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ANTIDIABETIC ACTIVITY OF ISOLATED PIPERINE FROM *DAUCUSCARATA* EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The root *Daucuscarota* belongs to family Araceae which is known as Carrot in Telugu. In the present study isolated piperine from *Daucuscarota* fruit were subjected to the phytochemical investigation and evaluated for antidiabetic activity on blood glucose level, lipid profiles and on the body weight in streptozotocin induced diabetic rats. Isolated piperine (100mg/kg) from Daucuscarota extract and Glibenclamide (10mg/kg) were administered orally in streptozotocin (50mg/kg, i.p.) induced diabetic rats. In this antidiabetic study, maximum reduction in blood glucose was observed in isolated piperine (160.8, 96.7mg/dl) at the dose of 100mg/kg on 21stday respectively. The isolated piperine showed the significant effect ($p<0.005$) in the various biochemical parameters like protein, triglycerides, cholesterol and total lipid levels. Isolated piperine (100mg/kg) was found to have significant ($p<0.001$) blood glucose lowering effect. Preliminary Phytochemical investigation revealed the presence of alkaloids, as the major constituents in the Daucuscarota plant. These results suggest that piperine (100mg/kg) showed antidiabetic activity in streptozotocin induced diabetic rats.

KEYWORDS

Daucuscarota, Streptozotocin, Glibenclamide, Lipid profiles, Blood glucose and Antidiabetic activity.

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INTRODUCTION

Diabetes is a condition in which the body does not produce enough insulin or cannot use insulin properly. Insulin is a naturally occurring hormone in the blood that is necessary for providing our cell with energy to function. Insulin helps sugar to move from the blood stream in to the cells. When glucose cannot enter our cell, it builds up in the blood (hyperglycemia) leading to damage of orangs including the eyes, kidneys, blood vessels and nerves. Most people with diabetes have type I

diabetes (juvenile-onset diabetes) have a condition where the body does not produce enough insulin at all. People with type I diabetes need insulin injection and close monitoring to control their blood sugar level. People with type II diabetes (adult-onset diabetes) which means that the body does not produce enough insulin or the insulin is not able to transfer glucose in to cell.

Classification of diabetes mellitus

Type I

Beta cells destruction, usually leading to absolute insulin deficiency,

Auto immune, Idiopathic.

Type II

Ranges from predominantly insulin resistant, with relative insulin deficiency, to predominantly insulin secretary defect, with or without insulin resistant.

Genetic defects in insulin action

Diseases of the exocrine pancreas

Endocrinopathies

Drugs or chemical induced diabetes

Other genetic syndromes associated with diabetes

GENERAL SYMPTOMPS

Polyuria (frequent urination)

Noctoria

Polydipsia (excessive thirst)

Polyphagia (excessive hunger and fatigue)

Symptoms of salt and water depletion: thirst, dizziness, cramps

Long term complication of diabetes include gangrene, retinopathy, myocardial in fraction, poly neuropathy and uremia.

Clinical features of type 2 diadetes

1. Usually affect over weigh persons (80%)
2. Most are over 40 years of age but now increasingly seen in children.
3. Common presentation are genital candidiasis (particularly in women)urinary track in fications or skin infections
4. Generally starts to 4 to 7 years before diagnosis is made.

The pathogenesis of diabetes mellitus is controlled by insulin and oral administration of antidiabetic drugs such as sulfonylurea as and biguanides. The essential value of some plants has been published and the large numbers of them remain unexplored as yet. One of such plant is *Daucuscarata* which consists of flavonoids, tannins, glycosides, alkaloids, terpenes, etc. *Daucuscarata* fruit is antidiabetic, anthelmintic, aphrodisiac, stimulant, diaphoretic, antidiarrhoeal, carminative, expectorant, tonic, antiprotozoal, anticancer, sharpening hearing, aphrodisiac, cardio tonic and appetite. It is also used in dysentery, asthma, troubles of the throat, rheumatism, asthma, worm infestations, helminthias is and bronchitis. Hence, the objective of the present study was designed to investigate the antidiabetic activity of isolated piperine from *Daucuscarata* fruit in STZ induced diabetic rats[1].

REVIEW OF LITERAURE

Daucuscarota, whose common names include wild carrot, bird's nest, bishop's lace, family Apiaceae, native to temperate regions of Europe and southwest Asia, and naturalized to North America and Australia. Domesticated carrots are cultivars of a subspecies, *Daucuscarota* subsp. *sativus*.

Description

The wild carrot is an herbaceous, somewhat variable biennial plant that grows between 30 and 60 cm (1 and 2 ft.) tall, and is roughly hairy, with a stiff, solid stem. The leaves are trip innate, finely divided and lacy, and overall triangular in shape. The leaves are bristly and alternate in a pinnate pattern that separates into thin segments. The flowers are small and dull white, clustered in flat. The umbels are terminal and approximately 3-4 inches (8-10 cm) wide[2]. They may be pink in bud and may have a reddish or purple flower in the center of the umbel. The lower bracts are three-forked or pinnate, which distinguishes the plant from other white-flowered umbel lifers. As the seeds develop, the umbel curls up at the edges, becomes more congested, and develops a concave surface. The fruits are oval and flattened, with short styles and hooked spines. The fruit is small, dry and bumpy with protective hairs surrounding it. The fruit of *Daucuscarota* has 2 mericarp. The endosperm of the fruit grows before the embryo. The dried umbels detach from the plant, becoming tumbleweeds. The function of the tiny red flower, coloured by anthocyanin, is to

attract insects. Wild carrot blooms in summer and fall. It thrives best in sun to partial shade. Daucuscarota is commonly found along roadsides and in unused fields.

Scientific classification

Uses

Like the cultivated carrot, the *D. carota* root is edible while young, but it becomes too woody to consume. The flowers are sometimes battered and fried. The leaves are also edible except in large quantities[2].

Extra caution should be used when collecting *D. carota* because it bears a close resemblance to poison hemlock. In addition, the leaves of the wild carrot may cause phytophotodermatitis. It has been used as a method of contraception and an abortifacient for centuries. If used as a dyestuff, the flowers give a creamy, off-white color.

D. carota, when freshly cut, will draw or change colour depending on the colour of the water in which it is held. This effect is only visible on the "head" or flower of the plant. Carnations also exhibit this effect. This occurrence is a popular science demonstration in primary grade school.

Diabetes mellitus (DM) is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications. Acute complications can include diabetic ketoacidosisor death. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes.

Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. There are three main types of diabetes mellitus:

- Type 1 DM results from the pancreas's failure to produce enough insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause is unknown.
- Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progresses a lack of insulin may also develop. This form was previously referred to as "non insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes". The most common cause is excessive body weight and not enough exercise.
- Gestational diabetes is the third main form and occurs when pregnant women without a previous history of diabetes develop high blood sugar levels.

MATERIAL AND METHODS

Collection of plant material

The *Daucuscarata* fruit were collected from the local area collected from the local market of Ongole, Andhra Pradesh state, India.

Isolation of piperinene from *Daucuscarata* fruit

Place 250g of grind *Daucuscarata* fruits in a 250ml round bottomed flask, add 2l of 95%ethanol, 5 boiling chips and reflex for 2h. Filter the mixture by suction filtration and concentrate the filtrate to a valum of 10-15 ml by simple distillation or by use of a rotory evaporator. To 250ml of a 10% Solution of KOH in 95% ethanol contained in a 125ml Erlenmeyer flask add the concentrated above alcoholic extract. The resulting solution was heated and add water drop wise. A yellow precipitate was formed. Add water until no more solid appears to form and allow the mixture to stand at least overnight. Collect the soild by suction filtration and recrystallize it by acetone[3].

Chemicals

Glucometer (Acucheck-Sensor) was purchased from Roche Diagnostics, Mumbai, India Glibenclamide was obtained as gift sample from IPCA Laboratories, Mumbai, India. Streptozotocin was purchased from Sigma, USA. Ethanol was purchased from Ranbaxy Fine Chemicals Ltd., New Delhi, India.

Qualitative chemical tests[8,9]

Isolated piperine was tested by the standard procedures. The isolated piperine showed the presence of alkaloids.

Animals[10,11]

Wister albino rats (150-200g) of either sex were used. Animals maintained under standard environmental conditions and have free access to

feed and water *ad libitum*. Acute toxicity study was carried out using albino mice.

ACUTE TOXICITY STUDY

The acute toxicity study was carried out by using Swiss albino mice of either sex, weighing about 25-30g. This study was performed as per OECD -423 guidelines. Animals were kept in a temp controlled environment 23 ± 2ºC) at 12hours light/dark cycle. It was found that the tolerated dose level is 2000 mg/kg bodyweight.

Streptozotocin-induced diabetes[4]

The albino rats weight of 150-200 g of either sex allowed to fast for 24 hours prior to experimentation and rendered diabetic by a single dose of intra peritoneal injection of streptozotocin 50 mg/kg body weight. 15 After 18 hours of injection of streptozotocin, diabetes was confirmed by testing blood sugar level more than 250 mg/dl were selected for the further study. Animals maintained for four days in diabetic condition for well establishment of diabetes.

Animal Grouping and drug administration[5,6,7]

They were divided into five groups.

Group 1 (control): Animals were administered distilled water orally.

Group 2 (diabetic control): Treated with streptozotocin (50mg/ kg, I.p)

Group 3 (standard): treated with standard glibenclamide (10mg/ kg, orally)

Group 4 (Test No.1): treated with isolated piperine from *Daucuscarata* fruit (50mg /kg b.w)

Group 5: (Test No.2): treated with isolated piperine from *Daucuscarata* fruit (100mg /kg b.w)

Assessment of Antidiabetic Activity

Effects of consumed piperine on blood-glucose level of rats

The blood samples were collected from the tail vein of the rats and blood glucose levels was estimated at 1st, 7th, 14th and 21st days after extract administration by using one touch basic glucose strips (Johnson and Johnson Ltd., Mumbai). The results were mentioned (Table No.2).

Effects of consumed piperine on body weight of rats[12]

The body weight of each animal in all groups were estimated after the 1st, 7th, 14th and 21st day intervals and the findings were mentioned (Table No.3).

Serum analysis[13]

On the twenty first day of experiment the animals were sacrificed and blood was collected from various groups by puncturing the retro-orbital plexus, keep aside for half an hour for clotting. Serum was separated by centrifugation the blood samples at 6000 rpm for 20mins and stored in the refrigerator until analyzed. The serum analyzed for various biochemical parameters such as protein, cholesterol, triglycerides and total lipids. The findings were mentioned (Table No.4)

Effects of consumed piperine on histopathology of pancreas (Histomorphologic Changes of Pancreas)[14]

The pancreas was removed for identifying histopathological changes. Pancreatic sections stained with hematoxylin and eosin (H and Ex40). The sections revealed that streptozotocin causes severe necrotic changes of pancreatic islets, especially in the centre of islets. Nuclear changes, karyolysis, disappearance of nucleus and in some places residue of destroyed cells were visible. The cellular integrity and architecture were intact in the non-diabetic control group (Figure No.1). Relative reduction of size and number of islets and severe reduction of beta cells were clearly seen in diabetic control group (Figure No.2). Pancreas of the diabetic group III which consumed 10mg/kg body wt Glibenclamide (Figure No.3), showed similarity to group I (Figure No.1). Examination of pancreas in treated diabetic groups IV and V showed increased size of islets and hyper chromic nucleus. There was also a relative increase of granulated and normal beta cells in the group V (Figure No.4) which consumed 50mg/kg body wt. piperin1, when compared with the diabetic group IV (Figure No.5) which consumed 200mg/kg piperine1309.

RESULTS

Anti- diabetic effect of piperine in Streptozotocin induced diabetic rats

In the Anti- diabetic study, repeated administration (once a day for 21 days) of the isolated piperine as well as Glibenclamide causes significantly ($p<0.001$) reduction in the blood glucose level as compared with diabetic control group. Maximum reduction in blood glucose level was observed (160.8, 96.7 mg/dl respectively) on 21st day in the diabetic rats treated with isolated piperine at 200mg/kg. Glibenclamide treated animals showed maximum reduction in blood glucose level (90.02 mg/dl) on 21st day (Table No.2). Sub-acute treatment of 14 days with the isolated piperine in the treated doses brought about improvement in bodyweights, indicating beneficial effect in preventing loss of body weight in diabetic rats. The ability of isolated piperine to prevent body weight loss seems to be due to its ability to reduced hyperglycaemia (Table No.3). The isolated piperine administered animals showed short onset and short duration of antihyperglycaemic action. Sub-acute treatment for 21 days with the in the isolated piperine treated doses brought about improvement in body weights indicating beneficial effect in preventing loss of body weight in diabetic rat. The isolated piperine showed the significant effect ($p<0.005$) in the various biochemical parameters like protein, triglycerides, cholesterol and total lipid levels. Flavonoids, alkaloids, tannins and phenolics are known to modulate the activities of various enzymes due to their interaction with various biomolecules.

The fruit of the plant *Daucuscarata* have been reported to contain alkaloids, flavonoids, saponin and tannins. Preliminary phytochemical analysis indicated that, *Daucuscarata* fruit contain flavonoids, alkaloids, phenolic compound and tannins. The antihyperglycaemic activity of isolated piperine may be due to the presence of several bioactive antidiabetic principles. It is thus apparent that piperine possesses antihyperglycaemic activity.

Statistical Analysis[15]

For *in-vivo* experiments values are represented by mean ± SEM. The mean values are analyzed by one way ANOVA followed by Dunnets test. The $p<0.05$ and $p<0.01$ was considered as statistically significant.

Histopatholgy

The effect of piperine at 100mg/kg dose on histopathlogical findings on the pancreas shown in plate 1-5. It is observed that diabetogenic agent streptozotocin produced lesion in the pancreatic islets as viewed by very scanty islets with acinar tissue. Treament with Glibenclamide has decreased the degree of lesions as indicated by partial intact pancreatic cells with acini. However attenuation of pancreatic degeneration was observed in diabetic animals treated with piperine 100mg/kg.

Kingdom:	**Plantae**
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Apiales
Family:	Apiaceae
Genus:	*Daucus*
Species:	***D. carota***
Binomial name	
Daucuscarota L.	

Table No.1: Percentage yield of isolated piperine from *Daucuscarata* fruit

S.No	Method	Colour	Percentage Yield
1	Isolation of piperine by using 95% alcohol reflex method	Red colored crystals	2.32%w/w

Table No.2: Effect of piperine on Blood glucose level against STZ induced Diabetic rats

Group	Treatment and Dose	Blood-glucose level (mg/dl)			
		Day 1	Day 7	Day 14	Day 21
I	Vehicle control (food and distilled water *ad libitum*, 10ml/kg/day orally)	207.40±3.21	205.3±2.33	206.8±2.43	208.43±2.32
II	Diabetic Control (STZ suspended in saline, 50mg/ kg i.p.)	214.20±4.8	218±6.79	216±4.32	212±2.36
III	Diabetic + Standard (Glibenclamide 10mg/kg/day orally	204.65±02.28*	194.32±1.49*	192.4±1.47*	191.7±1.48**
IV	Diabetic + piperine 50 mg/kg/day orally	205.72±2.41*	207.46±0.21*	196.46± 0.23*	194.19±2.19*
V	Diabetic + piperine 100 mg/kg/day orally	210.23±2.26*	202.02±2.16	190.46± 0.24*	189.19±1.47*

Values are expressed as mean ± SD (n=6).
Student T test followed by one way ANOVA using Dunnett's
Statistical significance was performed by one way ANOVA using Dunnett's
*p value<0.05
**p value<0.01

Table No.3: Effect of piperine on Body weight of STZ-induced Diabetic rats

Group	Treatment and Dose	Body weight (g)			
		Day 1	Day 7	Day 14	Day 21
I	Vehicle control (food and distilled water *ad libitum*, 10ml/kg/day orally)	201.50±3.31	202.2±2.31	204.7±2.33	206.8±1.94
II	Diabetic control (STZ suspended in saline, 50mg/kg i.p.)	206.30±4.88	175.21±7.16a*	162.2±3.54a*	149.79±2.31a*
III	Diabetic + Standard (Glibenclamide 10mg/kg/day orally)	205.66±2.48	196.2±1.48	192.2±1.23	191.7±1.49
IV	Diabetic + piperine 50 mg/kg/dayorally	206.81±2.31	185.56±0.21	181.18±2.14	179.8±0.31b*
V	Diabetic + piperine 100 mg/kg/day orally	205.72±2.33	193.02±2.36	191.28±2.41	189.21±1.48b

Values are expressed as mean ± SEM (n=6); *P<0.05
* is used to indicate the significance, a is used to indicate the significance between Group II VS Group I b is used to indicate the significance between Group II VS Group IV and V
Data were analyzed by One-way ANOVA followed by Dunnett's t-test

Table No.4: Effect of piperine on biochemical parameters

Group	Treatment and Dose	Parameters at Day 21st			
		Protein (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Total Lipids
I	Vehicle control (food and distilled water *ad libitum*, 10ml/kg/day orally)	2.56±0.07	151.51±1.11	86.85±5.6	143.88±0.59
II	Diabetic control (STZ suspended in saline, 50mg/kg i.p.)	0.55±0.02a*	269.32±12.5a*	201.82±9.2a*	285.13±0.34a*
III	Diabetic + Standard (Glibenclamide 10mg/kg/day orally)	1.87±0.02b*	147.81±7.01b*	98.15±4.78b*	146.75±0.42b*
IV	Diabetic + piperine 50 mg/kg/day orally	1.52±0.02b*	173.82±4.7b*	127.46±0.48b*	176.93±0.66b*
V	Diabetic + piperine100 mg/kg/day orally	1.76±0.05b*	156.51±6.7b*	108.33±0.41b*	153.11±0.45b*

Values are expressed as mean ± SEM (n=6); *P<0.05

* is used to indicate the significance

a is used to indicate the significance between Group II VS Group I

b is used to indicate the significance between Group II VS Group IV and V

Data were analyzed by One-way ANOVA followed by Dunnett's t-test

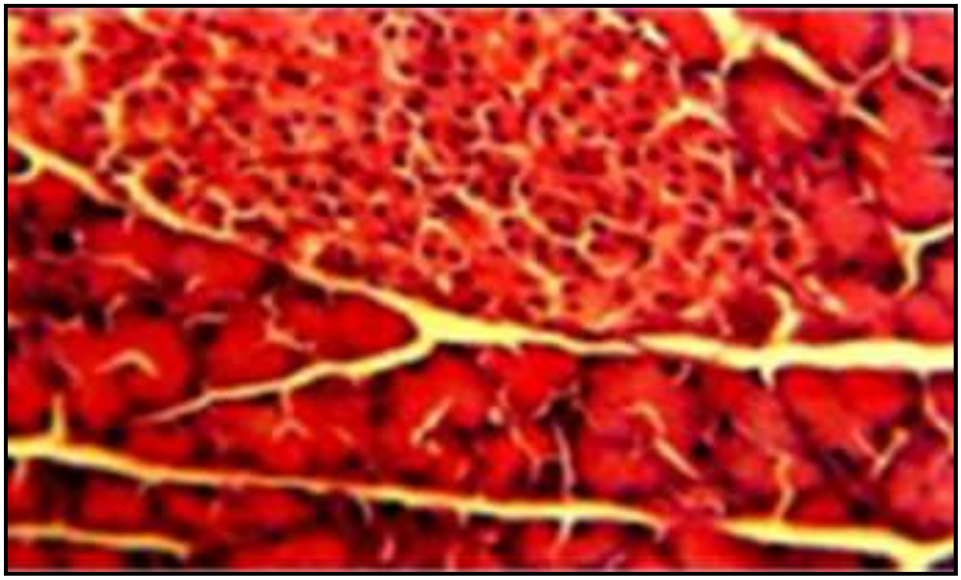

Figure No.1: Normal Pancreas, H and E Staining (40X) Section shows degeneration of β-cells granules in β-cells

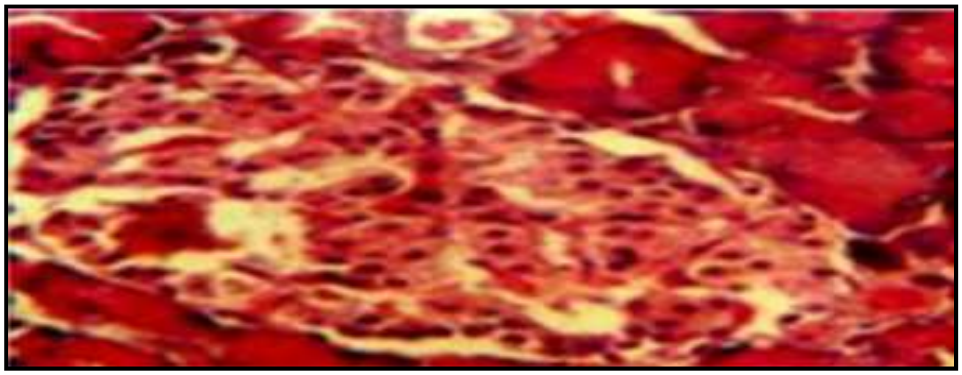

Figure No.2: Diabetic pancreas H and E Staining (40X) Section shows normal pancreas with insulin in pancreas

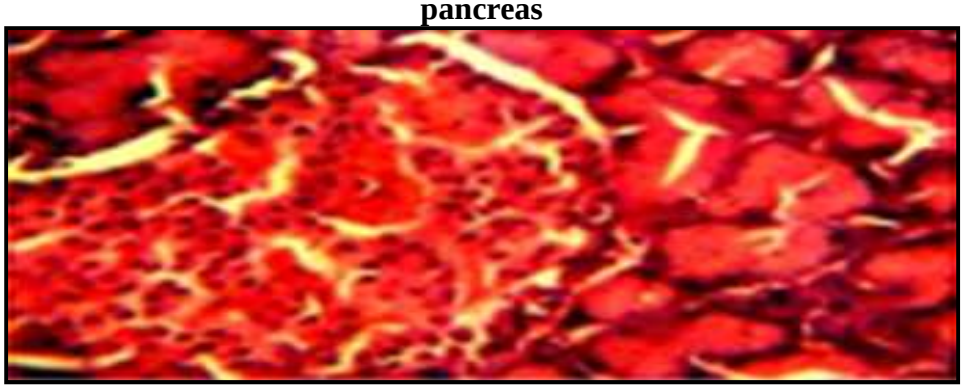

Figure No.3: Pancreas treated with standard (Glibenclamide10mg/kg) H and E Staining (40X) Section shows pancreas with mild damage

Figure No.4: Pancreas treated with test drug (piperine 50mg/kg) H and E Staining (40X) Section shows increased size of islets

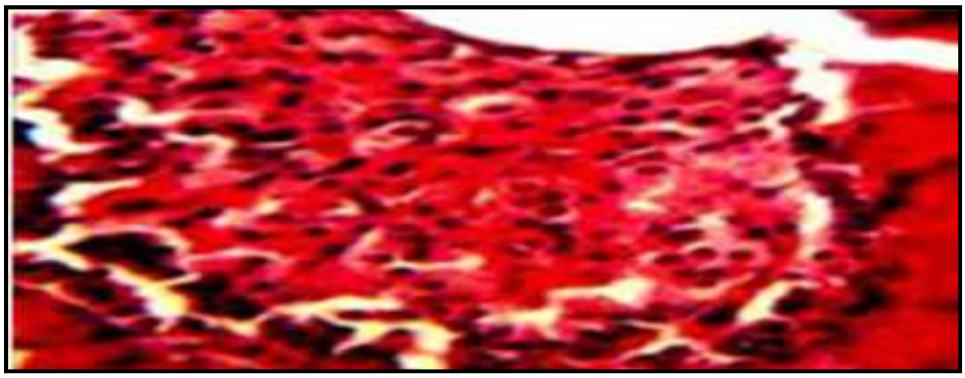

Figure No.5: Pancreas treated with test drug 1 (piperine100mg/kg) H and E Staining (40X) Section shows increase in granulated and normal beta cells

CONCLUSION

The root *Daucuscarata* (Roxb.) belongs to family Araceae is known as Carrot in Telugu. The findings of antidiabetic study support the traditional use of *Daucuscarata* fruit for controlling hyperglycemia in diabetics. Further characterization of active principles flavonoids, alkaloids, tannins in Scindapsus and studies are in progress to isolate, identify and characterize such active components.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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