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PHYTOPHARMACOLOGICAL ASSESSMENT OF ANTIDIABETIC AND ANTIHYPERLIPIDEMIC POTENTIAL OF *PSIDIUM CATTLELIANUM* SABINE LEAVES EXTRACT THROUGH *IN VIVO* METHODS

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ABSTRACT

The present study evaluates the antidiabetic and antihyperlipidemic potential of *Psidium cattleianum* Sabine leaves extracts on Streptozotocin and Dexamethasone induced type 2 diabetic rats. In STZ induced study, β-cell destruction is caused by single i.p. injection of Streptozotocin 40mg/kg for 28 days using Glibenclamide 5mg/kg as standard. In the second study, insulin resistance is induced by s.c. injection of dexamethasone 10mg/kg for a period of 11 days using Metformin 50mg/kg as standard. In both the studies the hypoglycemic and hypolipidemic potential of HAEPC were evaluated at doses of 200 and 400mg/kg by analysing body weight, blood glucose level and serum lipid profiles. The effect of HAEPC on peripheral glucose uptake is studied by using isolated rat diaphragm. The extracts exhibited significant anti-diabetic as well as hypolipidemic effects by lowering FBS, TC, TG, LDL, and VLDL levels but also with elevation of HDL level. Potent hypoglycemic activity was observed with 400mg/kg extract. Histopathological study on pancreatic tissues showed severe degeneration of β-cells in the STZ induced diabetic groups whereas regeneration of β-cells were observed in extract treated groups.

KEYWORDS

Psidium cattleianum, Hyperglycemia, Hyperlipidemia, Streptozotocin, Dexamethasone and Insulin resistance.

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INTRODUCTON

Diabetes mellitus (DM) is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism, glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonaemia[1]. Hyperlipidemia or dyslipidemia is characterized by presence of excess lipids, largely cholesterol and triglycerides in blood[2]. The occurrence of cardiovascular diseases is

more in patients with either type 1 or type 2 diabetes. Among the metabolic abnormalities that commonly accompany diabetes are disturbances in the production and clearance of plasma lipoproteins. Moreover, development of dyslipidemia is a harbinger of future diabetes[3]. The currently available therapies which are mainly targeted towards reducing hyperglycemia, are not able to maintain the normoglycemic state in long run and they are more often associated with various side effects. Hence there is a need for an effective antidiabetic agent, which not only controls the hyperglycemia but also reduces diabetic complications[4]. The herbal medicines are widely used for the treatment of disease because of their effectiveness, safety, affordability and acceptability. *Psidium cattleianum* Sabine or strawberry guava is a shrub or small tree of Myrtaceae family. The plant is used not only as food but also as folk medicine in subtropical areas around the world because of its pharmacological activities. The shoots, leaves and bark of the plant are extensively used in Brazilian traditional medicine to treat diseases such as diabetes, diarrhoea and also as a prophylactic hepatoprotective agent. In particular, the leaf extract of guava has traditionally been used for the treatment of diabetes in East Asia[5]. Several studies had already reported the chemical composition of *Psidium cattleianum* leaves and corroborated the presence of flavonoids, saponins, cardiac glycosides, phenolic compounds, anthraquinones, tannins and catechins in phytochemical screening. Many literatures claims that the antihyperglycemic and antihyperlipidemic potential of strawberry guava is mainly related to the phenolic compounds especially the catechins present in the plant. However, no scientific study has been conducted on the antidiabetic and antihyperlipidemic activity of this plant. The present study therefore was designed to evaluate the antidiabetic and hypolipidemic activities of the *Psidium cattleianum* leaves extract in Streptozotocin induced diabetic rats.

MATERIAL AND METHODS

Collection and authentication of plant material

The fresh leaves of *Psidium cattleianum* Sabine were collected in the month of December 2017 from Ambalavayal, Wayanad District, Kerala. The plant specimen (No: 148219) was authenticated by Dr. A. K. Pradeep, Assistant Professor and Head, Department of botany, University of Calicut, Kerala, India. The leaves were dried under shade and finally pulverised into coarse powder with the help of a mechanical grinder and then stored in a well closed container.

Extraction

The leaf powder was extracted by continuous hot percolation process (Successive solvent extraction) by using Soxhlet apparatus with different solvents of increasing order of polarity, started with a nonpolar solvent n-Hexane followed by mid-polar Chloroform and highly polar ethanol(70%)[6].

Phytochemical screening

The n-Hexane, Chloroform and 70% ethanol extracts of *Psidium cattleianum* Sabine were subjected to qualitative chemical test for the detection various plant constituents and which showed the presence of alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, saponins, carbohydrates, proteins, amino acids, glycosides, vitamin C, starch, pectin and tryptophan.

Estimation of total phenol content

The total phenolic content of the samples was determined by the Folin-Ciocalteu's reagent method. An aliquot (1 ml) of extract (1mg/ml) was added to 9 ml water and 1ml of the Folin-Ciocalteu reagent in a volumetric flask. After 5 min, 10 ml of the sodium carbonate solution was added to the mixture and carefully agitated for 10 min. The mixture was allowed to stand in the dark for 90 min at room temperature. The absorption was measured at 750 nm using UV spectrophotometer. Different concentrations of gallic acid dissolved in pure ethanol were used to prepare the calibration curve (R^2 = .9672). The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE)/mg of extract[7].

Estimation of total flavonoid content

Total flavonoid content (TFC) of the extracts was measured according to the colorimetric assay. One milliliter of the ethanolic extract (1:10 w/v) was added to 300 µl sodium nitrite solution (5%) followed by 300µl aluminum chloride (10%). The mixtures were incubated at room temperature for 5 min, and then, 2 ml of 1 mol/L sodium hydroxide was added. Immediately, the volume of reaction mixture was made to 10 ml with distilled water and then thoroughly vortexed. The absorbance of the mixture was determined at 510 nm. A calibration curve was prepared from different concentrations of quercetin (R^2 = .974). Total flavonoid content was reported as milligrams of quercetin equivalents per g dry weight sample (mg CE/g DW)[8].

Estimation of total vitamin C content

The total vitamin C concentration in the extract was determined by Redox titration method using iodine solution[9].

Animals

Wistar albino rats (150-200 g) were used for *in-vivo* antidiabetic and antihyperlipidemic studies. They were housed in polypropylene cages as small groups and maintained at 22 ± 2ºC under 12 hours light/dark cycles and are fed ad libitum with standard pellet diet and has free access to water. The animals acclimatized to laboratory condition for 15 days before commencement of experiments. The animal experimental protocol has been approved by Institutional Animal Ethics Committee (IAEC) with approval no: DAMCOP/IAEC/038[10].

***In vivo* antidiabetic study**

Induction of Diabetes

Diabetes was induced to overnight fasted rats by intraperitoneal injection (i.p.) of freshly prepared STZ (40mg/kg BW) in 0.1M citrate buffer (pH 4.5). After 72 hrs of STZ administration, glucose levels were measured by collecting blood from the animal tail. Animals with blood glucose level higher than 200mg/dl were considered diabetic and used for the experiment[11].

Experimental Design

The rats were divided into five groups of six each randomly:

Group I

Normal control received 0.2% carboxy methyl cellulose (CMC) (1ml/100g) (p.o.).

Group II

Diabetic control received 0.2% CMC (1ml/100g) (p.o.).

Group III

Diabetic rats received Glibenclamde (5mg/kg) (p.o.).

Group IV

Diabetic rats received hydroalcoholic extract of *P. cattleianum* leaves (HAEPC) (200mg/kg) (p.o.).

Group V

Diabetic rats received (HAEPC) (400mg/kg) (p.o.).

The extracts and Glibenclamide were suspended in 0.2% CMC and administered for 28 days once daily to the respective groups. Blood samples were collected from the tip of rat tail and blood glucose levels were estimated at 0^{th}, 7^{th}, 14^{th}, 21^{th} and 28^{th} days of treatment using one touch glucometer. Body weights were measured initially and during the treatment period. On the 29^{th} day blood is collected by retro-orbital puncture from the inner can thus of the eye under mild anesthesia (Thiopentone sodium 40mg/kg, i.p.) and the biochemical parameters (blood glucose, total cholesterol, triglyceride, LDL, VLDL and HDL) were analysed[12,13].

Induction of Insulin Resistance

The insulin resistance in Wistar albino rats was induced by subcutaneous (s.c.) administration of Dexamethasone at a dose of 10mg/kg once daily for a period of 10 days.

Experimental Design

The animals were randomly divided into five groups, six animals in each group.

Group I

Normal control 11ml/100g normal saline (p.o.).

Group II

Dexamethasone 10mg/kg (s.c.) + vehicle 1ml/100g (p.o.) (Diabetic control).

Group III

Dexamethasone 10mg/kg (s.c.) + Metformin 50mg/kg (p.o.).

Group IV

Dexamethasone 10mg/kg (s.c.) + HAEPC (200mg/kg) (p.o.).

Group V

Dexamethasone 10mg/kg (s.c.) + HAEPC (400mg/kg) (p.o.).

All the animals received their respective assigned dose of treatments once daily for a period of 10 days. Body weight and blood glucose concentrations of the animals were measured at initial and at the final day of treatment period. On day 11, the overnight fasted animals were anesthetized with Thiopentone sodium (40mg/kg, i.p.) and blood was collected from the retro-orbital plexus and biochemical parameters such as blood glucose, total cholesterol, triglyceride, LDL, VLDL and HDL were estimated by using respective kits[14,15].

Biochemical Analysis

The biochemical parameters such as blood glucose concentration, total cholesterol, triglycerides, lipoprotein phospholipids, high density (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) were estimated using appropriate kits (Agappe diagnostic Ltd., Eranakulam) with the help of an auto-analyzer (Benesphera clinical anlyyzer C 611, Avantor.)

***Ex-vivo* anti-diabetic study**

Glucose uptake by isolated rat hemi-diaphragm method

All the tested Wistar albino rats were killed by cervical dislocation. The diaphragms were dissected out quickly with minima trauma and divided into two halves and weighed. The diaphragms were then rinsed in cold Kreb's Ringer bicarbonate buffer without glucose and the blood clots were removed. It is then placed in small culture tubes containing 5ml of Kreb's Ringer bicarbonate buffer with 5.55Mm glucose and incubated at 37°C for a period of 30 min. Five sets containing six number of graduated tubes were used for each group.

Group I

Normal control rat's diaphragm in Kreb's Ringer bicarbonate buffer with 5.55mM glucose.

Group II

Diabetic controlrat's diaphragm in Kreb's Ringer bicarbonate buffer with 5.55mM glucose.

Group III

Standard treated rat's diaphragm in Kreb's Ringer bicarbonate buffer with 5.55Mm glucose.

Group IV

Hydroalcoholic extract of *P. catteleianum* leaves treated (200mg/kg) rat's diaphragm in Kreb's Ringer bicarbonate buffer with 5.55mM glucose.

Group V

Hydroalcoholic extract of *P. catteleianum* leaves treated (200mg/kg) rat's diaphragm in Kreb's Ringer bicarbonate buffer with 5.55mM glucose.

Following incubation the hemi-diaphragm were taken out and the glucose content of the incubated medium was measured by GOD-POD method. The glucose uptake by the hemi-diaphragms was then calculated as the difference between the initial and final glucose content in the medium. The uptake of glucose was calculated and expressed as mg/g of tissue[16,17].

Statistical Analysis

The data were expressed as mean ± standard error mean (SEM). Different groups were assessed by One-way analysis of variance (ANOVA) for multiple comparisons followed by Dunnet's test (Graph Pad Prism 6 software, La Jolla CA. Trial version 5). The criterion for statistical significance was set at $p < 0.05$.

RESULTS

Total phenolic content

The concentration of phenolic content in the extract was interpolated from the calibration curve of gallic acid. The results obtained are given below (Figure No.1).

The concentration of phenolic content in the extract was found to be 41.45µg/ml of the test solution (1mg/ml), i.e. 41.45µg (GAE)/mg of extract.

Total flavonoid content

The concentration of flavonoid content in the extract was interpolated from calibration curve of standard quercetin (Figure No.2).

The total flavonoid content in the extract was found to be 31.73µg/ml of test solution (1mg/ml), i.e. 31.73µg (QE)/mg of extract.

Total vitamin C content

The vitamin C content in the HAEPC was determined by redox titration method using iodine solution. Ascorbic acid is taken as the standard and the total vitamin C content was found to be 11.22µg/mg of extract.

***In-vivo* antidiabetic and antihyperlipidemic studies**

Streptozotocin induced β-cell destruction in rats

Effect of *P.catteleianum* on body weight

The values are mean ± SEM. The body weight of diabetic control group was compared with control group and that of all other treatment groups were compared with diabetic control group animals by One-way ANOVA (Tukey test). The significance levels were expressed as *.*P<0.05, **P<0.01, ***P<0.001 and ns – not significant.

An increase in the body weight of control group (C) was observed from initial day to day 29. The body weight of the diabetic control group (DC) showed significant (*P* <0.001) reduction after the administration of STZ compared to normal control group. Administration of HAEPC at doses of 200 and 400mg/kg (LD and HD) produced a significant (*P* <0.001) improvement in weight reduction as compared to the diabetic control group (DC). The effect of HAEPC at 400mg/kg on body weight were found to be almost similar to that of the standard drug Glibenclamide

Effect of *P.catteleianum* on blood glucose level

The values are mean ± SEM. The blood glucose levels of diabetic control group were compared with control group and that of all other treatment groups were compared with diabetic control group animals by One-way ANOVA (Tukey test). The significance levels were expressed as *. ***P<0.001 and ns – not significant.

After the administration of STZ, the blood glucose levels of rats in all groups except the control group were found to be significantly higher when compared to the normal glucose level (>200mg/kg). During the study, there was a gradual significant (*P*<0.001) increase in blood glucose level were observed in diabetic control group (DC) when compared with normal control group (C). When compared to the diabetic control group, administration of HAEPC at doses of 200 (LD) and 400mg/kg (HD) and Glibenclamide 5mg/kg (S) produced an extremely significant (*P*<0.001) decrease in blood glucose levels of treated groups.

Effect of *P.catteleianum* on lipid profile

The values are mean ± SEM. The lipid profiles of diabetic control group were compared with control group and that of all other treatment groups were compared with diabetic control group animals by One-way ANOVA (Tukey test). The significance levels were expressed as*. *P<0.05, ***P<0.001 and ns – not significant.

After administration of STZ, profound alterations of the lipid profiles were seen in diabetic rats (DC). The treatment with standard drug Glibenclamide and different concentrations of HAEPC showed a significant (*P*<0.001 and *P*<0.05) reduction in elevated TC, TG, LDL and VLDL levels and showed a significant (*P*<0.001 and *P*<0.05) increase HDL level when compared to the diabetic control group (DC). The HAEPC at a dose of 400mg/kg (HD) were found to be more effective in improvement of lipid profile when compared to a dose of 200mg/kg (LD).

Dexamethasone induced insulin resistance

Effect of *P. cattelianum* on body weight and blood glucose level

The values are mean ± SEM. The body weights and blood glucose levels of diabetic control group were compared with control group and that of all other treatment groups were compared with diabetic control group animals by One-way ANOVA (Tukey test). The significance levels were expressed as *. *P<0.05, ***P<0.001, ns – not significant.

Initial body weights of rats in all the groups were almost similar. After 10 days, the dexamethasone treated rats (DC) lost the body weight significantly (*P*<0.001) compared to control rats (C). Treatment with metformin (S) produced a slight improvement (*P*<0.05) in weight reduction whereas the treatment with HAEPC (LD and HD) did not showed any significant improvement in weight loss

Effect of *P. cattelianum* on lipid profile

The values are mean ± SEM. The lipid profiles of diabetic control group were compared with control group and that of all other treatment groups were

compared with diabetic control group animals by One-way ANOVA (Tukey test). The significance levels were expressed as *. **P<0.01 and ***P<0.001.

There is a significant (p<0.001) increase in the levels of triglycerides, total cholesterol, LDL, VLDL and significant (p<0.001) decrease in HDL level were observed in dexamethasone induced diabetic rats (DC) when compared with vehicle treated rats (C). The HAEPC significantly (p<0.001) decreased the level of triacylglycerides, total cholesterol, LDL, VLDL and significantly (p<0.01 and p<0.001) increased the level of HDL in two different doses compared to the diabetic control group. The standard group (S) treated with Metformin showed more significant (p<0.001) changes in the level of triacylglycerides, total cholesterol, LDL, VLDL and HDL than the extract.

Glucose uptake by isolated rat hemi-diaphragm

The values are mean ± SEM. The glucose uptake of diabetic control group was compared with control group and that of all other treatment groups were compared with diabetic control group animals by Tukey test. The significance levels were expressed as*. **P<0.01 and ***P<0.001.

The glucose uptake of diabetic control groups (DC) were found to be significantly (P<0.001) lower when compared to the control group (C). The glucose uptake of dexamethasone induced diabetic control group animals were observed much better than that of STZ induced diabetic control group. The uptake was found to be significantly (P<0.001) higher with both the doses of HAEPC (LD and HD) and with standard drugs Glibenclamide and Metformin (S) when compared to the diabetic control group. The HAEPC at a dose of 400mg/kg produced a higher effect in glucose uptake and which can be comparable with that of the standard drugs.

Histopathological studies

Histopathology of pancreas

Histopathological study of pancreas was carried out on STZ induced diabetic rat models. Pancreatic sections from Group I (C) showed abundant patches of normal pancreatic structure, the specimen was observed with normal islets of Langerhans and normal acini tissues. Pancreatic sections from Group II (DC) which is treated with STZ showed disorganization of the structure of the endocrine and exocrine cells, illustrated less number of Langerhans cells with damaged and necrotic pancreatic acini. Lytic and vascular changes of cellular components, small and shrunken islets and destruction of β-cells were observed in the diabetic condition. On the other hand sections from Groups IV and V (HD and LD), treated with two different concentrations of HAEPC showed restoration of pancreatic endocrine cells and regeneration of some of the pancreatic acini. More over pancreatic cells of group III (S) treated with Glibenclamide shows that it have much better effect. The cells showed healthy structure, enlarged atrophied islet, infiltration of inflammatory cells and blood cells through islets of Langerhans.

Histopathology of liver

The histopathological changes of liver in both STZ and dexamethasone induced diabetic rats were studied.

In case of STZ induced diabetes, examined sections of normal rats (Group I, C) showed the presence of normal hepatocytes with most of the cells contained a central rounded nucleus while some are binucleated. The blood sinusoids are present between the cords. They are observed with normal sinusoidal spaces and normal vein lumen. Liver sections from diabetic rats (Group II, DC) showed severe injury. The sinusoidal spaces and vein lumen were appeared enlarged. The wall of blood vessels and capillaries were found thickened. Fatty changes (lipoma) could also be seen in the DC section. Examined sections of group III (S) which is treated with Glibenclamide showed restoration of hepatocytes and blood sinusoids. Most of the cells were healthy and seem to be normal. On the other hand sections from groups IV and V (LD and HD) that is treated with HAEPC illustrated the gradual restoration of hepatocytes in 200mg/kg and 400mg/kg respectively. In HD most of the hepatocytes were relatively in normal state with still dilated blood vessels, hydropic degeneration of some of the hepatocytes was still observed.

In dexamethasone induced diabetic models, the liver of the normal control rats treated with vehicle (C) showed normal architecture of the hepatocytes. The liver of the rats treated with dexamethasone (DC) showing degeneration of hepatocytes and disorganization of hepatic cords and inflammation. They also observed with steatosis, i.e., accumulation of fats in the liver. The metformin treated group's liver showing the normal architecture of the hepatocytes without inflammation. The rats treated with HAEPC showing the regeneration hepatocytes and rearrangement of hepatic cords with minimal inflammation (Figure No.6).

DISCUSSION

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism that affects nearly 10% of the population every year. Both STZ and dexamethasone induced diabetes characterized severe weight reduction during the study. In STZ induced diabetes the rats showed symptoms like polyuria, polydipsia and polyphagia. In contrast to this the rats treated with dexamethasone showed decreased food and water intake. In both the cases HAEPC showed an improvement in these diabetic complications. The *P. cattleianum* leaves are good source of vitamin C, dietary fibres and proteins which may regulate the metabolic disturbances associated with hyperglycemia and hyperlidemia and may improve body weight by reducing blood glucose level. The fibre present in the plant retards glucose diffusion and reduces GI glucose absorption. The protein content in the leaves may compensate protein breakdown and contribute in reconstruction of building blocks. In overall which made a positive response in weight reduction in diabetic condition[18,19].

In STZ and dexamethasone induced diabetes the normal glucose homeostasis in the body gets altered by insulin deficiency and impaired insulin action respectively. The high levels of glucose in blood in turn cause major metabolic disturbances in carbohydrates, fats and protein systems due to metabolic and oxidative stress. Insulin deficiency and resistance leads to decreased uptake of glucose by the peripheral tissues which cause energy lack in the cells. The increased levels of c-AMP in cells enhance glycogenolysis over glycogenesis and gluconeogenesis which cause further increase in blood glucose level. As an adaptive response to high blood glucose concentration the pancreas try to release more and more insulin into the blood and this over workload causes degeneration of β-cells. The increased blood glucose concentration also promotes the release of reactive oxygen species (ROS) which also contributes to cells death and apoptosis[19].

The HAEPC produced a significant effect in lowering blood glucose level in both STZ and dexamethasone induced diabetes. The plant *P.cattleianum* leaves bears an extremely high concentration of phenolic, flavonoid, tannins, vitamins C and β-carotene. The phenolic compounds play a significant role in management of diabetes mellitus. They have reported with declining the levels of glycated haemoglobin and fructosamine as well as producing a significant reduction in the glycemic levels in diabetes. The phenolic compounds, gallic acid, catechins and quercetin in guava leaves are reported to inhibit the glycation of proteins suggesting its use for the prevention of diabetes complications. Catechins are important as a preventive treatment for diabetes type 2[20].

The polyphenols have also been reported to possess antioxidant activity. The catechins have strong antioxidant action. They reduce the development of body fat, and protect the body against diabetes, heart diseases and inflammation. They also stimulate insulin secretion from β-cells by protecting them from destructive effects of ROS[21].

The flavonoids such as reynoutrin, guajaverin, quercetin, morin, myricetin, luteolin and kaempferol isolated from *P. cattleianum* leaves are reported to have powerful antioxidant and free radical scavenging activity[21]. Guajaverin have been tested and proven for its inhibitory activity against aldose reductase, an enzyme involved in diabetes mellitus. In the presence of hyperglycemia high glucose levels saturate the hexokinase pathways and glucose

is then metabolized by the polyol pathway. This then has a knock-on effect for other metabolic processes. Increased aldose reductase activity and accumulation of sorbitol have been found in diabetic animal models. As sorbitol does not easily dissolve across cell membranes this increases cellular osmolarity, ultimately leading to cell damage. Thus, aldose reductase inhibitors can reductase the hyperglycemic-induced polyol pathway, controlling to the treatment and prevention of diabetic complications[22].

It is well known that diabetes is associated with hyperlipidemia, since insulin activates the enzyme lipoprotein lipase, which hydrolyzes triglyceride under normal condition. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesteremia. In the present study diabetic rats exhibited marked hypercholesterolemia, hypertriglyceridemia, increased levels of LDL. VLDL, with concomitant decrease in HDL. The treatment with HAEPC produced marked decrease in serum TC, TG, LDL, and VLDL levels and a significant increase in HDL in diabetic rats. Studies have shown that gallic acid, catechin, and epicatechin inhibit pancreatic cholesterol esterase, which decreases cholesterol levels. Catechins are important as a preventive treatment for diabetes type 2 and obesity. Quercetin has been associated to decreased mortality from heart disease and decreased incidence of stroke. Quercetin presents hypocholesterolemic and antioxidant activity[23]. The plant sterols stand out for reducing the uptake of low density lipoprotein cholesterol (LDL-C) levels. Consequently, they prevent the development of coronary heart diseases. Long-term administration of ascorbic acid depresses cholesterol levels in blood serum in the majority of hypercholesterolemic diabetics.

The STZ and dexamethasone treated groups showed a significantly lower glucose uptake as compared to the normal rats. The standard and extract treated rats showed higher glucose uptake which may be due better insulin secretion and improved insulin sensitivity. The presence of polysaccharides enhances insulin secretion from the β-cells and the constituents like gum and mucilage showing antidiabetic activity by acting on glucose transporter systems. These information suggest that the improved glucose uptake in HAEPC treated groups may be due to the presence of phytoconstituents having a positive impact on insulin secretion, insulin action and glucose transporter system[24].

Besides from these findings the histopathological observations conjointly support the concept that HAEPC reduces the burden of hyperglycemia, hyperlipidemia and associated oxidative stress and protects the hepatic and pancreatic tissues from diabetic complications.

Table No.1: Absorbance values for estimation of total phenolic content

S.No	Concentration (µg/ml)	Absorbance at 750 nm (Mean± SEM)
1	10	2.1812±0.0034
2	20	2.4034±0.0061
3	30	2.6840±0.0147
4	40	2.9902±0.0100
5	50	3.2083±0.0048
6	1 mg/ml of extract	2.9961±0.0545

The absorbance values are expressed as mean ± SEM

Table No.2: Absorbance values for estimation of total flavonoid content

S.No	Concentration (µg/ml)	Absorbance at 415 nm (Mean± SEM)
1	10	0.1166±0.0034
2	20	0.1701±0.0060
3	30	0.3118±0.0056
4	40	0.4603±0.0013
5	50	0.5952±0.0035
	1 mg/ml of extract	0.3524±0.0032

The absorbance values are expressed as mean ± SEM

Table No.3: Body weight changes in STZ induced diabetes

S.No	Groups	Body weight (g)				
		Day 0	Day 7	Day 14	Day 21	Day 28
1	Control (C)	181.6± 4.89	189.6± 4.82	197.6± 5.17	204.2± 5.21	214.75± 6.38
2	Diabetic control (DC)	182.16± 3.62	166± 3.76**	145.33± 3.29***	128.5± 2.93***	108± 4.06***
3	Standard (S) Glibenclamide 5mg/kg	179.66± 4.65	173.5± 4.81ns	171.16± 5.14**	174.66± 5.47***	177.8± 5.84***
4	Lower dose (LD) HAEPC 200mg/kg	180.33± 4.38	167.33± 4.27ns	159.5± 4.91ns	159.66± 5.18***	161.4± 3.17***
5	Higher dose (HD) HAEPC 400mg/kg	180.33± 3.82	171.83± 4.04ns	168± 4.10*	170.5± 4.05***	174.8± 4.92***

Table No.4: Changes in blood glucose levels in STZ induced diabetes

S.No	Groups	Blood glucose concentration (mg/dl)				
		Day 0	Day 7	Day 14	Day 21	Day 28
1	Control (C)	92.2± 3.48	95± 5.51	92± 3.78	93± 4.83	98± 6.6
2	Diabetic control (DC)	262.33± 33.32	322.83± 36.29***	407.66± 39.41***	478.83± 32.65***	535.8± 26.46***
3	Standard (S) Glibenclamide 5mg/kg	280.83± 21.24	234.33± 18.39ns	200.5± 6.50***	161± 11.45***	118.2± 7.88***
4	Lower dose (LD) HAEPC 200mg/kg	282± 18.45	251± 18.00ns	220.83± 12.85***	200.16± 8.65***	180.2± 10.35***
5	Higher dose (HD) HAEPC 400mg/kg	273.66± 26.68	245± 20.79ns	206.16± 14.88***	176.66± 11.75***	135.6± 11.39***

Table No.5: Changes in serum lipid profiles in STZ induced diabetes

S.No	Groups	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
1	Control (C)	109.8± 6.30	116.4± 6.46	34± 1.58	60.44± 6.34	21.96± 1.26
2	Diabetic control (DC)	178.8± 7.45***	219± 10.36***	16± 0.70***	168± 9.52***	34.96± 1.28***
3	Standard (S) Glibenclamide 5mg/kg	122.8± 3.15***	138.6± 3.35***	29± 0.77***	85.04± 3.67***	24.56± 0.63***
4	Lower dose (LD) HAEPC 200mg/kg	155.8± 2.74*	186± 7.66*	21± 0.71*	135.32± 8.54*	31.88± 0.87ns
5	Higher dose (HD) HAEPC 400mg/kg	127.8± 2.59***	148.2± 4.22***	26.4± 0.92***	96.24± 3.90***	25.56± 0.51***

Table No.6: Changes in body weight and blood glucose level in dexamethasone induced diabetes

S.No	Groups	Body weight (g)		Blood glucose (mg/dl)	
		Day 0	Day 11	Day 0	Day 11
1	Control (C)	160.5± 4.14	177.83± 4.72	89.66± 1.82	87.33± 1.38
2	Diabetic control (DC)	172.33± 3.98	119.75± 1.65***	91± 3.19	178± 2.83***
3	Standard (S) Metformin 50mg/kg	173± 6.70	140± 5.06*	90.16± 3.09	121.5± 1.38***
4	Lower dose (LD) HAEPC 200mg/kg	168± 6.03	127.4± 3.4ns	92.66± 1.80	145.8± 1.71***
5	Higher dose (HD) HAEPC 400mg/kg	169.16± 4.64	138± 3.61ns	90.16± 3.37	128.83± 2.00***

Table No.7: Changes in serum lipid profiles in dexamethasone induced diabetes

S.No	Groups	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
1	Control (C)	118.6± 3.16	125.66± 3.74	34.5± 2.26	67.53± 5.21	23.63± 0.63
2	Diabetic control (DC)	287± 3.87***	221.5± 5.23***	17.25± 1.25***	146.85± 5.89***	57.4± 0.77***
3	Standard (S) Metformin 50mg/kg	134.16± 2.00***	133.33± 2.88***	29.66± 1.22***	76.83± 3.71***	26.83± 0.40***
4	Lower dose (LD) HAEPC 200mg/kg	178.6± 4.38***	156.8± 3.24***	22.6± 0.92**	98.48± 3.31***	35.72± 0.87***
5	Higher dose (HD) HAEPC 400mg/kg	149.83± 2.77***	145.83± 2.27***	28± 0.96***	87.86± 2.72***	29.96± 0.55***

Table No.8: Glucose uptake in isolated rat hemi-diaphragm

S.No	Groups	Glucose uptake (mg/g) Mean ± SEM	
		STZ induced	Dexamethasone induced
1	Control (C)	8.49±0.23	8.49±0.23
2	Diabetic control (DC)	0.75±0.08***	2.79±0.20***
3	Standard (S)	8.14±0.18***	7.62±0.12***
4	Lower dose (LD) HAEPC 200mg/kg	4.87±0.12***	4.15±0.18***
5	Higher dose (HD) HAEPC 400mg/kg	7.89±0.16***	7.37±0.18***

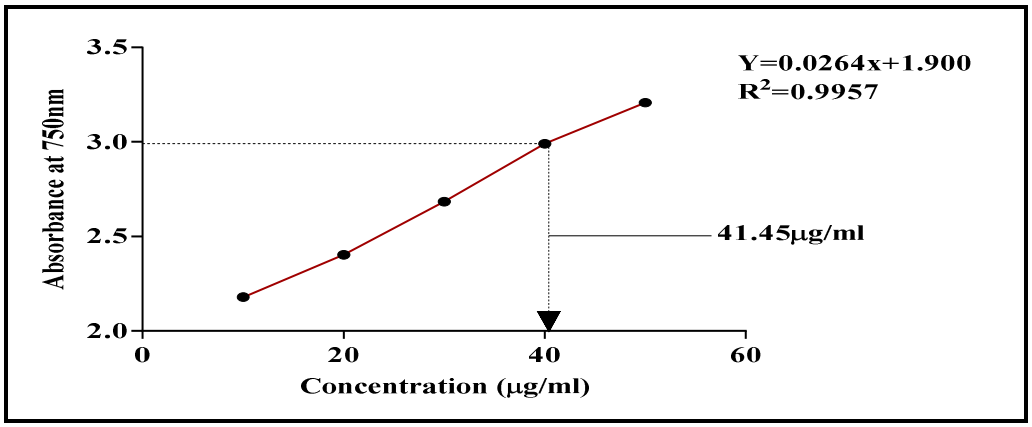


Figure No.1: Calibration curve for estimation of total phenolic content

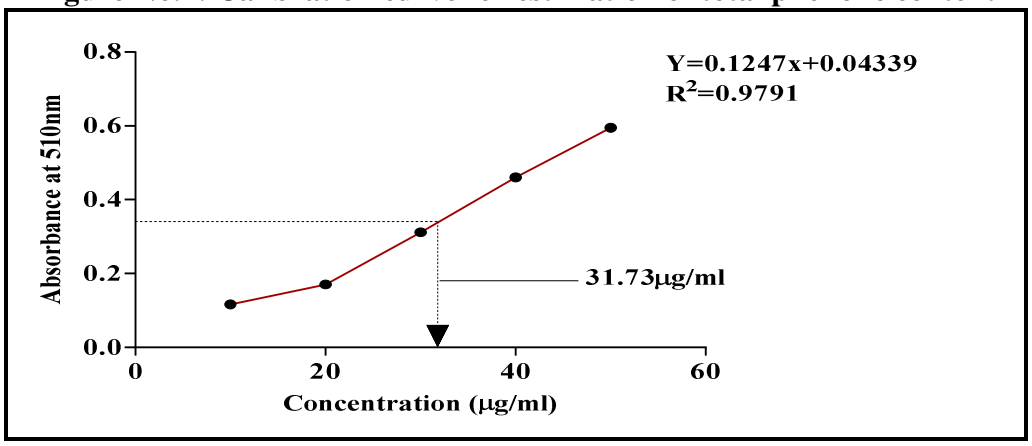


Figure No.2: Calibration curve for estimation of total flavonoid content

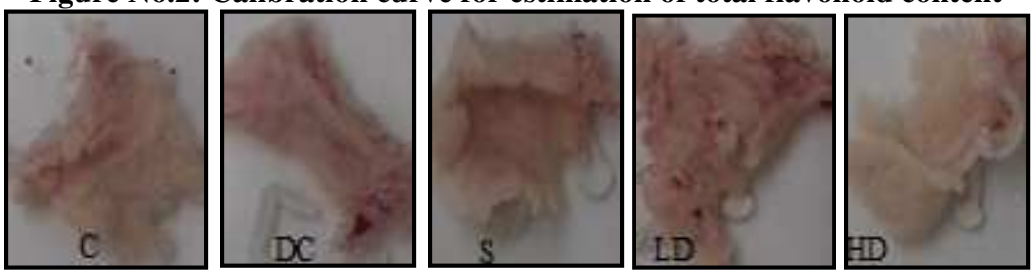
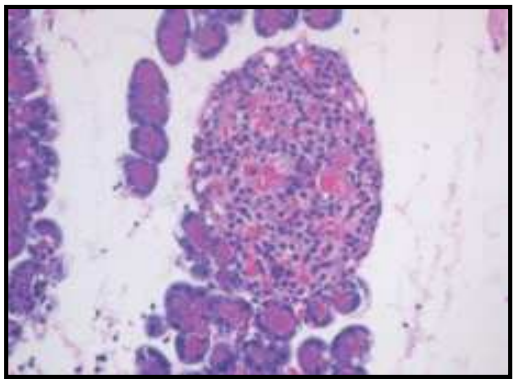
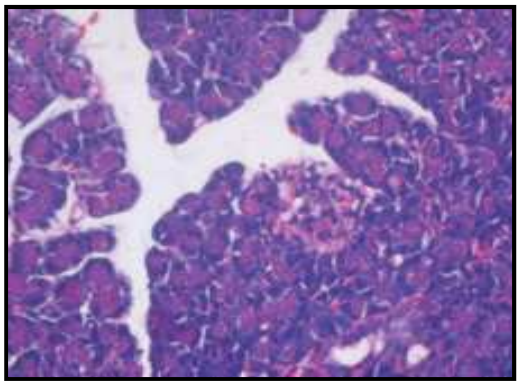


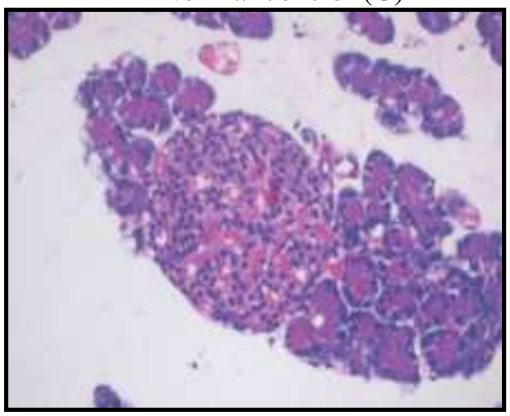
Figure No.3: Isolated samples of pancreas in STZ induced diabetes



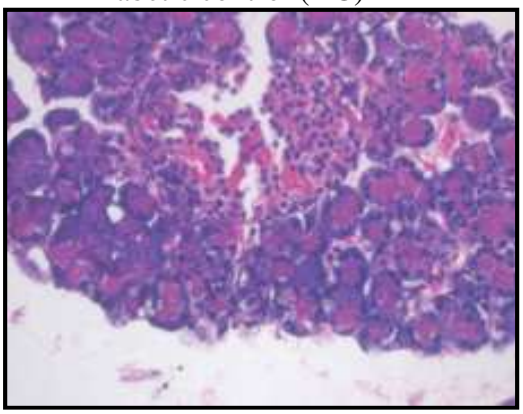
Normal control (C)



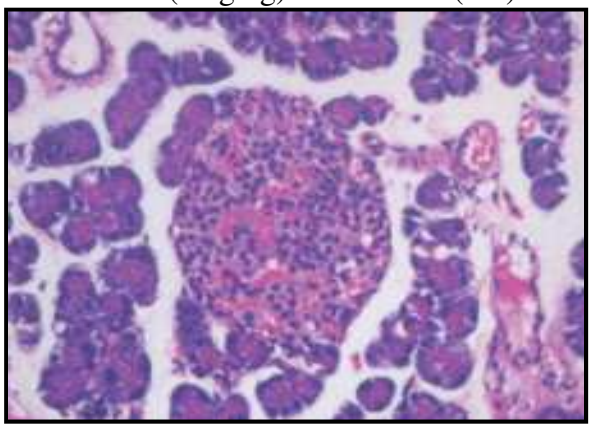
Diabetic control (DC)



Standard (S) Glibenclamide (5mg/kg)

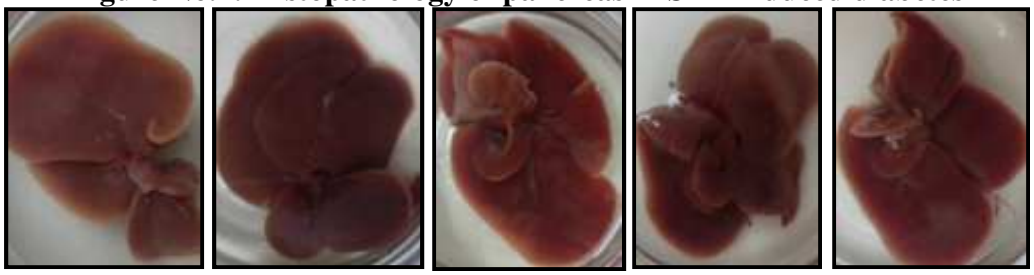


Lower dose (LD) HAEPC (200mg/kg)



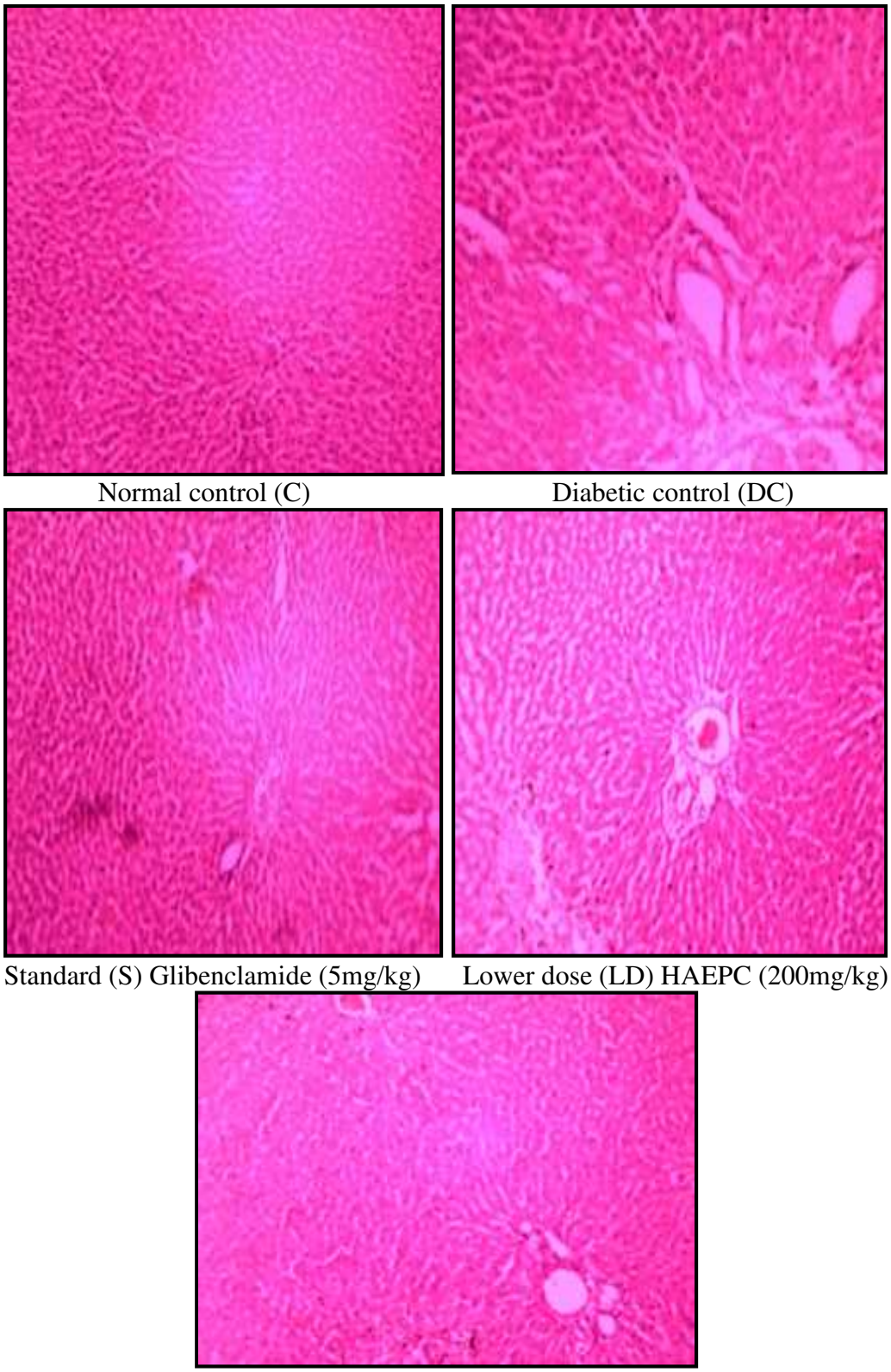
Higher dose (HD) HAEPC (400mg/kg)

Figure No.4: Histopathology of pancreas in STZ induced diabetes



C DC S LD HD

Figure No.5: Isolated samples of liver in STZ induced diabetes

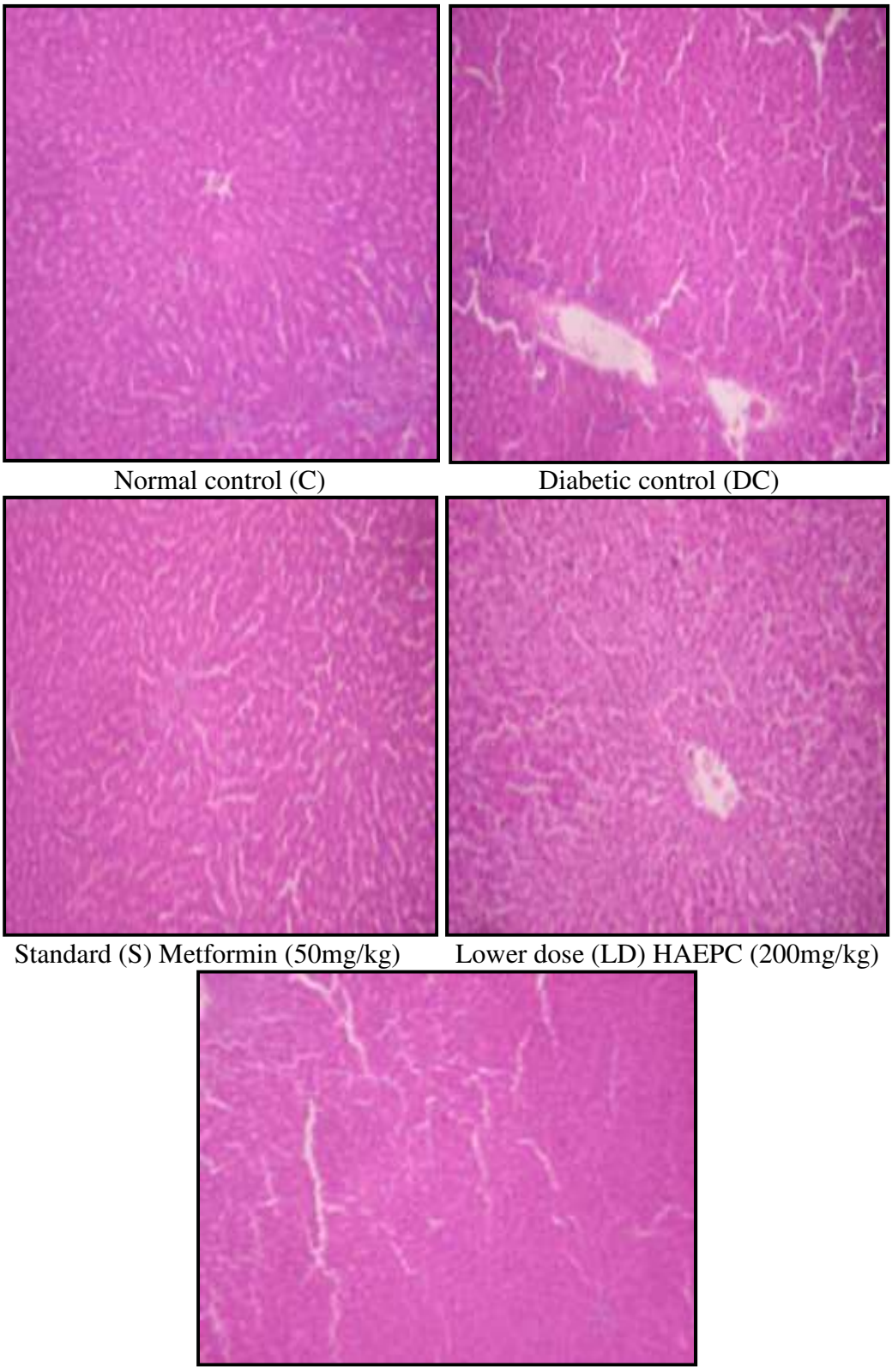


Normal control (C) Diabetic control (DC)

Standard (S) Glibenclamide (5mg/kg) Lower dose (LD) HAEPC (200mg/kg)

Higher dose (HD) HAEPC (400mg/kg)

Figure No.6: Histopathology of liver in STZ induced diabetes



Normal control (C) Diabetic control (DC)

Standard (S) Metformin (50mg/kg) Lower dose (LD) HAEPC (200mg/kg)

Higher dose (HD) HAEPC (400mg/kg)

Figure No.7: Histopathology of liver in dexamethasone induced diabetes

CONCLUSION

The present study concluded that the hydroalcoholic extract of *P.cattleianum* Sabine leaves exhibits significant protection against hyperglycemia and hyperlipidemia. The antidiabetic activity of the plant could be due to its stimulatory effect on insulin secretion, regenerative effect on β-cells, and inhibitory effect on carbohydrate digesting enzymes, control over glycolysis and gluconeogenesis, antioxidant and free radical scavenging activity, insulin sensitizing effect also due its effects on lipid metabolism.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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