Research Paper

Antihyperglycemic and Antihyperlipidemic Activity of *Plectranthus Amboinicus* on Normal and Alloxan-Induced Diabetic Rats

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The present study was undertaken to investigate the antihyperglycemic and antihyperlipidemic effects of ethanol extract of *Plectranthus amboinicus* in normal and alloxan-induced diabetic rats. Diabetes was induced in Wistar rats by single intraperitoneal administration of alloxan monohydrate (150 mg/kg). Normal as well as diabetic rats were divided into groups (n=6) receiving different treatments. Graded doses (200 mg/kg and 400 mg/kg) of ethanol extract of *Plectranthus amboinicus* were studied in both normal and alloxan-induced diabetic rats for a period of 15 days. Glibenclamide (600 µg/kg) was used as a reference drug. Oral administration with graded doses of ethanol extract of *Plectranthus amboinicus* exhibited hypoglycemic effect in normal rats and significantly reduced the peak glucose levels after 120 min of glucose loading. In alloxan-induced diabetic rats, the daily oral treatment with ethanol extract of *Plectranthus amboinicus* showed a significant reduction in blood glucose. Besides, administration of ethanol extract of *Plectranthus amboinicus* for 15 days significantly decreased serum contents of total cholesterol, triglycerides whereas HDL-cholesterol, total proteins and calcium were effectively increased. Furthermore, effect of ethanol extract of *Plectranthus amboinicus* showed profound elevation of serum amylase and reduction of serum lipase. Histology examination showed ethanol extract of *Plectranthus amboinicus* exhibited almost normalization of damaged pancreatic architecture in rats with diabetes mellitus. Studies clearly demonstrated that ethanol extract of *Plectranthus amboinicus* leaves possesses hypoglycemic and antihyperlipidemic effects mediated through the restoration of the functions of pancreatic tissues and insulinotropic effect.

Key words: Antihyperlipidemic, glibenclamide, insulinotropic, *Plectranthus amboinicus*

Diabetes mellitus is characterized by hyperglycemia with disturbances of carbohydrate, lipid and protein metabolism. Obesity and lack of exercise play an important role in diabetes[1]. According to World Health Organization projections, around 3.2 million deaths every year worldwide are attributable to complications of diabetes, characterized by retinopathy, nephropathy, neuropathy, microangiopathy, diabetic ketoacidosis[2] which equates to six deaths every minute. Increased production of superoxides and lowered antioxidant enzyme activities compromising with body antioxidant defense systems in hyperglycemia is associated with the pathogenesis of diabetic dyslipidaemia, micro- and macrovascular complications[3]. Currently available drugs have side effects and failure of response after prolonged use. Plant based medicines are gaining prominence in treatment of metabolic diseases like diabetes. Many flavonoid containing plants serve as a hidden wealth of potentially useful natural products for diabetes control[4].

Plectranthus amboinicus (Lamiaceae) is an aromatic shrub widely distributed in India. Phytochemical analyses reported the presence of phytochemical constituents like flavonoids, terpenoids, saponins, steroids, tannins and volatile oil[5]. The literature survey revealed *P. amboinicus* leaves extract to have an antioxidant property[6]. Hence, the present study has been planned to evaluate the antihyperglycemic effects of ethanol extract of *P. amboinicus* (PAEE) in albino rats.

MATERIALS AND METHODS

Plant Material and Extract:

Fresh leaves of *P. amboinicus* were collected from the botanical garden of S. K. Arts and H. S. K.

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Science Institute, Vidyanagar, Hubli, Karnataka, in the month of June. The leaves of *P. amboinicus* were cleaned, shade-dried for 30 days at room temperature, crushed to a coarse powder and subjected to exhaustive extraction using a Soxhlet apparatus. Powder weighing 70 g was extracted with 600 ml of 95% ethyl alcohol for 72 h for each batch. The solvent was recovered using rotovapour (Buchi, Switzerland). The semisolid mass obtained was concentrated under reduced pressure and stored in an air tight container.

Animals:
Wistar albino rats (150-200 g) and mice (20-30 g) of either sex were procured from animal house of KLES College of Pharmacy, Hubli and kept for one week to acclimatize to laboratory conditions before starting the experiment. Animals were fed with standard diet and water *ad libitum*, but 12 h prior to an experiment; the animals were deprived of food but not water.

Acute Toxicity Test:
The acute oral toxicity[7] study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), the study was approved by the Institutional Animal Ethics Committee (IAEC). No mortality and no signs of toxicity were found even after administration of a limit dose of 2000 mg/kg body weight of extract; hence 1/10th of the dose was taken as effective dose. Two doses, 200 and 400 mg/kg were selected for the present study to evaluate antihyperglycemic and antihyperlipidemic activity.

Hypoglycemic effect of ethanol extract of *P. amboinicus* (PAEE) in normal rats:
Overnight fasted rats were divided into four groups of six animals each. The first group served as a control group received distilled water (5 ml/kg). Group II and III received PAEE 200 and 400 mg/kg, respectively. Glibenclamide (GLB) 600 μg/kg was administered to group IV as a reference standard drug[8] suspended in vehicle. Suspensions were prepared using 0.3% w/v sodium carboxy methylcellulose in distilled water[9]. The baseline fasting blood glucose was determined before oral administration of respective treatment.

Hypoglycemic effect of PAEE in glucose-loaded normal rats:
Overnight fasted animals were divided into four groups of six animals per group. The group I, served as a control group received distilled water. Group II and III received PAEE 200 mg/kg and 400 mg/kg respectively. GLB was administered to group IV as a reference drug (600 μg/kg). The treatment was administered orally, 30 min before the glucose load (2 g/kg)[10]. Blood samples were taken before and 30, 60 and 120 min after glucose intake and analyzed for glucose level[11].

Induction of hyperglycemia with alloxan:
The selected rats were weighed, marked for individual identification and fasted for 16 h[12]. The rats were injected with alloxan monohydrate dissolved in sterile saline (0.9% NaCl) at a single dose of 150 mg/kg intraperitoneally. The baseline fasting blood glucose was determined before intraperitoneal administration of alloxan. After 6 h alloxan administration, 5% glucose solution was infused orally in feeding bottle for a day to overcome the early hypoglycemic phase as a result of acute massive pancreatic release of insulin[13]. Hyperglycemia was confirmed by elevated serum glucose level, determined at 3rd day post-induction. All the rats became consistently hyperglycemic and stable by 5th day post-induction. Rats showing fasting blood glucose level around 400-450 mg/dl were selected for the study[14].

Experimental protocol:
The animals were randomly divided into five groups of six animals each. Group I and Group II served as normal control and diabetic control treated with 5 ml/kg of distilled water, respectively. Group III and IV diabetic rats treated with PAEE 200 and 400 mg/kg respectively. Group V diabetic rats orally treated with GLB 600 μg/kg. The daily oral treatment was administered in between 08.00 to 09.00 h for 15 days.

Biochemical analysis:
Blood was withdrawn retro-orbitally from the inner canthus of the eye with the help of capillary tube under mild ether inhalation anesthesia[15] at between 08.00 to 09.00 h. Blood samples were collected in Eppendorff's tubes and allowed to clot for 10 min. Serum was separated by centrifuging the samples at 3000 rpm for 10 min and stored in a refrigerator until analyzed. Glucose estimation along with their body weight was done in all the groups prior to treatment and 1 h after the respective treatment on first, fourth, seventh, tenth and fifteenth day of the experiment[16]. Blood glucose was determined by Trinder's glucose oxidase method[17]. Serum contents of total cholesterol

(TC), triglycerides (TGs), HDL-cholesterol, total protein, calcium and amylase were estimated using commercial diagnostic kits (ERBA Diagnostics Mannheim GmbH Ltd., India). Measurements of lipase enzyme activities were done by one hour period of hydrolysis method[18]. All estimations were performed according to the kit manufacturer's instructions. The animals were sacrificed after blood collection by cervical dislocation on the day 15. The pancreas was then quickly dissected out, washed in ice-cold saline and stored in 10% formalin for tissue characterization and further organ identification. Histological specimens were examined to evaluate the details of pancreatic architecture in each group microscopically.

Statistical analysis:

The results were expressed as the mean±SEM. The results obtained from the present study were analyzed using One-way ANOVA followed by Dunnett's multiple comparison tests. Data was computed for statistical analysis using Graph Pad Prism Software. Differences between the data were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Alloxan-induced hyperglycemic rats showed a significant decrease ($P<0.05$) in body weight on days 7, 10 and 15 of the experiment. Daily oral treatment with PAEE showed significant increase ($P<0.05$) in body weight at the end of the experiment as compared to diabetic control group. The most pronounced effect was obtained with dose of 400 mg/kg as shown in Table 1. Effect of graded doses of PAEE in fasting normal rats showed significant reduction ($P<0.05$) on blood glucose after 15 days of oral treatment, with 400 mg/kg showed profound hypoglycemia. While the control rats did not exhibit any significant alterations in their glucose levels throughout the experimental studies as shown in Table 2. The blood glucose levels reached a peak at 30 min and gradually decreased to attain basal glucose level. Pretreatment with graded doses of PAEE showed significant reduction ($P<0.05$) in blood glucose at 60 and 120 min as compared to the control group as shown in Table 3.

Diabetic control rats showed significant elevation ($P<0.05$) in fasting blood glucose on successive days of the experiment as compared to their basal values, which was maintained over a period of 2 weeks. Daily oral treatment with PAEE showed significant reduction ($P<0.05$) in blood glucose on successive days of the experiment as compared to their basal values. The most pronounced antihyperglycemic effect was obtained with dose of 400 mg/kg as shown in Table 4. Alloxan-induced hyperglycemic rats showed a significant elevation ($P<0.05$) in serum

TABLE 1: EFFECT OF PAEE ON BODY WEIGHT IN NORMAL AND DIABETIC RATS

Treatment	Normal	Diabetic control	PAEE 200	PAEE 400	GLB
Basal values	179.3±2.060	163.5±5.045	162.2±2.242	165.2±2.151	165.7±4.104
After treatment					
1st day	182.8±2.915	160.7±4.447	164.3±2.319	167.2±4.757	167.0±1.983
4th day	186.7±1.706	154.2±3.868	167.2±1.887	172.3±3.127	172.8±2.120
7th day	189.3±3.333	148.2±3.591[a]	171.7±5.346*	178.8±1.621*	178.5±2.643*
10th day	195.3±2.404	142.5±3.181[a]	175.0±2.129*	182.2±1.400*	184.0±3.454*
15th day	201.8±2.358	135.8±2.257[a]	180.5±2.579*	187.5±4.264*	189.5±2.446*

PAEE was administered at two doses, 200 and 400 mg/kg. Body weights are expressed as g and each value is mean±SEM of 6 observations, [a]$P<0.05$ compared to normal and *$P<0.05$ compared to diabetic control.

TABLE 2: EFFECT OF PAEE ON BLOOD GLUCOSE LEVEL IN NORMAL RATS

Treatment	Normal	PAEE 200	PAEE 400	GLB
Basal values	85.18±0.5958	86.78±1.774	84.43±0.7085	87.82±1.125
After treatment				
1st day	82.45±0.3253	84.75±1.049	77.38±0.8180*	81.92±1.625*
4th day	83.47±0.2679	77.09±0.5772*	72.61±0.9854*	75.31±1.244*
7th day	83.67±0.2629	71.94±0.5550*	64.21±1.527*	67.76±0.8717*
10th day	84.40±0.4906	64.57±1.420*	57.09±0.9828*	56.83±0.1365*
15th day	85.45±1.214	54.21±1.336*	49.33±1.003*	47.48±0.3915*

PAEE was administered at two doses, 200 and 400 mg/kg. Blood glucose values are as expressed as mg/dl and each value is mean±SEM of 6 observations, *$P<0.05$ compared to normal.

TABLE 3: EFFECT OF PAEE ON BLOOD GLUCOSE IN NORMAL RATS (OGTT)

Treatment	Normal	PAEE 200	PAEE 400	GLB
Basal values	84.42±1.354	85.92±1.077	84.33±1.588	85.59±1.189
After glucose loading				
30 min	147.4±1.299*	145.3±1.957*	143.7±1.261*	146.2±1.468*
60 min	136.1±1.880*	131.2±1.900*	121.2±0.6421*	110.7±1.283*
120 min	125.3±0.7450*	121.3±1.716*	112.8±1.105*	101.6±1.682*

PAEE was administered at two doses, 200 and 400 mg/kg. Blood glucose values are as expressed as mg/dl and each value is mean±SEM of 6 observations,*P<0.05 compared with their basal values of respective groups.

TABLE 4: EFFECT OF PAEE ON BLOOD GLUCOSE LEVEL IN DIABETIC RATS

Treatment	Normal	Diabetic control	PAEE 200	PAEE 400	GLB
Basal values	85.14±0.5962	384.4±0.9036	363.6±1.117	438.6±1.002	426.6±1.215
After treatment					
1st day	82.45±0.3253	435.8±0.7685*	306.3±1.120*	355.0±1.025*	365.9±1.135*
4th day	83.47±0.2679	493.3±1.161*	274.8±1.079*	287.8±0.7758*	283.3±0.7027*
7th day	83.67±0.2629	503.1±0.173*	236.3±1.060*	206.7±1.118*	206.5±0.8087*
10th day	84.40±0.4906	519.4±1.116*	196.4±1.469*	148.0±0.6750*	135.0±0.7436*
15th day	85.45±1.214	544.0±1.545*	126.3±0.7676*	101.9±1.730*	95.18±1.413*

PAEE was administered at two doses, 200 and 400 mg/kg. Blood glucose values are as expressed as mg/dl and each value is mean±SEM of 6 observations,*P<0.05 compared with their basal values of respective groups.

TABLE 5: EFFECT OF PAEE ON LIPIDS, TOTAL PROTEIN AND CALCIUM IN NORMAL AND DIABETIC RATS

Treatment	Total cholesterol	Triglycerides	HDL-cholesterol	Total protein	Calcium
Normal	82.79±1.615	87.60± 2.000	28.05±0.9097	8.302±0.6466	9.862±0.3676
Diabetic control	151.3±2.793#	124.9±2.203#	16.35±0.7931#	4.067±0.5077#	5.972±0.6706#
PAEE 200	111.5±3.993*	111.1±2.229*	19.12±1.056	6.028±0.3457*	8.385±0.6600
PAEE 400	91.92±1.914*	100.5±1.839*	22.97±0.8593*	7.233±0.2565*	9.062±0.7820*
GLB	86.57±1.910*	94.57±2.667*	25.50±0.5933*	7.798±0.7074*	9.323±0.5104*

PAEE was administered at two doses, 200 and 400 mg/kg. Protein levels were expressed as g/dl, while lipids and calcium levels were expressed as mg/dl. Each Value is mean±SEM of 6 observations. #P<0.05 compared with normal. *P<0.05 compared with diabetic control.

TABLE 6: EFFECT OF PAEE ON AMYLASE (G/DL) AND LIPASE (UNITS) IN NORMAL AND DIABETIC RATS

Treatment	Amylase	Lipase
Normal	784.1±7.291	9.217±1.062
Diabetic control	134.6±4.674#	17.47±0.6070#
PAEE 200 mg/kg	478.6±9.211*	13.32±0.4126*
PAEE 400 mg/kg	688.3±7.142*	11.60±0.8140*
GLB	719.6±8.922*	9.650±0.3819*

PAEE was administered at two doses, 200 and 400 mg/kg. Amylase levels were expressed as g/dl, while lipase levels in units. Each Value is mean±SEM of 6 observations. #P<0.05 compared with normal. *P<0.05 compared with diabetic control.

contents of TC, TG whereas HDL-cholesterol, total proteins and calcium were significantly decreased (P<0.05) as compared to the control group. Daily oral treatment with PAEE showed significant reduction in serum contents of total cholesterol, triglycerides and simultaneously increased the HDL-cholesterol, total proteins and calcium levels as compared to the diabetic control group as shown in Table 5.

Amylase was significantly decreased (P<0.05) whereas serum lipase enzyme showed a significant elevation (P<0.05) in alloxan-induced hyperglycemic rats as compared to the control group. The PAEE showed significant elevation (P<0.05) for amylase activities and also showed a marked decrease in lipase enzyme activities as compared to the diabetic control group as shown in Table 6.

Increased oxidative stress as one of the unpredicted participants in the progression of diabetes and its squeal is widely accepted[19]. The alloxan rats exhibited severe glucose intolerance and metabolic stress as well as hyperglycemia due to a progressive oxidative insult interrelated with a decrease in endogenous insulin secretion and release[20]. Treatment with antioxidants might be an effective strategy for reducing diabetic complications due to disproportionate generation of free radicals[21]. *P. amboinicus* leaves are known to contain several flavonoids, terpenoids, saponins, tannins and steroids[22] which are known to be bioactive antidiabetic principles[23].

Weight loss is a very serious issue in the management of diabetes mellitus may be due to degeneration of

the adipocytes and muscle tissues to make up for the energy lost from the body due to frequent urination and over conversion of glycogen to glucose[24]. Diabetic animals treated with graded doses of PAEE continued to gain weight as compared to diabetic control.

High levels of oxidative cytotoxicity have been linked to glucose oxidation, lipid abnormalities and nonenzymatic glycation of proteins which contribute to the development of diabetic complications[25]. Increased gluco-oxidation results due to increased aldose reductase pathway activity lead to accumulation of sorbitol and fructose, NADP redox imbalances as well as alterations in signal transduction[26]. In this study diabetic rats exhibited a severe hyperglycemia. Treatment with PAEE has successively reduced serum glucose level measured on days 1, 4, 7 and 10 and at the end of study both in normal and diabetic rats. Decrease in serum glucose level in normal rats was found to be an indication of hypoglycemic action of PAEE treatment. The 400 mg/kg dose shows consistent decrease in serum glucose level. In oral glucose tolerance test, PAEE has significantly decreased elevated serum glucose after glucose load. These two studies suggest that the hypoglycemic agent reduces the basal and post-prandial blood glucose levels[27]. In this study, PAEE does not produce a dose dependent glucose reduction in normal and diabetic group. The extract showed optimum reduction in serum glucose level at 200 mg/kg but at a higher 400 mg/kg, it did not show a matching decrease in blood glucose level.

Lipid profile has been shown to be the important predictor for metabolic disturbances including diabetes. Higher concentration of serum TC and TG in diabetes may be attributed to inhibition of cholesterol catabolism or may be due to insulin deficiency and mobilization of fatty acids from adipose tissue by lipolysis. Administration of PAEE showed a significant decrease in TC and TG with increased HDL-cholesterol significantly as compared to diabetic control rats. The increase in HDL-cholesterol is accompanied by increased catabolism of VLDL and replacement of TG in the core of HDL with cholesterol[28,29]. This hypolipidemic effect of extract has prognostic significance as free fatty acids are atherogenic in diabetes[30]. Excessive catabolism of protein further contributes in micro- or macrovascular complications[31]. PAEE 200 and 400 mg/kg increased total protein content, but significant increase was shown by 400 mg/kg.

Acute regulation of systemic calcium has invoked its role in insulin secretion, appropriate response to glucose, catabolic effects on cortical bones, osteoporosis and hypertension[32]. The study was in agreement with previous reports that total serum calcium decreases during the course of diabetes. Administration of PAEE has resulted in significant increase in serum calcium. Increased serum calcium may be responsible for the insulin release by exocytosis which may explain sulphonylurea like action[33].

Insulin deficiency follows a close relationship with pancreatic enzyme abnormalities[34]. Serum levels of pancreatic enzymes alter with the degree of diabetic disequilibrium and may involve in functional damage to the pancreas by ischemia and oxidative stress[35]. Serum amylase and serum lipase were evaluated in the present study. Initial drop in the serum amylase activity in diabetes may be due to impaired pancreatic exocrine secretion or lack of insulin stimulation on synthesis in exocrine cells[36]. PAEE has shown profound elevation for amylase. Lipase is more specific than amylase. Lipases functions as a lipolytic enzyme that hydrolyzes TGs and phospholipids in circulating plasma lipoproteins. High blood TG and low HDL-cholesterol levels are associated with high lipase activity[37]. Administration of PAEE to alloxan-induced diabetic rats produced considerable reduction in serum lipase level. Insulin secretion may be enhanced by improved pancreatic exocrine function[38].

In conclusion, the present study indicates treatment of alloxan-treated rats with ethanol extract of *P. amboinicus* for two consecutive weeks could restore the normal biotransformation by shifting the balance of lipid and carbohydrate metabolism. The extract showed significant hypoglycemia with very crucial effects on lipids and total protein levels. The extract also exhibited an enhancement in serum calcium, which may elevate the intracellular Ca^{2+} concentration and releases insulin by exocytosis. Improved pancreatic exocrine activities can be ascribed to insulin secretion from existing residual β-cell of islets or due to enhanced transport of blood glucose to peripheral. Histopathological report of PAEE 400

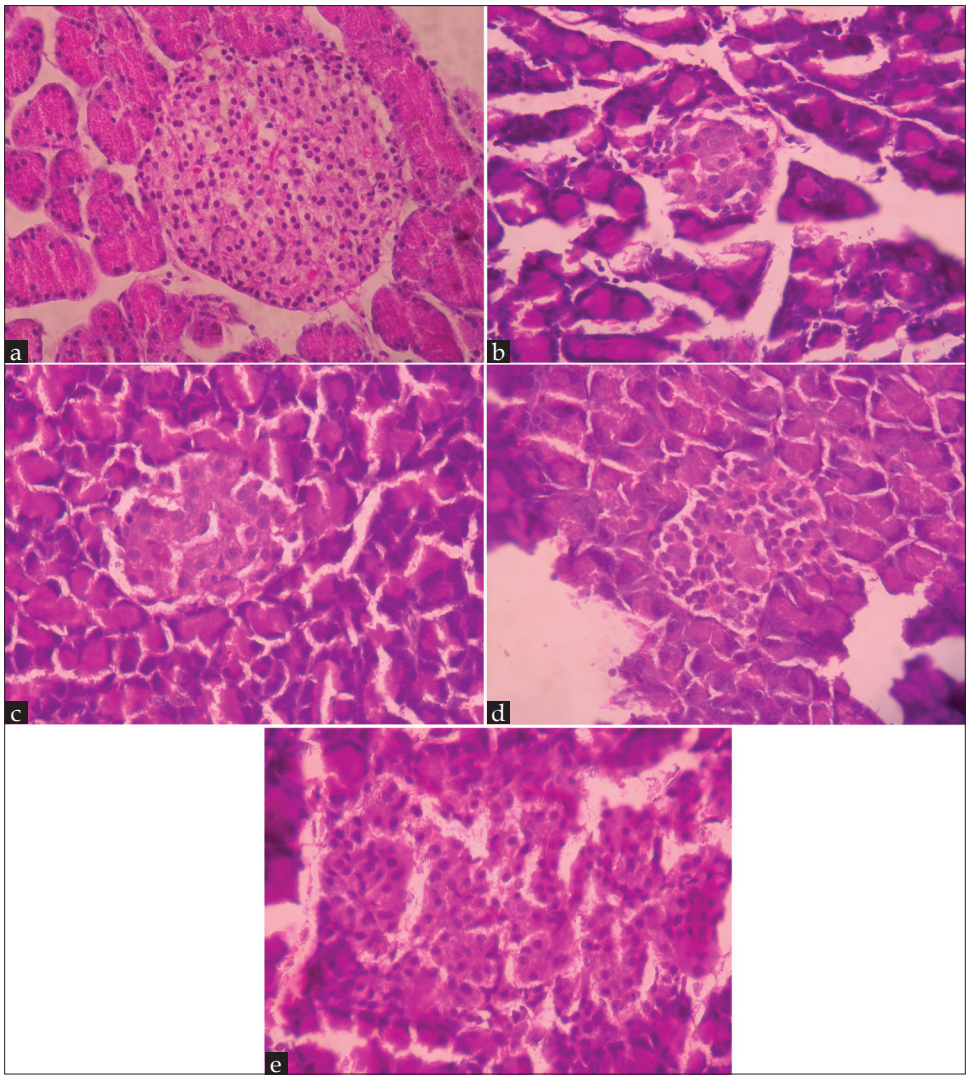

Fig. 1: Photomicrograph of pancreatic tissues
(a) normal rat showing normal acini and normal cellular population in islets of Langerhans and absence of both damage to islets and hyperplasia, (b) diabetic control rat showing damaged islets and reduced islet size, (c) diabetic rat treated with PAEE 200 mg/kg showing restoration of normal cellular population size of islets of Langerhans and absence of islet damage and presence of hyperplasia, d. diabetic rat treated with PAEE 400 mg/kg showing restoration of normal cellular population size of islets of Langerhans and absence of islet damage and presence of hyperplasia, (e) diabetic rats treated with GLB 600 µg/kg showing restoration of normal cellular population size of islets of Langerhans and absence of islet damage and presence of hyperplasia.

mg/kg treated pancreas showed the maintenance of normal architecture of pancreatic β-cells as shown in fig. 1. Thus the attributed antihyperglycemic effects of PAEE were partly due to their ability to restore the functions of pancreatic tissues and insulinotropic effect is very similar to sulphonylureas.

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