration in the perfusate after the incubation period was determined in terms of mg per g of dry weight of liver [18].

2.8.8 Histopathological studies of pancreatic tissues

Pancreatic tissues isolated from rats were used for histopathological studies. The tissue in each group was cut into small portions measuring 1 cm, fixed with 10 % formaldehyde solution, dehydrated in gradually increasing concentrations of ethanol (50–100 %), cleared in xylene and embedded in paraffin. Sections of 5 µm thickness were prepared. Haemotoxylin and eosin were used for staining and later the microscopic slides of pancreatic tissue were photographed under 100x magnifications.

2.8.9 Statistical analysis

All determinations for *in vitro* study were carried out in triplicate and the values are expressed as mean ± SEM and inhibitory concentration (IC_{50}) was carried out with GraphPad Prism. *In vivo* study the results were expressed as mean ± SEM for 6 rats in each group. Statistical analysis of the results was carried out using GraphPad InStat software by one-way analysis of variance (ANOVA) followed by Dunnett's test. The level of significance was set at $P<0.05$.

3. RESULTS

3.1 *In vitro* inhibition of α-amylase

The fractions of EAFES and NBFES, elicited a dose dependent inhibition of α-amylase enzyme activity. The α-amylase inhibitory effect of the EAFES was found to be ranging from 15.21 % to 85.86% when studied at concentrations 5–1000 µg/ml. At the same concentration range the inhibitory effect of NBFES was found to be ranging from 13.36% to 82.63% whereas the effect of standard drug acarbose ranged from 20.12% to 89.17%. The IC50 of EAFES was found to be 117.20±1.43µg/ml, whereas NBFES showed at 150.10±1.22 µg/ml. The IC50 of acarbose was found to be 94.47±1.61µg/ml. (Fig. 1).

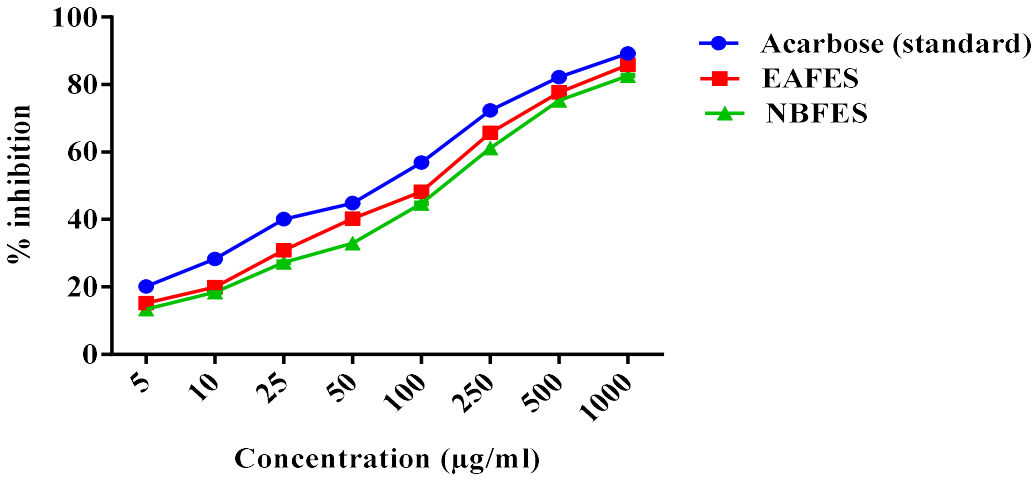

Fig. 1: *In vitro* α-amylase inhibitory activity of Ensete *superbum* fraction Values are expressed as mean± SEM of triplicate measurement. EAFES – Ethyl acetate fraction of *Ensete superbum*; NBFES – N-butanol fraction of *Ensete superbum*

3.2 *In vitro* inhibition of α-glycosidase

The fractions of EAFES and NBFES, elicited a dose dependent inhibition of α-glycosidase enzyme activity. The α-glycosidase inhibitory effect of the EAFES was found to be ranging from 12.74 % to 83.90% when studied at concentrations 5–1000 µg/ml. At the same concentration range, the inhibitory effect of NBFES was found to be ranging from 11.48% to 81.63% whereas the effect of standard drug acarbose ranged from 17.95% to 88.83%. The IC50 of EAFES was found to be 105.80 ±1.65µg/ml whereas NBFES showed at 135.40±1.13 µg/ml. The IC50 of acarbose was found to be 95.55±2.06µg/ml. (Fig. 2).

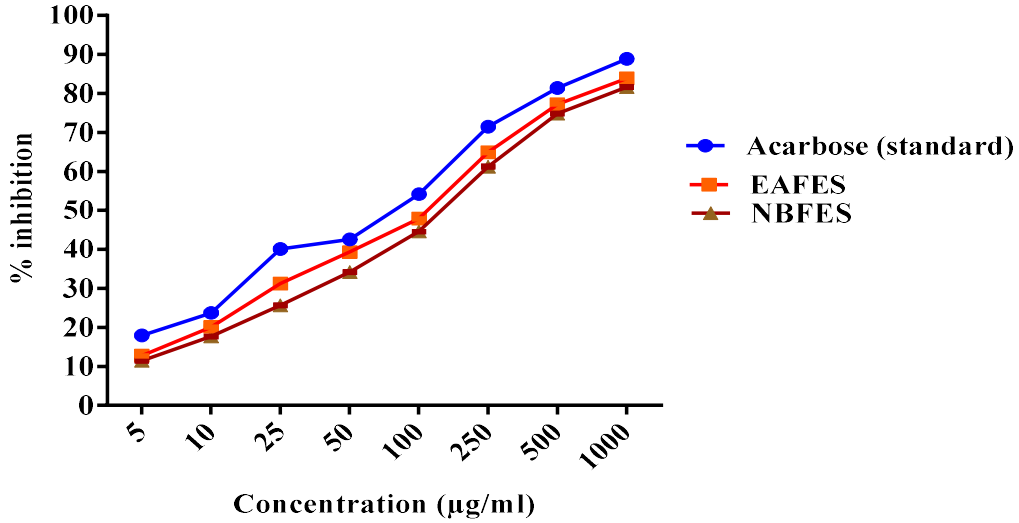

Fig. 2: In vitro α-glucosidase inhibitory activity of *Ensete superbum* fraction. Values are expressed as mean± SEM of triplicate measurement. EAFES – Ethyl acetate fraction of *Ensete superbum*; NBFES – N-butanol fraction of *Ensete superbum*

3.3 Selection of active fraction used in vivo anti diabetic studies

The fractions of EAFES and NBFES showed the dose dependent inhibition in α-glycosidase and α-amylase enzyme. These fractions were comparatively EAFES fraction shows less IC_{50} value and preferable effect than NBFES, so EAFES was selected *in vivo* antidiabetic studies.

3.4 *In vivo* antidiabetic studies

3.4.1 Oral glucose tolerance test (OGTT)

The EAFES doses of 100, 200 and 400mg/kg have shown increase the tolerance for glucose and blood glucose levels were significant reduced in the dose dependent after oral administration (2g/kg). The tested drug of EAFES 400mg/kg, standard drug ($p < 0.01$) and EAFES 200mg/kg ($p < 0.05$) showed significant activity at 30 minutes onwards and at the time of 120 minutes all the dose of drug shows significant ($p < 0.01$) activity (Table 1).

3.4.2 Effect of ethyl acetate fraction on STZ induced diabetic rats

The body weight was slightly increased in the normal control and High fat diet control significantly increased compared to initial body weight, whereas in the diabetic control rats there was a significant decrease in the body weight. Standard drug, Glibenclamide, EAFES 400 ($P<0.01$) and EAFES 200 ($P<0.05$) treatment significantly reversed this body weight changes to normal. Although there was marginal reduction in the weight of animals compared to initial body weights. (Table 2)

The diabetic rats showed a significant increase in the fasting blood glucose levels and the HFD control rats showed slightly significant increase when compared to normal control. The treatment of diabetic rats with the standard and EAFES resulted significant ($P<0.01$) decrease in the fasting blood glucose levels from the 7^{th} day onwards and the values were compared to diabetic control (Table 3).

Table 1: The effects of EAFES on oral glucose tolerance test (OGTT) in rats

Groups	Fasting blood glucose (mg/dl) at different time (min) after the treatment				
	0	**30**	**60**	**90**	**120**
Normal control	76.50±1.89	136.16±1.62	131.66±1.22	124.00±1.46	111.16±2.12
Glibenclamide (10mg/kg)	77.00±1.43	119.50±2.02**	107.33±0.95**	97.16±0.79**	85.16±1.30**
EAFES (100mg/kg)	75.00±2.14	133.83±1.24	125.83±0.70*	118.33±1.35*	105.15±1.35*
EAFES (200mg/kg)	77.16±1.88	129.83±1.40*	123.00±0.96**	115.00±1.36**	101.33±1.68**
EAFES (400mg/kg)	75.83±3.06	125.33±1.94**	120.83±2.05**	109.83±1.44**	93.66±1.28**

*Values are given in mean ±SEM for groups of six animals each. **p < 0.01, * p < 0.05 denotes when compared to control (One way ANOVA followed by Dunnett test). EAFES – Ethyl acetate fraction of Ensete superbum.*

Table 2: The effects of EAFES on body weight in HFD/STZ -induced diabetic rats

Groups	Body weight (g)		
	Initial	**Final**	**(% Change)**
Normal control	168.95±1.80	178.16±1.39	(+09.21)
HFD control	182.86±1.22	198.76±2.45**	(+15.90)
Diabetic control	190.11±1.71	150.50±1.01**	(-39.61)
Diabetic + Glibenclamide (10mg/kg)	179.70±1.26	158.95±2.28 **	(-20.75)
Diabetic + EAFES (200mg/kg)	178.78±1.72	158.5±1.47*	(-20.28)
Diabetic + EAFES (400mg/kg)	179.83±1.48	159.13±1.05**	(-20.70)

*Values are given in mean ±SEM for groups of six animals each. **p < 0.01, * p < 0.05 denotes when diabetic control and HFD control was compared with the Normal control and treated groups were compared with the diabetic control.*

The untreated diabetic and HFD control group showed a significant (P<0.01) increase in the levels of cholesterol, triglycerides, LDL but decrease in HDL when compared to normal control group. Treatment of Glibenclamide and EAFES showed a marked (P<0.01) reversal of changes in the serum lipid parameters as compared to diabetic rats. (Table 4).

The levels serum enzymes such as ALP, AST and ALT were significantly (P<0.01) increased in diabetic control when compared to normal control rats. But HFD control rats were not shown any significant increase in these levels when compared to control group. The Glibenclamide and EAFES treated rats showed a significant (P<0.01) reduction in the serum enzymes when compared to diabetic control rats (Table 5).

Table 3: The effects of EAFES on fasting blood glucose levels in HFD/STZ -induced diabetic rats

Groups	Fasting blood glucose (mg/dl)				
	0 day	7 day	14 day	21 day	28 day
Normal control	80.66 ±2.83	73.00±2.56	77.00±3.30	76.50±3.56	74.66±3.04
HFD control	95.83 ±2.76**	104.83±2.04**	108.83±2.31**	111.83±1.86**	113.83±2.42**
Diabetic control	272.33±0.76**	278.66±3.59**	276.33±4.17**	286.83±3.46**	293.83±2.34**
Diabetic + Glibenclamide (10mg/kg)	273.50±1.40	201.00±1.48**	186.00±2.19**	172.66±2.95**	154.00±3.76**
Diabetic + EAFES (200mg/kg)	278.5±1.56	251.5±5.13**	237.83±3.79**	226.50±3.30**	213.00±2.73**
Diabetic + EAFES (400mg/kg)	279.50±1.89*	235.33±2.95**	218.00±3.05**	207.83±2.49**	180.00±3.41**

*Values are given in mean ±SEM for groups of six animals each. **p < 0.01, * p < 0.05 denotes when diabetic control and HFD control was compared with the Normal control and treated groups were compared with the diabetic control.*

Table 4: The effects of EAFES on serum lipid profile in HFD/STZ -induced diabetic rats

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Normal control	55.66±2.77	52.50±1.05	49.33±2.57	40.16±1.42
HFD control	121.83±2.99**	115.33±1.38**	91.16±2.76**	35.83±1.25*
Diabetic control	173.00±2.63**	167.00±2.04**	112.50±2.55**	27.50±0.56**
Diabetic + Glibenclamide (10mg/kg)	151.16±2.88**	117.66±1.92**	59.50±2.86**	38.50±0.71**
Diabetic + EAFES (200mg/kg)	162.33±1.66*	135.16±2.24**	73.83±2.48**	37.33±0.76**
Diabetic + EAFES (400mg/kg)	155.50±1.80**	119.83±1.35**	60.66±2.89**	38.16±0.79**

*Values are given in mean ±SEM for groups of six animals each. **p < 0.01, * p < 0.05 denotes when diabetic control and HFD control was compared with the Normal control and treated groups were compared with the diabetic control.*

Serum urea, serum creatinine and HbA1c levels were significantly (P<0.01) decreased and serum insulin levels were significantly (P<0.01) increased by Glibenclamide and EAFES treated rats when compared to diabetic rats. Diabetic control rats were reversal changes when compared to normal control (Table 6).

Table 5: The effects of EAFES on serum marker enzymes in HFD/STZ -induced diabetic rats

Groups	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Normal control	61.66±1.58	68.33±1.66	53.66±1.14
HFD Control	71.0±2.20**	72.66±1.68	61.5±1.72*
Diabetic control	147.33±1.74**	137.00±2.01**	126.16±2.37**
Diabetic + Glibenclamide (10mg/kg)	68.83±1.83**	66.00±1.21**	58.66±1.47**
Diabetic + EAFES (200mg/kg)	93.00±1.89**	76.16±1.64**	70.83±2.44**
Diabetic + EAFES (400mg/kg)	75.83±1.24**	65.83±2.63**	64.66±1.66**

*Values are given in mean ±SEM for groups of six animals each. **p < 0.01, * p < 0.05 denotes when diabetic control and HFD control was compared with the Normal control and treated groups were compared with the diabetic control.*

Table 6: The effects of EAFES on serum profile in HFD/STZ -induced diabetic rats

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Insulin(µ IU/ml)	HbA_1c (%)
Normal control	26.00±0.73	0.50±0.01	3.48±0.10	6.07±0.04
HFD control	31.66±0.95**	0.66±0.01	3.13±0.16	6.82±0.05*
Diabetic control	63.33±1.17**	1.35±0.17**	1.55±0.75**	10.48±0.35**
Diabetic + Glibenclamide (10mg/kg)	35.50±0.76**	0.59±0.01**	2.91±0.09**	6.67±0.12**
Diabetic + EAFES (200mg/kg)	40.66±1.05**	0.73±0.03**	2.36±0.08**	7.75±0.13**
Diabetic + EAFES (400mg/kg)	35.66±0.61**	0.61±0.01**	2.81±0.09**	7.02±0.16**

*Values are given in mean ±SEM for groups of six animals each. **p < 0.01, * p < 0.05 denotes when diabetic control and HFD control was compared with the Normal control and treated groups were compared with the diabetic control.*

There was a marked significant reduction (P<0.01) in the liver glycogen levels of diabetic rats from 4.10gm/100gm tissue (in normal control rats) to 2.34gm/100gm tissue. Glibenclamide treatment elicited 3.84gm/100gm increase in liver glycogen levels, EAFES 200 mg/kg treatment showed 3.34gm/100gm increase while at dose level 400 mg/kg there was 3.78gm/100gm increase in liver glycogen levels when compared with the diabetic control rats. Hemidiaphragms taken from rats treated with EAFES and standard drug Glibenclamide showed a significant (P<0.01) facilitation of the glucose uptake process as compared to diabetic untreated rats. Also, both EAFES and Glibenclamide treated rats showed a significant (P<0.01) inhibitory effect on glycogenolysis in liver tissue (Table 7).

3.4.3 Histopathological study

The histopathological studies of the pancreatic tissues are shown in (Fig. 3). Normal rats showed normal architecture of the pancreas with preserved islet of Langerhans cells (Fig. 3A). HFT control rats showed mild oedema of islets of langerhans cells, thick walled and congested vessels, and focal lymphocytic infiltrate exhibiting focal oedematous changes (Fig. 3B). Induction of diabetes using Streptozotocin resulted in hyalinization of islets of langerhans cells with focal necrotic degenerative changes, mild fibrosis, dilated and congested vessels along with focal chronic inflammatory cell infiltrate in diabetic rats (Fig. 3C). The abnormal histopathology of

pancreas due to Streptozotocin-induced diabetes was reversed in the Glibenclamide and EAFES treated diabetic animals. The recovery of standard Glibenclamide treated group is evident as near normal architecture with preserved islets cells and mild oedema (Fig. 3D). The resulted in EAFES (200mg/kg & 400mg/kg) treated groups mild hyalinization islets of langerhans cells with focal mild degenerative changes when compared to untreated diabetic rats. It can be noted that the islets of langerhans cells regenerated in the treatment groups (Fig. 3E, 3F & 3G).

Table 7: The effects of EAFES on Liver glycogen, Glucose uptake by hemi diaphragm and Glucose transport by liver in HFD/STZ - induced diabetic rats

Groups	Liver glycogen (g/100g of wet tissue)	Glucose uptake by hemi diaphragm (mg/100mg)	Glucose transport by liver (mg/g)
Normal control	4.02±0.10	16.78±0.30	27.05±0.22
Diabetic control	2.27±0.15**	4.76±0.20**	42.03±0.85**
HFD control	3.98±0.06	15.51±0.40*	28.83±0.35
Diabetic + Glibenclamide (10mg/kg)	3.77±.073**	16.31±0.24**	19.13±0.30**
Diabetic + EAFES (100mg/kg)	2.83±0.05**	9.46±0.27**	26.36±0.51**
Diabetic + EAFES (200mg/kg)	3.17±0.11**	12.61±0.22**	23.53±0.50**
Diabetic + EAFES (400mg/kg)	3.72±0.09**	15.83±0.44**	19.36±0.28**

*Values are given in mean ±SEM for groups of six animals each. **p < 0.01, *p < 0.05 denotes when diabetic control and HFD control was compared with the Normal control and treated groups were compared with the diabetic control.*

4. DISCUSSION

The *in vitro* α-amylase and α- glucosidase inhibitory studies demonstrated EAFES had inhibitory activity of intestinal digestive enzyme. The percentage inhibition showed a concentration dependant reduction. These enzymes are responsible in hydrolysing dietary starch into maltose which then breaks down to glucose prior to absorption. Since α-amylases play an important role in starch break down in human beings and animals, the presence of such inhibitors in food stuffs may be responsible for impaired starch digestion. α-amylase inhibitor may be of value as novel therapeutic dietetic agents [19].

Acarbose-like drugs, that inhibit α- glucosidase present in the epithelium of small intestine, have been demonstrated to decrease postprandial hyperglycaemia, and improve impaired glucose metabolism without promoting insulin secretion in type 2 diabetic patients. These medications are most useful for people who have just been diagnosed with type-II diabetes and who have blood glucose levels slightly above the level considered serious for diabetes [20]. They are also useful for people taking sulfonylurea medication, who need an additional medication to keep their blood glucose level within safe range. Therefore, the retardation and delay of carbohydrate absorption with a plant-based α-glucosidase inhibitor offers a prospective therapeutic approach for the management of type-II diabetes mellitus.

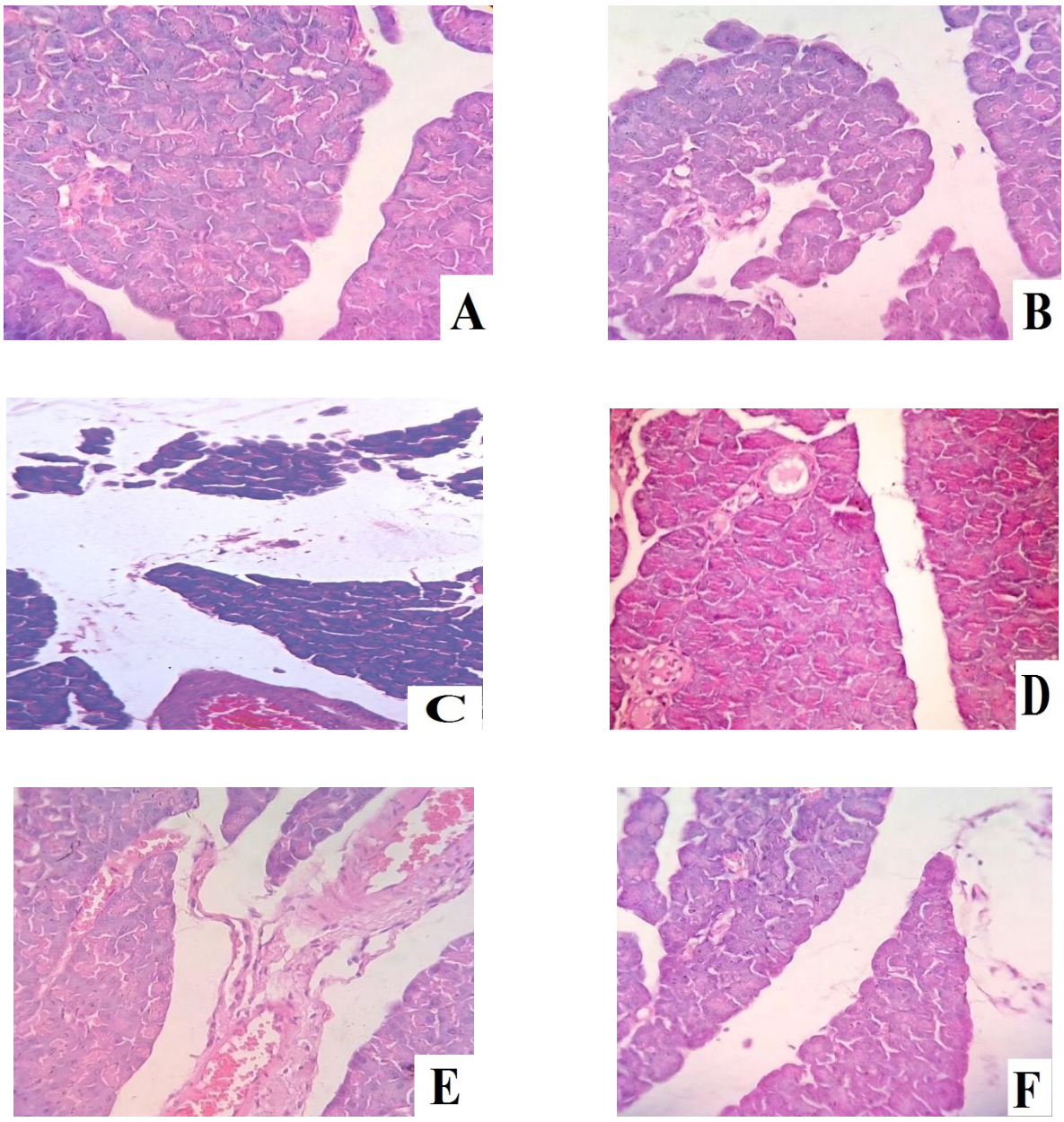

Fig.3: Histology of control and treated rat pancreas of antidiabetic studies. A) Control rat, B) HFT control, C) Diabetic control, D) Standard, E) EAFES (200mg/kg) and F) EAFES (400mg/kg)

The metabolic syndrome is characterized pathological changes including obesity, hypertriglyceridemia, impaired glucose tolerance and insulin resistance. A modified high fat diet was adopted to induce insulin resistance because the role of HFT in the development of diabetic complications and injection of a single dose of STZ induced a type 2 diabetic state similar to prediabetic, insulin resistant state in humans.

In the *in vivo* study the reduction in the body weight in Streptozotocin- induced diabetic rats can be attributed to muscle wasting, dehydration, reduced body fat, reduction in adipose tissue and excessive breakdown of proteins [21]. There was reversal of this loss in body weight in the diabetic animals treated with the EAFES and Glibenclamide.

The mechanism in diabetes involves the over production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by tissue. STZ is a slightly cytotoxic agent of pancreatic β cells and selectively destroys the pancreatic insulin

secreting β cells, leaving less active cells and resulting in a diabetic state. Hence in this study, observed an increasing level of blood glucose in STZ induced rats. The hyperglycaemic activity of EAFES has shown significant ($p<0.01$) fall in blood glucose level from 7th day onwards in all the doses of 100mg/kg, 200mg/kg and 400mg/kg per oral [22].

Hyperglycaemia is accompanied with dyslipidaemia and represents a risk factor for coronary heart diseases. The abnormality high level of serum lipids is mainly due to the uninhibited action of lipolytic hormones on the fat depots, mainly due to the action of insulin. Under normal condition, insulin activates the enzyme lipoprotein lipase which hydrolysis triglycerides. However in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hyper triglyceridemia and insulin deficiency is also associated with hyper cholesterolemia due to metabolic abnormalities [23]. The dyslipidaemia is characterized by increase in TC, TG, LDL and fall in HDL. This altered serum lipid profile was reversed towards normal after treatment with EAFES.

The excess of free fatty acids during diabetes is found to be directly toxic to hepatocytes [24]. As a result the enzyme like ALP, AST and ALT may leak from the hepatocytes into the circulation where their levels become elevated [25]. The elevation of ALT is attributed to impairment in insulin signalling rather than hepatocyte injury. In the present study increase the levels of these enzymes in diabetic control rats were decrease when treated with the EAFES similar to Glibenclamide. EAFES may bring about the hypoglycaemic effects through insulin secretion from the remnant β cells and regenerate β cells due to increased peripheral glucose utilization and insulin stimulatory effects [26,27]. Increase the glycogen in liver can be brought about by an increase in glycogenesis and decrease in glycogenolysis. Hemidiaphragms taken from rats treated with EAFES and Glibenclamide showed a significant enhancement of the glucose uptake and significant inhibitory effect on glucose transport (glycogenolysis) in liver compared to diabetic control.

STZ administration results in DNA damage in β-cells of pancreatic islets due to its potent alkylating properties [28]. Histopathologically the damage was seen in untreated diabetic rats. There was regeneration of β-cells in EAFES and Glibenclamide treatment groups since the cells recovered from the initial injury. The regeneration of the β-cells destructed by STZ is probably due to the fact that pancreas contains stable cells which have the capacity to regenerate. Therefore, the surviving cells can proliferate to replace the lost cells [29].

5. CONCLUSION

In conclusion, the result of this present study suggests that HFD is one of the complication induced type 2 diabetes. EAFES is a potent hypoglycaemic agent and beneficial in the control of diabetes related abnormalities such as hyperglycaemia, lipids abnormalities, hepatic changes and histopathological changes in pancreas of HFD/STZ-induced non-genetic rat model of type 2 diabetes mellitus. However, these findings make the EAFES is safe for oral consumption and elicit hypoglycaemic activity, further research and would result in discovery of lead compounds useful in treating diabetes mellitus.

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REFERENCES

1. American Diabetes Association Diagnosis and classification of diabetes mellitus. Diabetes Care 2008; 31:S55–S60.

2. International Diabetes Federation IDF Diabetes Atlas.5th ed. International Diabetes Federation. Brussels: 2011.

3. Jing M, Rayner CK, Jones KL, Horowitz Z. Insulin secretion in healthy subjects and patients with Type 2 diabetes—role of the gastrointestinal tract. Best Pract Res Clin Endocrinol Metab 2009; 23:413–424.

4. Maarel MJEC, Veen B, Uitdehaag JCM, Leemhuis H, Dijkhuizen L. Properties and applications of starch converting enzymes of the α-amylase family. J Biotechnol 2002; 94:137–155.

5. Powers AC, D'Alessio. Endocrine pancreas and pharmacotherapy of diabetes mellitus and hypoglycaemia. In: Brunton LL, Chabner BA, Knollmann BC, editors. Goodman and Gillman's: The Pharmacological basis of therapeutics. 12th ed. New York: McGrawHill Medical Publishing Division; 2011, p.1237– 1274.

6. Sos skovso. Modeling type 2 diabetes in rats using high fat diet and Streptozotocin. Journal of diabetic investigation 2014; 5(4):349-358.

7. De Smet PAGM. Health risk of herbal remedies. Drug safety 1995; 13: 81-93.

8. Cardellina JH. Challenges and opportunities confronting the botanical dietary supplement industry. J Nat Prod 2002; 65: 1073-84.

9. Constantine, D. (1999) The Musaceae — an annotated list of the species of Ensete, Musa and Musella.

10. H.M Patil, V.V Bhaskar. Medicinal uses of plants by tribal medicine men of nandurbar district in Maharashtra. Explorer research article. 2005;125-130.

11. Monica Kachroo, S.S. Agrawal. Isolation, characterization & anti-fertility activity of the active moiety from the seeds of *Ensete superbum* Cheesm (Banakadali). Journal of natural remedies, Vol. 9/1 (2009) 12 - 20

12. Rao TN, Kumarappan C, Thilagam E, Mohanalakshmi S, Subash MC. Inhibition of carbohydrate digestive enzymes by Talinum portulacifolium (Forssk) leaf extract. J Complement Integr Med 2008; 5:1–10.

13. Subramanian R, Asmawi MZ, Sadikun A. *In vitro* α-glucosidase and α-amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide. Acta Biochim Pol 2008; 55:391–398.

14. PHS. Public health service policy on human health care and use of laboratory animals. Washington D.C: U.S department of health and human services; revised 2015.

15. Gandhi GR, Stalin A, Balakrishna K. Insulin sensitization via partial agonism of PPARgamma and glucose uptake through translocation and activation of GLUT4 in PI3K/p-Akt signaling pathway by embelin in type 2 diabetic rats. Biochim Biophys Acta 2013; 1830: 2243–2255.

16. Prem kumar N, Annamalai A R, Thakur R S. Antinociceptive property of Emblica officinalis Gaertn (Amla) in high fat diet fed/low dose Streptozotocin induced diabetic neuropathy in rats. Indian Journal of Experimental Biology 2009; 47:737-742.

17. Carroll VV, Longly RW, Joseph HR. Determination of glycogen in liver and muscle by use of anthrone reagent. J Biol Chem 1956; 220:583-93.

18. Nafisa PC Fernandes, Chakradhar V Lagishetty, Vandana S Panda and Suresh R Naik. An experimental evaluation of the antidiabetic and antilipidemic properties of a standardized Momordica charantia fruit extract. BMC Complementary and Alternative Medicine 2007;29(7):1-8.

19. Andichettiar Thirumalaisamy Sivashanmugam & Tapan Kumar Chatterjee. *In vitro* and *in vivo* antidiabetic activity of Polyalthia longifolia (Sonner.) Thw. Leaves. Orient Pharm Exp Med 2013; 13:289–300.

20. N. V. L. Suvarchala Reddy, Sneha J. Anarthe and N. M. Raghavendra. *In vitro* Antioxidant and Antidiabetic activity of Asystasia gangetica (Chinese Violet) Linn. (Acanthaceae). International Journal of Research in Pharmaceutical and Biomedical Sciences 2010; 1 (2):72-75.

21. Pushparaj PN, Low HK, Manikandan J, Tan BK, Tan CH. Antidiabetic effects of Cichorium intybus in Streptozotocin induced diabetic rats. J Ethnopharmacol 2007; 111:430–434.

22. Krishnamurthy Girija, Kuruba Lakshman, Nagaraj Pruthvi, Pulla Udaya Chandrika. Antihyperglycemic and hypolipidemic activity of methonalic extract of amaranthus viridus leaves in experimental diabetics. Indian Journal of Pharmacology 2011; 43(4): 450-454.

23. Murali B, Upadhyaya UM, Goyal RK. Effect of chronic treatment with Enicostemma littorale in non-insulin dependent diabetic (NIDDM) rats. J Ethnopharmacol 2002; 81:199-204.

24. Harris EH. Elevated liver function tests in type 2 diabetes. Clin Diabetes 2005; 23(3):115–119.

25. Erejuwa OO, Sulaiman SA, Wahab MS, Sirajudeen KNS, Salleh MS, Gurtu S. Hepatoprotective effect of tualang honey supplementation in streptozotocin-induced diabetic rats. Int J App Res Nat Prod 2012; 4(4):37–41.

26. Somani R, Kasture S, Singhai AK. Antidiabetic potential of Butea monosperma in rats. Fitoterapia 2006; 77 (2): 86-90.

27. Sathishsekar D, Subramanian S. Beneficial effects of Momordica charantia seeds in the treatment of STZ-induced diabetes in experimental rats. Biol Pharm Bull 2005; 28: 978–983.

28. Szkudelski T. The mechanism of alloxan and streptozotocin action β cells of the rat pancreas. Physiology Res 2001; 50:536-546.

29. Yazdanparast R, Esmaeili MA, Helan JA. Teucrium polium extract effects pancreatic function of streptozotocin diabetic rats: a histopathological examination. Iran Biomed J 2005; 9(2):81–85.