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In-vitro and *In-vivo* antidiabetic activity of *Spathodea campanulata*. P. Beauv flower extract.

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ABSTRACT

The study was designed to determine the *in-vitro* and *in-vivo* of the antidiabetic activity of *Spathodea campanulata*.P.Beauv flower extract. The *in-vitro and in-vivo* antidiabetic activity of *Spathodea campanulata*.P.Beauv flower extract was studied by alpha amylase and alloxan induced diabetic respectively. In alloxan induced diabetic rats various biochemical markers like fasting blood sugar, serum lipid profile, serum urea, serum creatinine, total serum protein were estimated to assess the antidiabetic activity. In addition, the histopathological studies were also studied. The *in vitro* Inhibition of hydrolyzing enzymes plays an important role in management of diabetes. In the present study, it can be concluded that treatment with 70%EESCF may inhibit alloxan–induced diabetes mellitus and also inhibit alpha- amylase which delays the absorption of carbohydrates. The results from the present study indicate that the 70%EESCF can reduce the levels of blood glucose, serum urea, serum creatinine, serum cholesterol, serum triglycerides, LDL, VLDL and increase the serum protein and HDL levels. Histopathological observations also confirmed. Antidiabetic potential of the test extracts may be attributed to the antioxidant principles present in the 70%EESCF. It has shown antidiabetic activity in a dose dependant manner.

Keywords *In vitro, in vitro* antidiabetic; alpha-amylase enzyme inhibitory; *Spathodea campanulata*.P.Beauv.

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INTRODUCTION

Diabetes mellitus (DM) is one of the most severe, multifactorial, incurable metabolic disorders characterized by hyperglycemia as a result of a relative, lack of insulin, or the action of insulin on its target tissue and disturbances of carbohydrate, lipid and protein metabolism. The raised basal metabolic rate, defect in reactive oxygen species scavenging enzymes and high oxidative stress induced damage to pancreatic beta cells. DM is ranked seventh among the leading causes of death and is considered third when its fatal complications are taken into account[1]. DM is also associated with long-term complications, including retinopathy, nephropathy, neuropathy and angiopathy and several others. It is caused by hereditary, increasing age, poor diet, imperfect digestion, obesity, sedentary lifestyle, stress, drug-induced, infection in pancreas, hypertension, high serum lipid and lipoproteins, less glucose utilization and other factors[2]. The vast majority of cases of diabetes fall into two broad etiopathogenetic categories. In one category, type 1 diabetes, insulin-dependent diabetes mellitus (IDDM).The cause is an absolute deficiency of insulin secretion. Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the pancreatic islets, leading to insulin deficiency. The cause is unknown. In the other, much more prevalent category, type 2 diabetes, "Non insulin-dependent diabetes mellitus" (NIDDM). The cause is a combination of resistance to insulin action and an inadequate compensatory insulin-secretory response. The worldwide increase in Type 2 DM becomes a most important health concern. Type 2DM is usually accompanied with insulin resistance in the skeletal muscles and the liver and the reduction in insulin production by the pancreas genetic susceptibility and various environmental factors are also involved in Type 2 DM. Patients with Type 2 DM are at risk of vascular complications such as coronary artery disease, stroke, hypertension, nephropathy, peripheral vascular disease, neuropathy and retinopathy. Although Type 2 DM was traditionally seen in individuals over the age of 40, recent data from several countries confirm that Type 2 DM occurs in younger people even in childhood[3].

Spathodea campanulata P.Beauv reported to be useful as diuretic, anti-inflammatory, antidote, enemas, antisecretolytic, antiparasitic, antimalarial, anti-HIV and anti-diabetic. It is further reported that the plant is useful in the treatment of kidney diseases, herpes, stomachache, urethral inflammations and fungal infections. The literature survey of this plant revealed that this plant possess quercetin caffeic acids, oleanolic acid, steroids, polyphones, flavonoids, tannins and cardiac glycosides[4,5]. Herbs are reported to contain phenolic compounds these phenolic components are known as antioxidants and antioxidants are reported to have organ protective properties. It is evident from the literature survey that the plant is reported to contain phenolic compounds these phenolic components are known as antioxidants and antioxidants are known for organ protective activity in nature[6]**.** So the present study was designed to screen for *in vitro* and *in vivo* antidiabetic activity of *Spathodea campanulata*.P.Beauv flower extract.

EXPERIMENTAL DESIGN

Experimental Animals

Either sex of *Swiss* albino mice and *Wister* albino rats were used for the pharmacological screening, which were procured from Sri Venkateshwara Enterprises, Bengaluru. All the animals were acclimatized and maintained as per the CPCSEA guidelines. They were provided with standard rat feed and water *ad libitium*. The husk in the cages was renewed thrice a week to ensure hygienity and maximum comfort for animals. Ethical clearance was obtained from the IAEC prior to the beginning of the research, the registered no is SCSCP/IAEC08.

In-vitro-antidiabetic activity by Amylase inhibition method[7]

The management of blood glucose level is a critical strategy in the control of diabetes complications. Inhibitors of carbohydrate hydrolyzing enzymes (such as α-amylase, α-glucosidase) have been useful as oral drugs for the control of hyperglycemia. Inhibition of these factors plays an important role in management of diabetes

Procedure

This study was performed by a modified starch iodine protocol[8]. In short 1 ml of ethanolic extract or standard of different concentration (0.5,1,1.5, 2,2.5 ,3, 3.5. mg/ml) was taken in pre-labeled test tubes. A

volume of 20 µL of 1% w/v α -amylase was added to each test tube and incubated for 10 min at 37 °C. After the incubation 200 µL of 1% starch solution was added to each test tube and the mixture was re-incubated for 1 h at 37 °C. Then 200 µL of 1% iodine solution was added to each test tube and after that, 4 ml distilled water was added. Absorbance was measured at 565 nm in UV-Visible spectroscopy. Sample, substrate and α-amylase blank were undertaken under the same conditions. Each experiment was done in triplicate. IC_{50} value was calculated by using regression analysis[9].

$$\text{Inhibition of alpha- Amylase (\%)} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample. All the experiments were carried in triplicates.

ii: *In-vivo* Antidiabetic activity by alloxan induced diabetic rat model [10]

Fasting blood glucose was determined after depriving food for 16 hrs. With free access of drinking water. Hyperglycemia was induced by a single i.p. injection of 120 mg/kg of alloxan monohydrate (S.D.fine-chem. Ltd., Mumbai, India) in sterile saline. After 5 days of alloxan injection, the hyperglycemic rats (glucose level >250 mg/dl) must be separated and divided into different groups comprising of 6 rats each for the anti-diabetic study. The treatment (P.O.) was started from the same day except normal control and diabetic control groups for a period of 15 days. During this period, animals in all groups have free access to standard diet and water. Body weight and blood glucose levels are estimated on 4^{th}, 7^{th}, 10^{th} and 15^{th} day of the treatment.

On the 15^{th} day, blood samples are collected from overnight fasted rats by cardiac puncture under mild ether anesthesia for biochemical estimations. The pancreas from all the animals were removed immediately and kept in 10% formalin solution for histopathological examination.

The various groups used in experiment

Group 1 : Served as normal control and did not receive any treatment.
Group 2 : Served as diabetic control and received alloxan monohydrate and vehicle
Group 3 : Alloxan monohydrate + Glibenclamide (10 mg/kg p.o.) and served as standard.
Group 4 : Alloxan monohydrate + 70%EESCF 250 mg/kg, p.o.
Group 5 : Alloxan monohydrate + 70%EESCF 500 mg/kg, p.o.

Care of Diabetic animals

Since diabetic animal's drunken large volume of water and produce large volume of urine, the bedding is changed frequently, usually every day and in some circumstances, more than once per day. Diabetic rats should have sufficient food and water.

Collection blood and serum sample

The blood was drawn from the retro orbital plexus of the rats (fasted for 14 h) under light ether anesthesia on different occasion, i.e, 0, 4^{th}, 7^{th}, 10^{th} and 15th day. The blood samples were allowed to clot for 30 min at room temperature and then they were centrifuged at 3000 rpm for 10 min. The resulting upper serum layer was collected in properly clean, labeled and dry micro-centrifuge tubes. The serum samples were stored at-40^0c

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RESULTS

In vitro Antidiabetic Activity

Alpha Amylase inhibition method

In the present study, the inhibitory activity of 70%EESCF at different concentration was investigated. The *in-vitro* alpha amylase inhibitory activity of 70%EESCF at different concentration was exhibited good antidiabetic activity. The per cent inhibition ranging from 1000µg/ml to 7000µg/ml concentration of 70%EESCF shows concentration dependent. The results revealed that 70%EESCF at a concentration of 7000µg/ml showed a maximum per cent inhibition 88.32 whereas in standard Acarbose 0.969. The IC50 value of 3.12 whereas standard was found 4.76µg/ml. Results are summarized in the table 01 and fig. 01.

In vivo antidiabetic activity by alloxan induced diabetic rat

Effect on body weight

During the treatment period there was change in body weight is given in table 02and fig. 02. This shows an increase in the mean body weight of normal control. Diabetic control group of rats have shown a change in body weight from a mean value of 180.6±4.905g to 155.4±3.865g in initial and final days respectively. These changes in the body weight illustrate that the diabetic rats show a progressive loss of body weight, which was found to be significant. The standard (glibenclamide) group of diabetic rats shown a mean body weight of 183.6±6.576 g in initial day and it was found to have been increased to 218.3±10.96 g on 15th day. These show that glibenclamide treatment has protected the diabetic rats from losing the body weight in a significant manner as compared to diabetic control group. Similarly the extract treated diabetic rat's shows reduction in body weight during the treatment period. Group 4 and Group 5 show a mean body weight of 185.0±10.96g and 185.6±13.05 g respectively in initial day, weight get increased to 215.4±6.356 g and 216.1±6.950 g in 15th day. Group 4 and group 5 reduction of weight is less compared to positive control group. This shows that extract treated has prevented diabetic rats from loosing body weight as compared to the diabetic control group of rats.

Effect on Fasting blood glucose levels

70%EESCF were subjected for anti-diabetic activity in rats where alloxan monohydrate (120 mg/kg b.w., i.p.) as the diabetogenic agent. A marked rise in fasting blood glucose (FBS) level observed in diabetic control compare to normal control rats. 70%EESCF shown anti-hyperglycemic activity on 5th, 10th and 15th day post treatment in alloxan induced diabetic rats. The FBS levels are shown in table 03 and fig. 03. The mean FBS in the diabetic control group was found to be 182.9±5.315, 159.0±5.915 and 158.2±4.862 on 0, 5th, 10th and 15th day respectively, which was found to be significantly higher as compared with the normal rats. These elevated FBS levels were found to be maintained throughout the treatment period indicating that the rats are rendered diabetic. Fasting blood glucose level in STD, group 4 and group 5 treated diabetic rats during treatment period significantly reduced its mean (± SEM) shown in the table 04. This reduction in FBG compared to Positive control group indicates the anti-hyperglycemic activity of reference standard glibenclamide and 70%EESCF.

Effect of 70%EESCF on biochemical parameters in alloxan induced diabetic rats

The biochemical parameters like serum urea, serum creatinine, serum triglyceride, serum HDL, serum LDL, serum VLDL are normal in negative control group during treatment period. Same parameters are significantly increased in positive control group ($p \leq 0.05$) compared to negative control group. The serum protein and hepatic glycogen concentration gets reduced in positive control group compared to negative control group. These parameters in other groups such as standard, group 4 and group 5 has shown significant as compared to positive control group with the mean (± SEM) is shown in table 04.

Histopathology reports of pancreas in alloxan induced diabetic rats

Negative control

Sections studied revealed the normal structure of acinar cells which were arranged in groups, clusters, sheets with intermixed varying sized normal group of Islet of Langerhans cells (Thick block white arrow) having normal morphology where in Islet cells are arranged in cords with admixed capillaries (Thin black arrow).

Positive control

Sections studied showed predominantly acinar cells with occasional very small sized clusters of Islet of Langerhans cells. Acinar cells surrounding these clusters were showing atrophic changes with few hydropic changes. Inside the Islet of Langerhans cell cluster was seen the areas of necrosis (White arrow) and degenerative changes. Also noted are the signs of irreversible cell injury in the form of karyolysis, karyorrhexis, and pyknosis (Thin black arrow) around the necrosed area in these small sized Islet of Langerhans cell cluster. Capillaries are also few in numbers which were obliterated. Photomicrograph of group 02 rat pancreas showing areas of necrosis (White arrow), degenerative changes in the form of karyolysis, karyorrhexis and pyknosis (Thin black arrow) and sparse capillaries.

Standard Group

Sections studied showed regenerative nodules of Islet of Langerhans cells with cells showing mild anisonucleosis, prominent nucleoli, and newly forming capillaries in between them (White arrow). Surrounding parenchymal acinar cells of pancreas are normal. (Thin black arrow). Photomicrograph of group 03 rat pancreas showing regenerative cluster of Islet of Langerhans cell groups (White arrow) and capillaries with normal surrounding acinar cells (Thin black arrow).

Lower dose (250mg/kg)

Sections studied showed small sized regenerative nodules of Islet of Langerhans cells comprising of moderate number of regenerative cells having mild anisonucleosis, prominent nucleoli, and few newly forming capillaries among them (White arrow). Few acinar cells of pancreas surrounding these clusters are showing atrophic changes. (Thin black arrow). Photomicrograph of group 04 rat pancreas showing small sized regenerative clusters of Islet of Langerhans cell groups (White arrow) and capillaries (Thin arrow) with few surrounding atrophied acinar cells.

Higher dose (500mg/kg)

Sections studied showed large number of predominantly large sized regenerative nodules of Islet of Langerhans cells comprising plenty of regenerative cells (Thin black arrows). Photomicrograph of group 06 rat pancreas showing predominantly large sized regenerative clusters of Islet of Langerhans cells comprising of plenty of Islet of Langerhans cells (Arrows). Reports are shown in fig. 04.a, b, c, d and e.

DISCUSSION

Diabetes is route cause to many complications which is the nature. Chronic diabetic syndrome may lead to cardiac, renal and hemo related problems including ishchemic stroke. The important enzyme in the diabetes pathogenesis and glucose metabolism is Alpha amylase is an enzyme that hydrolyses polysaccharide with large alpha linked like glycogen and starch thereby yield glucose and maltose from the process. These enzyme inhibitors bind to alpha-bond and prevent lysis of polysaccharide into mono and disaccharide. The pancreatic and intestinal glycosidase are the key enzymes of dietary carbohydrate digestion and inhibitors of these enzymes may be effective in retarding glucose adsorption. This is because only monosaccharides are readily taken up from the intestine and all other carbohydrates have to be broken-down enzymatically before they can be absorbed.

In this study, the β cells of pancreatic were destroyed using alloxan a toxic substance (chemical diabetogen), which accumulates in beta cells of the pancreatic via GLUT 2 glucose transporters. In the presence of thiols, including glutathione (GSH), alloxan produces reactive oxygen species (ROS) in cyclic redox reactions. The production of reduced product from alloxan is dialuric acid. Dialuric acid generates ROS by auto oxidation, and it is responsible for the necrotic death of the β cells. The inducing agent also inhibits glucose-induced insulin secretion and causes inhibition the β cell glucose sensor, glucokinase. Unfortunately irregular and inappropriate activation by ROS may start a cascade of events that result in an inflammatory and autoimmune response in pancreas, so the inhibition by antioxidants could improve the severity of type 1 diabetes[11].

In any dietary supplements, carbohydrates are the major source of energy. They are polyhydroxy carbonyl moeties, alcohols and acids of single monomeric sized units (monosaccharides) to polymers (polysaccharides). Before being absorbed by the body, carbohydrates may underso lysis in order to form into monosaccharide's. This lysis reaction occurs due to two major enzymes: amylase and Glucosidase. Naturally occuring inhibitors of amylase and glucosidase from plant origin offer an commendable therapeutic approach towards the treatment of post-prandial hyperglycemia by lowering glucose release from starch and slowed carbohydrate absorption by inhibiting its activity by hydrolyzing enzymes in the small intestine and may have good potential for use implementing in the treatment of diabetes mellitus.

There are regular dietary supplements from natural origin which are the sources which can inhibit the α-glucosidase and few of them are quite specific to inhibit sucrose rather than maltase. The collective inhibition of amylase, sucrose and maltase brought by polyphenolic entities of green tea leaves has been reported in literature[12]. Our present research suggests that, the potentially pivotal role in management of diabetes via the inhibition of α- inhibition of amylase. The EESCF was showed this confirmed in this study.

In the present study, in light of the results, it can be concluded that treatment with 70%EESCF may inhibit alloxan–induced diabetes mellitus and also inhibit alpha- amylase which delays the absorption of carbohydrates. The results from the present study indicate that the 70%EESCF can reduce the levels of blood glucose, serum urea, serum creatinine, serum cholesterol, serum triglycerides, LDL, VLDL and increase the serum protein, HDL, hepatic glycogen and confirms the possibility that the major function of the extract are on the protection of vital tissue (pancreas), thereby reducing the causation of diabetes in the experimental animals. From the qualitative studies, spectroscopic and HPLC analysis it has confirmed that the study plant rich in antioxidant phytoconstituents like flavanoids, polyphenols and tannin. Therefore antidiabetic potential of the test extracts may be attributed to the antioxidant principles present in the aerial 70%EESCF. It has shown antidiabetic activity in a dose dependant manner.

Table. 01 Effect of 70%EESCF on *in vitro* alpha amylase inhibition method.

Concentration (µg/ml)	Standard Acarbose	% of inhibition of 70%EESCF
1000	0.175	20.68
2000	0.232	42.52
3000	0.348	65.05**
4000	0.506	69.13**
5000	0.644***	73.04***
6000	0.750***	76.34***
7000	0.969***	88.32***

Each value is expressed as mean ± SEM (n = 6), where, NS represents non significant; ***P<0.001 – highly significant; **P<0.01- very significant; *P<0.05- significant, when compared to control. One-way ANOVA followed by Dunnett's comparison test.

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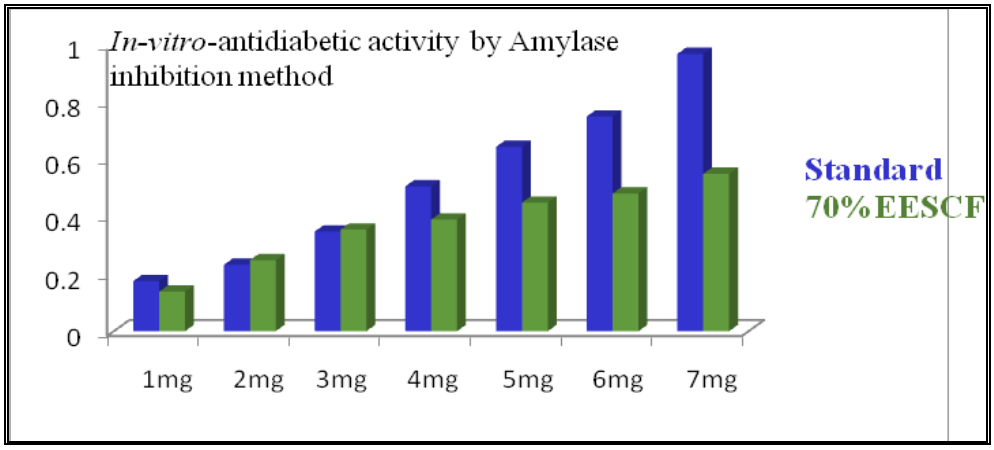

Fig. 01. Alpha-amylase inhibition of 70%EESCF at various concentrations.

Table 02. Effect of 70%EESCF on body weight in alloxan induced diabetic rats

Dose (mg/kg)	0 day	5th day	10th day	15th day
	Mean± SEM	Mean± SEM	Mean± SEM	Mean± SEM
Vehicle	213.3±6.009	230.0±7.638	240.8±9.347	235.0±4.830
Alloxan (120mg/kg)	180.6±4.426	178.3±5.650	159.0±5.824	155.4±3.865
Alloxan (120mg/kg) + Glibenclamide (10mg/kg)	183.6±6.576	198.6±7.690	203.9±8.636 **	218.5±10.96 ***
Alloxan (120mg/kg) + 70%EESCF (250mg/kg)	185.0±10.96	193.0±9.95	182.0±4.920 **	215.4±6.356 ***
Alloxan (120mg/kg) + 70%EESCF (500mg/kg)	185.6±12.49	183.2±13.93	199.0±8.438 **	216.1±6.950 ***

Each value is expressed as mean ± SEM (n = 6), where, NS represents non significant; ***P<0.001 – highly significant; **P<0.01- very significant; *P<0.05- significant, when compared to alloxan treated rats. One-way ANOVA followed by Dunnett's comparison test.

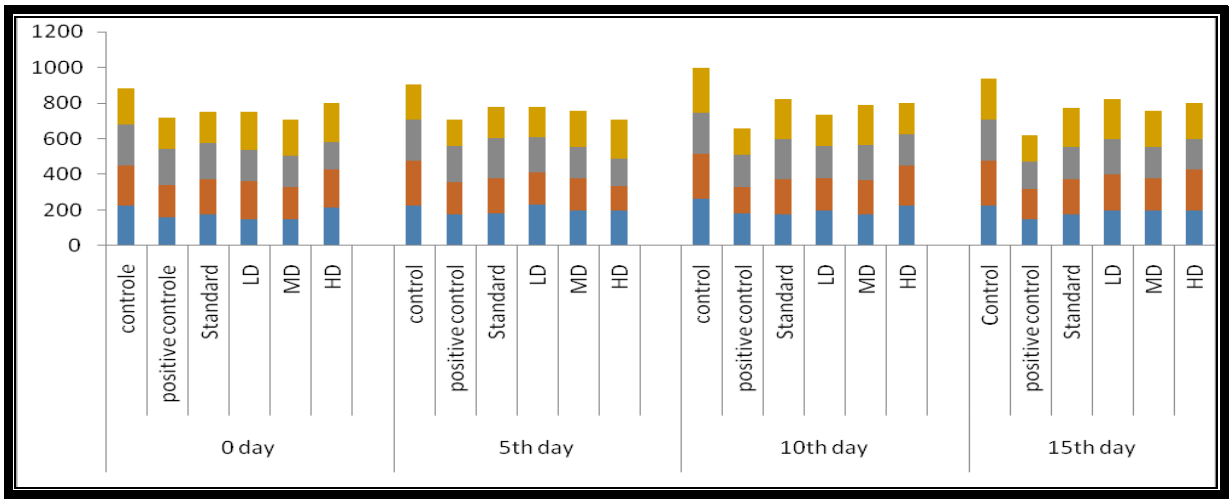

Fig. 02. Effect of 70%EESCF on body weight in alloxan induced diabetic rats

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Table 03. Effect of 70%EESCF on blood glucose level in alloxan induced diabetic rats

Dose (mg/kg)	0 day	5th day	10th day	15th day
	Mean± SEM	Mean± SEM	Mean± SEM	Mean± SEM
Vehicle	182.9±5.315	179.5±6.850	159.0±6.325	158.2±4.862
Alloxan(120mg/kg)	213.3±6.009	230.0±7.638	240.8±9.347	235.0±4.830
Alloxan(120mg/kg) + Glibenclamide(10mg/kg)	196.7±6.593	193.8±8.960	190.2±8.267**	168.4±10.65***
Alloxan(120mg/kg) + 70%EESCF(250mg/kg)	189.0±10.98	186.0±10.96	184.0±4.760**	180.4±5.345***
Alloxan(120mg/kg) + 70%EESCF(500mg/kg)	194.5±13.56	190.4±13.65	180.0±8.359**	172.2±5.656***

Each value is expressed as mean ± SEM (n = 6), where, NS represents non significant; ***P<0.001 – highly significant; **P<0.01- very significant; *P<0.05- significant, when compared to alloxan treated rats. One-way ANOVA followed by Dunnett's comparison test.

Table 04. Effect of 70%EESCF on biochemical parameters in alloxan induced diabetic rats

Dose (mg/kg)	Serum urea	Serum Creatinine	Triglycerides	Total protein	Hepatic Glycogen content	Pancreas weight	Total Cholesterol	HDL	LDL	VLDL
Vehicle	26.96 ± 8.533	0.3625± 0.02452	0.1982 ± 0.01785	10.69± 0.3396	0.07375± 0.004715	0.9250± 0.02952	65.02± 5.397	5.189 ± 0.1055	59.86 ± 5.472	0.03887 ± 0.003481***
Alloxan (120mg/kg)	161.6 ± 14.59	0.5817± 0.07439	0.7154 ± 0.0953	12.00± 0.6030	0.02375± 0.0005737	0.5108± 0.01938	82.81± 4.522	6.458 ± 0.4573	69.35 ± 5.974	0.1342± 0.02033
Alloxan(120mg/kg) + Glibenclamide(10mg/kg)	59.04 ± 17.55***	0.3665± 0.02928**	0.3694 ± 0.04335***	4.595± 0.1885***	0.06658± 0.009496***	0.7950± 0.06989**	46.36± 8.537***	4.393 ± 0.4988**	46.31 ± 2.582**	0.06876 ± 0.007198**
Alloxan(120mg/kg) + 70%EESCF(250mg/kg)	12.07 ± 6.352***	0.3502± 0.02693***	0.3537 ± 0.03256***	7.349± 0.3442***	0.0580± 0.006914**	0.7650± 0.09926**	47.11± 5.004***	3.581 ± 0.3932***	46.98 ± 3.455**	0.0707± 0.006520**
Alloxan(120mg/kg) + 70%EESCF(500mg/kg)	19.88 ± 10.81***	0.3741± 0.01591**	0.2336 ± 0.06555***	4.215± 0.2125***	0.0775± 0.006302***	0.7650± 0.008466**	46.10± 2.607***	3.931 ± 0.5227***	42.96 ± 2.408**	0.06483 ± 0.01088***

Each value is expressed as mean ± SEM (n = 6), where, NS represents non significant; ***P<0.001 – highly significant; **P<0.01- very significant; *P<0.05- significant, when compared to alloxan treated rats. One-way ANOVA followed by Dunnett's comparison test.

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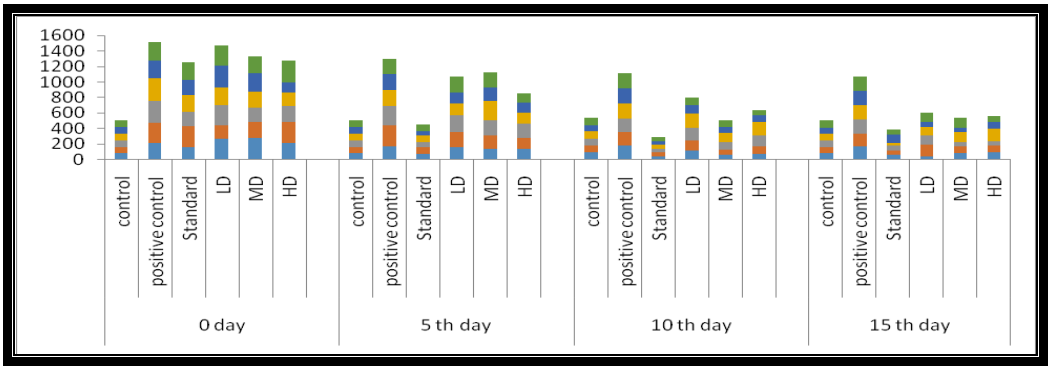

Fig.03. Effect of 70%EESCF on blood glucose level in alloxan induced diabetic rats

Histopathology reports of pancreas in alloxan induced diabetic rats

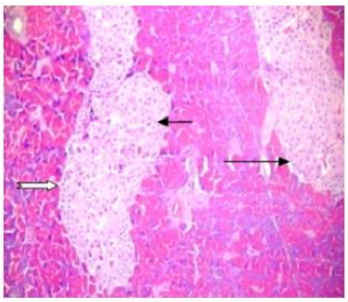

Fig. 04.a Normal Control

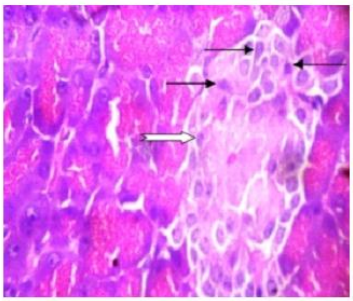

Fig. 04.b Alloxan treatment

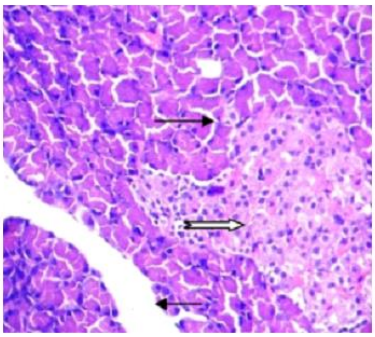

Fig. 04.cAlloxan + Glibenclamide(10mg/kg)

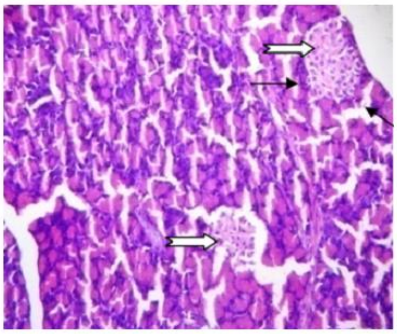

Fig. 04.d Alloxan + 70%EESCF(250mg/kg)

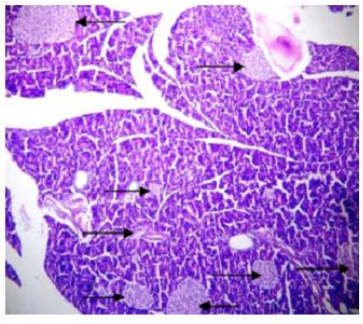

Fig. 04.e Alloxan + 70%EESCF(500mg/kg)

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CONCLUSIONS

The 70%EESCF exhibited an appreciable antidiabetic activity. The study plant possess very good percentage of alpha amylase inhibition in dose depended manner. Form the results it can be concluded that treatment with 70%EESCF shown reduction in blood glucose level, serum urea, serum creatinine, serum cholesterol, serum triglycerides, LDL, VLDL and increase the serum protein, HDL and confirms the possibility that the protection of pancreas, thereby reducing the causation of diabetes in the experimental animals.

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