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ENHANCED GLYCEMIC CONTROL, PANCREAS PROTECTIVE, MODULATED CARBOHYDRATE METABOLIC ENZYME ACTIVITIES BY ZINGERONE IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: To investigate the glycemic control, pancreas protective, modulated carbohydrate metabolic enzyme activities by Zingerone in STZ induced diabetic rats by administering oral doses (10mg/kg body weight). **Methods:** Blood glucose levels were measured using Liqui CHEK glucose assay kit (Agappe Diagnostic Ltd., India) at weekly intervals (i.e. 0, 7, 14, 21 and 28 days) till the end of study. Serum insulin levels were measured by Enzyme Linked Immuno Sorbent Assay (ELISA) using the rat insulin ELISA kit (BioGenes GmbH, Germany). Carbohydrate metabolic enzymes hexokinase, glucose-6-phosphatase, glucose-6-phosphate dehydrogenase and fructose-1,6-bisphosphatase were evaluated. Histopathological changes in diabetic rat organ (pancreas) were also observed after the 30 days treatment. **Results:** Diabetes mellitus was induced by a single intraperitoneal administration of streptozotocin (STZ) (40 mg/kg body weight). Five days after STZ administration, diabetic rats received zingerone (10 mg/kg body weight) orally for 30 days. Metformin (Met) was used as reference drug. Zingerone treatment significantly reduced blood glucose level with an increase in the level of insulin. The zingerone-treated diabetic rats sustained their initial weights during the treatment period. We observed increase in the activity of carbohydrate metabolizing enzymes including

hexokinase, glucose-6-phosphate-dehydrogenase and glycogen content in liver of Zingerone treated rats with reduction in the levels of glucose-6-phosphatase and fructose-1,6-bisphosphatase. **Conclusion:** These findings substantiated the beneficial effects of Zio in the treatment of diabetes through exhibiting antihyperglycemic effects as well as restoring the function of pancreas. Thus, zingerone may have the potential in managing the effects of diabetic complications in human subjects.

1. INTRODUCTION

Diabetes mellitus is the heterogeneous chronic metabolic disorder that rises to an alarming epidemic level, and it is considered to be the biggest health catastrophe globally with escalating morbidity and mortality rates.[1] The global prevalence of diabetes was 382 million in 2013 and is expected to rise to 592 million by 2035.[2] The disease arises either from a deficiency of pancreatic β-cells or insulin resistance resulting in hyperglycemia affecting essential biochemical pathways such as carbohydrate, protein and lipid metabolism in the body leading to the development of complications including renal disorders, neuropathy, retinopathy, ketoacidosis, and cardiovascular diseases.[3] The treatment objectives include prevention of imminent mortality, alleviate symptoms and morbidity, and normalize glucose levels with the intent of averting diabetic complications. Current commercial oral hypoglycemic drugs such as sulphonylureas, thiazolidinediones, and bioguanides instigate undesirable side effects that include hypoglycemia, diarrhea, hypertension, hypercoagulability, lactic acidosis, hepatotoxicity, and dyslipidemia.[4] Due to the concerns of synthetic antidiabetic drugs such as adverse effects, cost and lack of cure, it is essential to search for novel therapeutic agents, preferably from natural sources. World Health Organization (WHO) is recommending the use of complementary and alternative approaches in combating diabetes through the utilization of herbal remedies due to their natural origin and non-toxicity.[5&6]

Medicinal plant based drug discovery continues to provide new leads against various pharmacological targets including diabetes as well as dietary supplements to existing therapies.[7] Wide array of plant-derived extracts as well as active principles has shown antidiabetic activity.[8-11] The main phytoactive constituents such as alkaloids, terpenoids, glycosides, steroids and polyphenols are reported to exhibit the following beneficial activities: manipulating the carbohydrate metabolism by multiple mechanisms to maintain glucose homeostasis, preventing and restoring integrity and functioning of pancreatic cells,

insulin release, improving glucose uptake and utilization, and antioxidant properties.[12-16]

Ginger, the rhizome of the plant Zingiber officinale, is a herbal dietary spice indigenous to India and its use has spread to most of the inhabited world due to the potent anti-inflammatory, antioxidative, antiarthritic, antithrombotic, anticancer, hypolipidaemic properties.[17-19] The herbal properties of ginger are similar to non-steroid anti-inflammatory drugs (NSAIDs), and hence, it can regulate biochemical pathways which are activated with chronic inflammation such as diabetes.[20] Ginger phytochemicals, upon oral consumption, are readily absorbed into the body where they can exert various activities and excreted after 48 to 60 h.[21] Various reports are available on the use of different preparations of ginger for antidiabetic property. A systematic review reported the analysis of randomized clinical trials (RCTs) conducted on type-2 diabetic human patients for examining the efficacy of ginger preparations against diabetes.[22] However, these studies ascribe the antidiabetic efficacy of ginger to the synergistic effects of phenolic phytochemicals mainly gingerols and their related dehydrated products, the shogaols and zingerone and it is not known that which bioactive compound of ginger is predominantly responsible for antidiabetic activity. Ginger extracts possess strong antioxidant radical activities as evidenced by the ABTS assay .Both aqueous and ethanol extracts of ginger have significant natural antioxidant activity. Therefore, consumption of ginger might be helpful in combating the progression of various diseases with oxidative stress components such as atherosclerosis, diabetes mellitus among others.[23] Further, there is no report on the antidiabetic effects of zingerone (4-(4-hydroxy-3-methoxy phenyl) butan-2-one), which is a stable active component of dry ginger rhizome and is known to have wide ranging pharmacological activities such as hepatoprotective[18], anti-oxidant[25], anti-inflammatory[25], antidiarrhoeal[26], antimicrobial[27], immunostimulant[28], and anticancer[29]

The present study is aimed to investigate the efficacy of zingerone for the antidiabetic properties in STZ-induced diabetic rats treated through oral administration for 30 days. Antidiabetic properties were validated by analyzing blood glucose, insulin levels, and carbohydrate metabolic enzymes activity, histological, immunohistochemical, & Scanning electron microscopy (SEM) studies on pancreas.

2. MATERIALS AND METHODS

2.1 Chemicals

Zingerone and streptozotocin were purchased from Sigma-Aldrich, St Louis, MO. Anti-insulin monoclonal antibodies and rat insulin ELISA kit were purchased from BioGenes GmbH, Germany. Glucose kits were purchased from Agappe Diagnostics Ltd., India. All other chemicals were obtained from Hi Media (Mumbai, India) and SD Fine Chemicals Limited (Mumbai, India).

2.2 Animals and Diet

Wistar albino male rats, weighing about 150-250 g, were obtained from King Institute of Preventive Medicine and Research, Chennai and maintained at animal house of Entomology Research Institute (ERI), Loyola College, Chennai. They were maintained under a constant 12 h light and dark cycle at 22–24 ^{o}C and at 45%-55% relative humidity in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. The study was approved by the Institutional Animal Ethical Committee (833/a/04/CPCSEA), Loyola College. Throughout the experimental period, the animals were fed with a balanced commercial pellet diet (protein, 21%; fat, 5%; nitrogen-free extract, 55%; fiber, 4%; adequate mineral and vitamin contents, 15%; Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

2.3 Experimental Induction of Diabetes

Rats were induced diabetes with an intraperitoneal injection of STZ ((40 mg/kg bw) freshly prepared in 0.1 M sodium citrate buffer) after overnight fasting [30]. The rats exhibited diabetes after 5 days (*i.e.*, fasting blood glucose concentration, >300 mg/dL) and were selected for the treatment with zingerone and with reference drug metformin separately.

2.4 Experimental Procedure

A total of 30 animals (6 normal and 24 diabetic rats) were used in the experiment. The rats were divided into the following 5 groups of 6 rats each: Normal control (Group I); Diabetic control (rats induced with STZ) (Group II); STZ-induced diabetic rats treated with zingerone (10 mg/kg bwt) orally for 30 days (Group III); STZ-induced diabetic rats treated with metformin (50 mg/b wt) orally for 30 days (Group IV); and Zio control (10mg/kg bwt) orally Zio only for 30 days (Group V) Animals were monitored for general health during the treatment period. No death of the animals was observed till the end of the study. At the end of the experimental period and after one day of last zingerone administration, the animals were

deprived of food overnight and sacrificed by decapitation. Blood was collected and serum was separated for the estimation of insulin and other biochemical parameters. Tissues such as pancreas & liver were dissected out, washed in ice-cold saline, patted dry, weighed snap-frozen in liquid nitrogen, and finally preserved at -80°C until further analysis.

2.5 Analytical Assays

Blood glucose levels were measured using Liqui CHEK glucose assay kit (Agappe Diagnostic Ltd., India) at weekly intervals (i.e. 0, 7, 14, 21 and 28 days) till the end of study. Serum insulin levels were measured by Enzyme Linked Immuno Sorbent Assay (ELISA) using the rat insulin ELISA kit (BioGenes GmbH, Germany). Carbohydrate metabolic enzymes hexokinase[31] glucose-6-phosphatase[32], glucose-6-phosphate dehydrogenase[33], and fructose-1,6-bisphosphatase[34] were evaluated.

2.6 Histopathological studies

The pancreas tissues obtained from all the experimental groups were washed immediately with saline and then fixed in 10% buffered neutral formalin solution for 24 h. The organs were dehydrated with a graded series of ethanol and embedded in paraffin wax. Sections of 5 µm were cut using a microtome (Leica RM2255 Rotary Microtome, USA), mounted on glass slides, stained with hematoxylin and eosin (HE) and photographed by microscope (Carl Zeiss, USA). The number and size of islets of Langerhans in pancreas were measured in 400x power fields.

2.7 Immunohistochemical studies on pancreas

Paraffin sections of 5 µm thickness were placed on microscope slides that had been sealed with poly-L-lysine (Sigma, USA) followed by fixing of tissue sections in a 37 ^{o}C oven for 1 h. Then, the tissues were deparaffinized by rinsing in xylene, dehydrated with absolute alcohol and finally washed with phosphate buffer saline (PBS) and distilled water. The tissues sections were then incubated for 15 min in 3% H_2O_2 in methanol to quench the endogenous peroxides. The sections were then washed in PBS for 5 min and excess PBS was wiped from around the tissues. The sections were blocked by incubating with diluted normal serum for 30 min and excess serum was blotted from the sections. For detection of insulin, sections were incubated with primary antibody (Mouse monoclonal anti-insulin antibody from BioGenes GmbH, Germany) diluted to 1:1000 in PBS for 60 min, and sections were washed with PBS for 5 min. Excess PBS was wiped from the slides. Then, the sections were processed by an indirect immunoperoxidase technique using a HRP detection kit (BioGene

GmbH, Germany) with secondary antibodies using hematoxylin as the counterstain. Evaluation of immunohistochemical staining was made by examination of 10 islets for each group of rats using a Confocal Scanning Microscope (Carl Zeiss, Germany).

2.8 Scanning Electron microscopy (SEM)

The differentiated ICAs were incubated in 2.5% phosphate buffer (0.1 M, pH 7.4) glutaraldehyde solution for 24 hrs. The tissue was post fixed in 1% OsO4 for 2 h at 4uC, dehydrated and dried. Specimens were then glued onto stubs, covered with gold in an S150 sputter coater and examined with CEG 15.0 kV 10.7 mm X200, X500 BSE3D scanning electron microscope operating at 15. 0 kV. (Anna university, Chennai).

2.9 Statistical analysis

All data are given as mean ± SD (standard deviation). Statistical analysis was performed with past (version 3) several sample tests (ANOVA, kru - wal) followed by Tukey's pairwise test for multiple comparisons. Values of $p<0.05$ were considered significant.

3. RESULTS

3.1. Effect of Zingerone on blood glucose level

The experimental rats showed a normal basal blood glucose level before the administration of STZ. After 5 days of STZ administration, the rats showed a significant increase ($p < 0.05$) in the blood glucose level. Oral administration of Zio (10 mg/kg bwt) reduced blood glucose levels in diabetic rats to almost the same degree as metformin (50mg/kg bwt) (Fig.1). The control rats showed a stable blood glucose level throughout the course of the study and there is no significant modulation in blood glucose level of Zio treated control rats (10 mg/kg bwt).

Figure: 1 Blood glucose was calculated 0, 7, 14, 21, 28 days intravel in normal control and experimental groups.

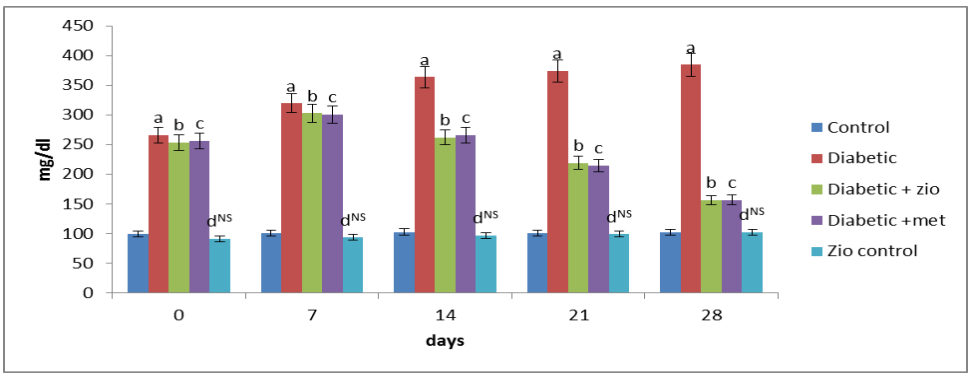

Figure 1: Blood glucose was calculated 0, 7, 14, 21, 28 days intravel in normal control and experimental groups.

Data are expressed as mean ± SD for six rats in each group. Values not sharing a common superscript letter (a-c) differ significantly at $P < 0.05$ (Tukey's pairwise test) NS- not significant. a-group I& II, b-group II& III, c-group II & IV, d-group I & V.

3.2 Effect of zingerone on body weight of STZ-induced diabetic rats

The body weight of rats was recorded every week and the data are shown in Fig. 2. Body weight was significantly reduced in the diabetic control group when compared to the normal untreated control and treated groups. Though there was no significant difference observed between zingerone and metformin treated groups, the treated groups showed slight body weight reduction when compared to control group and this could be due to induction of diabetic condition. The diabetic animals continued to lose weight till the end of the study while zingerone administration for 30 days showed significant improvement in the gain of body weight when compared with diabetic group.

Table 1: Animal body weight was calculated 0, 7, 14, 21, 28 days intravel in normal control and experimental groups.

		Body weight (g)			
Groups/Days	**0**	**7**	**14**	**21**	**28**
Control	179.66±2.86	186.66±2.98	195.16±2.54	203.3±3.59	213.83±3.02
Diabetic	170.33±2.86	165.83±3.28 [a]	161.66±2.74 [a]	155.16±2.91 [a]	146.66±2.62 [a]
Diabetic + zio	173.83±2.26	168.83±2.26 [b]	173.66±4.64 [b]	179.83±3.23 [b]	188.33±4.45 [b]
Diabetic +met	171.83±2.26	169.33±1.97 [c]	174.50±2.81 [c]	179.33 ±2.42 [c]	186.33±4.37 [c]
Zio control	170.5±3.40	176.83±4.05 [d]	182.83±4.13 [d]	188.66±4.18 [d]	198.83±3.28 [d]

Data are expressed as mean ± SD for six rats in each group. Values not sharing a common superscript letter (a-d) differ significantly at $P < 0.05$ (Tukey's pairwise test). a-group I& II, b-group II& III, c-group II & IV, d-group I & v.

3.3 Effect of Zingerone on serum insulin level

STZ caused a significant decrease ($p < 0.05$) in the serum insulin level and administration of Zio (10 mg/kg bwt) for 30 days considerably increased the level of insulin at the end of the study when compared with the diabetic control rats. In addition, there was no significant difference observed in the level of insulin between the normal and Zio control rats (Fig. 2). Five days after STZ administration, all animals have displayed a moderate loss of body weight. The changes in body weight in normal and experimental diabetic rats are presented in (Table 1). STZ produced significant loss in body weight as compared to normal rats during the study. Diabetic control continued to lose weight until the end of the study while Zio administration (10 mg/kg bw) for 28 days showed significant improvement in body weight

compared to diabetic control group. There was no significant difference between the Zio and metformin-treated groups.

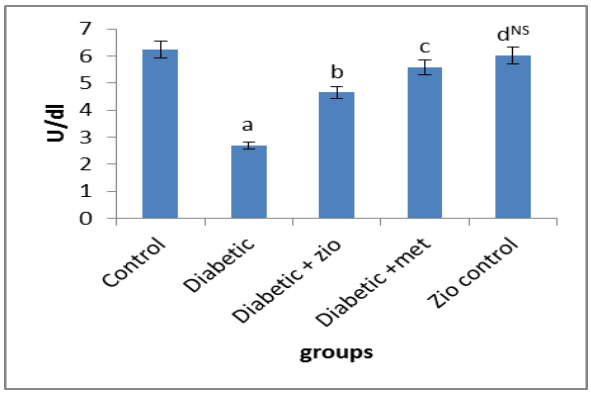

Figure 2: Effect of Zio on serum insulin level.

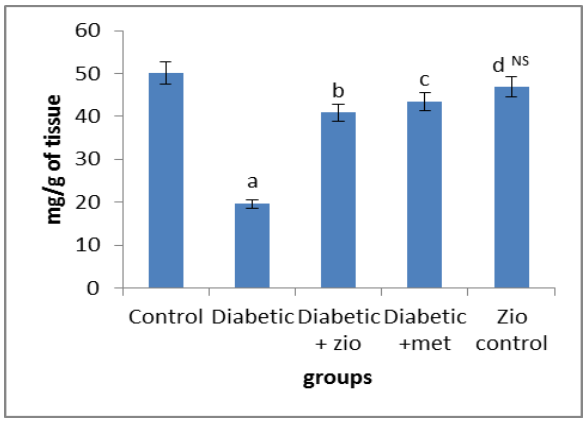

Figure 3: Effect of Zio on liver glycogen level.

Data are expressed as mean ± SD for six rats in each group. Values not sharing a common superscript letter (a-c) differ significantly at $P < 0.05$ (Tukey's pairwise test), NS-not significant. a-group I& II, b-group II& III, c-group II & IV, d-group I & v.

3.4 Effect of Zingerone on carbohydrate metabolic enzymes

The effect of Zio on carbohydrate metabolic enzymes in liver of normal, STZ-induced diabetic and treated rats are represented in table 2. The activity of hexokinase and G6PD was significantly decreased ($p < 0.05$) in liver of the diabetic rats. These enzyme activities were restored in Zio treated rats (10 mg/kg bwt) to that of the normal and were comparable to metformin treated rats. On the other side, the activities of G6P and F1,6 BP were significantly increased ($p < 0.05$) in the liver of diabetic rats when compared to control and Zio control rats. These enzyme activities were inclined to decrease to normal level in Zio treated and metformin rats. All these enzyme levels were found to be normalized after Zio treatment and were comparable with metformin.

Table 2: Changes in the activity of Hexokinase, glucose-6- phosphatase, glucose-6-phosphate dehydrogenase, fru-1,6 bisphosphatase in liver of normal control and experimental groups.

Groups	Hexokinase (units[#]/g protein)	Glucose-6-phosphatase (units[#/] mg protein)	Glucose-6-phosphate dehydrogenase (×10−4 IU/mg protein)	Fru-1,6 bisphosphatase (units[$]/mg protein)
Control	103.25±7.49	0.171±0.04	4.46±0.16	0.326±0.06
Diabetic	74.57±6.78 [a]	0.341±0.11 [a]	1.11±0.15 [a]	0.664±0.11 [a]
Diabetic + zio	91.03±3.15 [b]	0.182±0.03 [b]	2.61±0.10 [b]	0.361±0.04 [b]
Diabetic +met	92.93±3.64 [c]	0.196±0.03 [c]	3.45±0.09 [c]	0.349±0.07 [c]
Zio control	102.34±4.39 [d NS]	0.174±0.03 [d NS]	3.98±0.46 [d NS]	0.334±0.05 [d NS]

[#] Unit – µmoles of glucose phosphorylated per minute.

[$] Unit – µmoles of inorganic phosphate liberated per hour

Data are expressed as mean ± SD for six rats in each group. Values not sharing a common superscript letter (a-c) differ significantly at $P < 0.05$ (Tukey's pairwise test), NS-not significant. a-group I& II, b-group II& III, c-group II & IV, d-group I & v.

3.5 Effect of zingerone on liver glycogen levels

Glycogen content in liver was decreased significantly ($p < 0.05$) in STZ treated diabetic rats compared to control rats. Administration of Zio for 30 days resulted in considerable increase in liver glycogen levels. Among that, Zio showed a significant increase in glycogen level whereas metformin and Zio control rats showed a normal liver glycogen level (figure 3).

3.6 Histopathological examination of pancreas

Histopathological sections of endocrine structures of pancreas of the STZ-induced diabetic rats showed beta cell hyper atrophy with a significant reduction in the number of islet cells as well as the size of islets due to necrosis. Nuclear changes, disappearing of nucleus and the residue of destructed cells were also seen in diabetic pancreas. Whereas the islets from the normal rats showed normal morphology with optimal number of β-cells distributed throughout the islet. It is noteworthy that the pancreas from zingerone treated diabetic rats showed recovered morphology with increased granulation, viable islets of Langerhans, regenerated β-cells and restoration of islet size when compared to that of the diabetic groups.

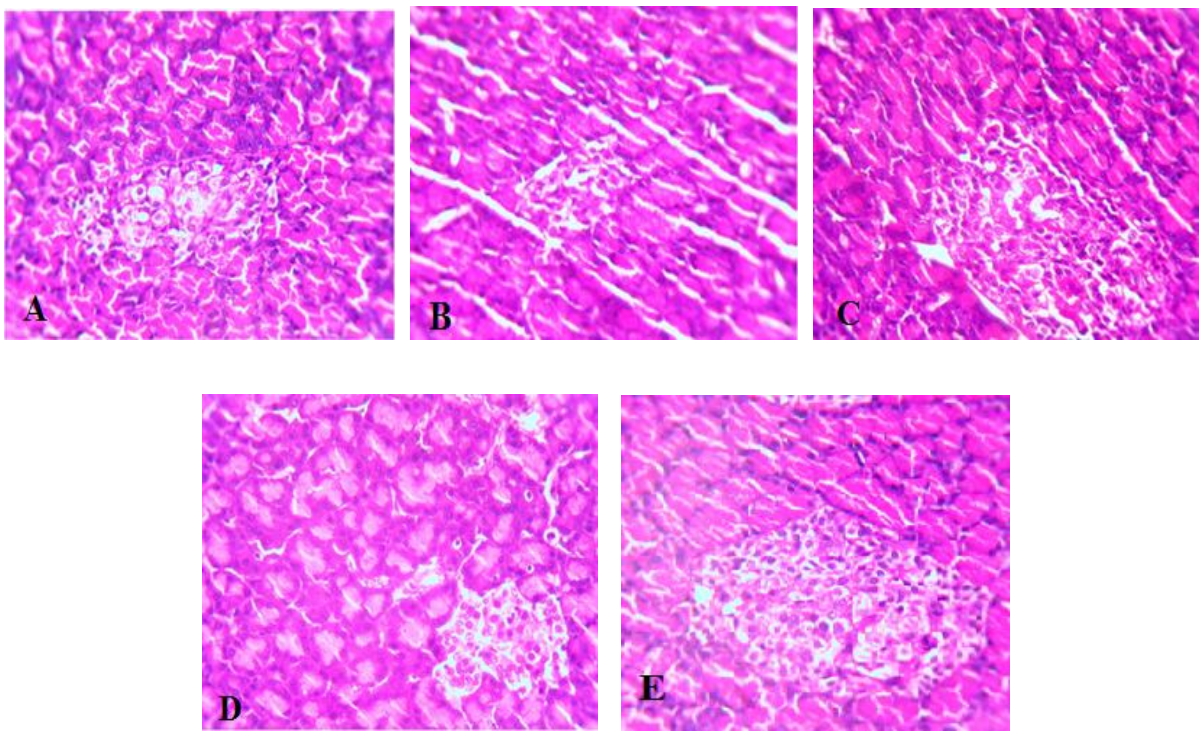

Figure 4: Histology of Pancreas.

Histopathological observations of Zio and metformin treated pancreas in STZ-induced diabetic rats after 30 days of treatment (H&E staining, 400×). (A) Normal control – presence of normal pancreatic islet cells; (B) Diabetic control – reduction in the size of islets, damaged

β-cell population and extensive necrotic changes followed by fibrosis and atrophy; (C) Diabetic + Zio 10mg/kg bw- restored necrotic and fibrotic changes and increased number and size of the islets; (D) Diabetic + Metformin 50 mg/kg bw) – absence of necrosis and fibrotic changes, increased number and size of the islets. (E) Zio control – presence of normal pancreatic islet cells.

3.7 Immunohistochemical studies on regeneration of β-cells in STZ-induced rats pancreas by zingerone treatment

Immunohistochemical studies of pancreas of normal rats revealed large area of islets of immunoreactive for insulin indicating the presence of cluster of viable β-cells. Similarly, pancreas of zingerone and metformin treated diabetic rats showed large area of intense insulin immunoreactivity and this suggested that the insulin secretion potential of β-cells in diabetic treated rats was restored to normal state. On contrary, pancreas of diabetic rats displayed fewer insulin-positive cells in the structurally deformed islets.

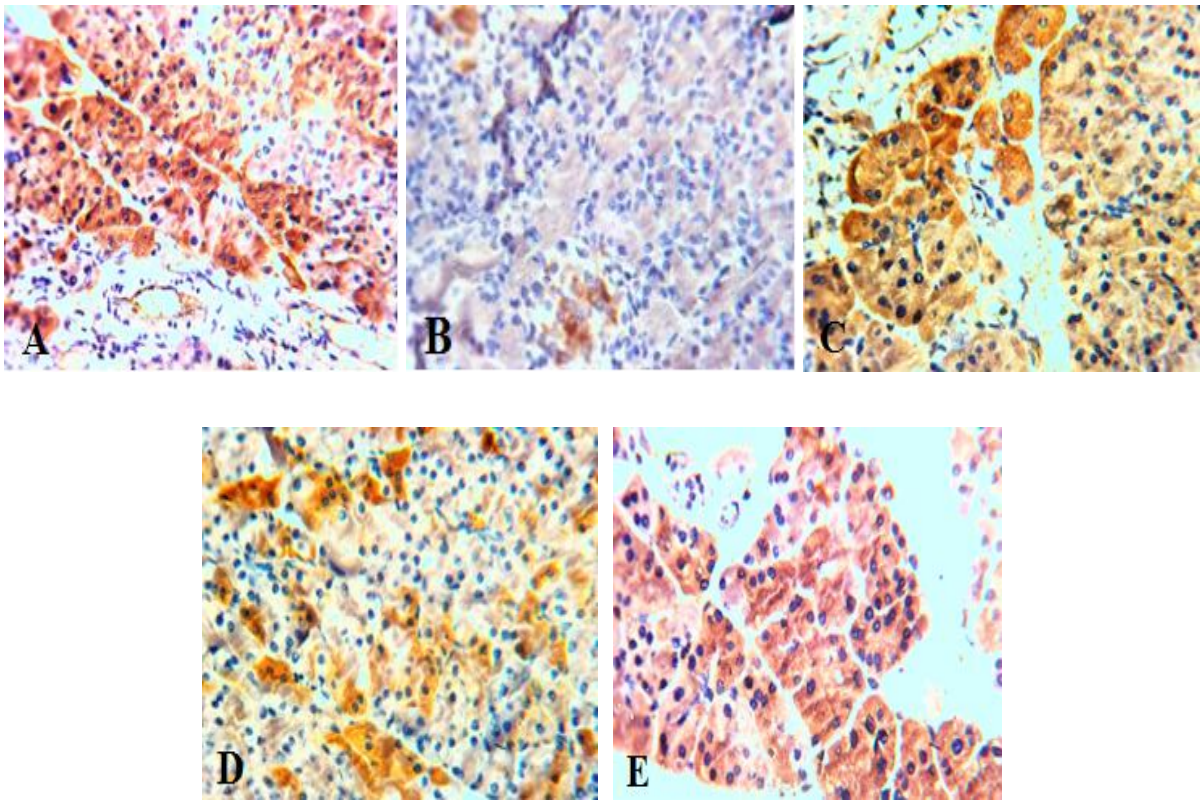

Figure 5 Immunohistochemistry of Pancreas.

Immunohistochemical analysis of insulin secreting cells in pancreatic islets of normal and experimental rats (400×): (A) Normal control – presence of normal structure of islets and β-cells; (B) Diabetic control – decrease in insulin immunoreactivity and number of

immunoreactive β-cells; (C) Diabetic + Zio – increase in insulin immunoreactivity and number of immunoreactive β-cells; (D) Diabetic + metformin – increase in insulin immunoreactivity and number of immunoreactive β-cells; (E) Zio control – presence of normal structure islets and β-cells.

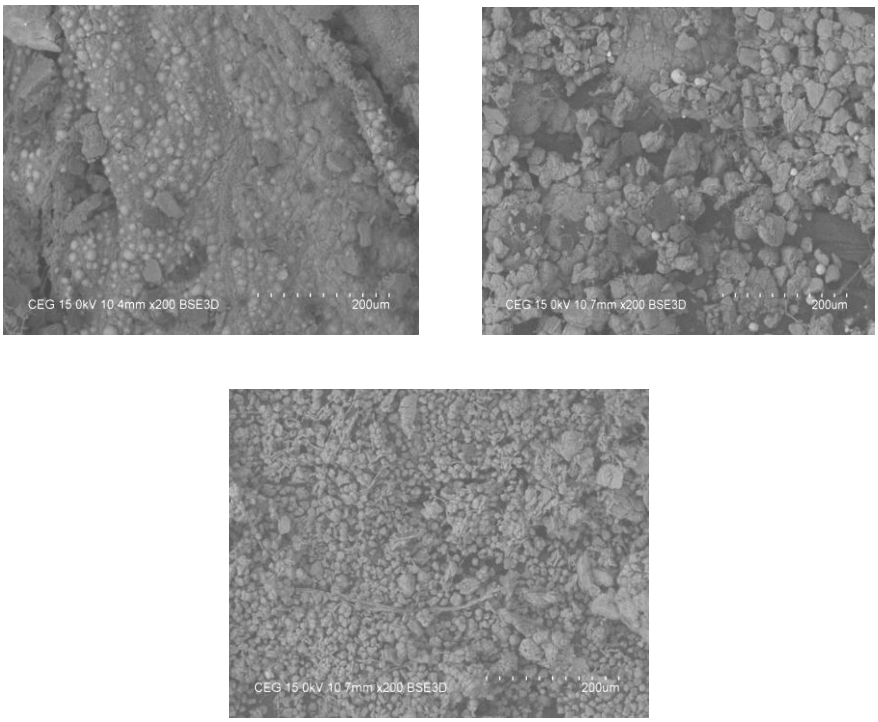

Figure 6: SEM image of pancreas- (A) 500X.

Control Diabetic Diabetic + Zio. Not in single line

1st- image-control

2nd image-Diabetic

3rd image-Diabetic+Zio

In SEM image was expressed in Normal control – presence of normal structure of pancreatic islets (B) Diabetic control – decrease and destroy the pancreatic islets (C) Diabetic + Zio – increase the number and regenerate the pancreatic islets.(Fig: 500X).

DISCUSSIONS

Zingerone have vanilloid[3-methoxy-4-hydroxy benzene] group in structural moiety and due to presence of hydroxyl group in zingerone structure, it belongs to antioxidant phenolic compounds. Zingerone has been shown to have wide range of pharmacological properties including antioxidant[35], anti-inflammatory[36], anticancer[29] and antimicrobial activity.[27] Studies regarding metabolism of zingerone showed that oral or intraperitoneal administration of zingerone resulted in side chain oxidation at all the available sites that led to excretion of

glucuronide and sulphate conjugates in urine with in 24 hour after consumption, without any side effects to vital organs.[37]

In the normal system insulin regulates the carbohydrate metabolizing enzymes in order to maintain glucose homeostasis. Hyperglycemia was characterized with the impairment in insulin secretion with excessive hepatic glycogenolysis and gluconeogenesis; and decreased glucose uptake by the tissues.[38] STZ is widely used for the induction of diabetes mellitus in animals and well-documented model of experimental diabetes.[39] It has been clearly demonstrated that the type of diabetes and characteristics differ with the employed dose of STZ and animal and species used.[40] STZ is a pancreatic β-cell toxin that induces rapid and irreversible necrosis of β-cells and increases the generation of free radicals thereby the result hyperglycemia. Clinically, symptoms of diabetes are clearly seen in rats within 5 days following single intravenous or intraperitoneal injection of 40 mg/kg STZ[41] Moreover, intraperitoneal injection of STZ led to physiologic alterations consistent with reports of spontaneous and chemically induced diabetes in other animals.[42]

Liver is vital organ that play an important role in defense of the postprandial hyperglycemia and involved in the glucose metabolism (synthesis of glycogen). In liver, glucose is converted into glucose-6-phosphate by the help of hexokinase.[43,44] STZ induced diabetic rat decreased glycolysis, disturb the capacity of the liver to synthesize glycogen and decreased the level of hexokinase. The decreased level of hexokinase showed an effect on glycolysis and inhibit the utilization of glucose for energy production.[45] The STZ induced diabetic rats treated with Zio brought back the activity of this enzyme near to normal control and increases the utilization of glucose for energy conversion. Another liver vital enzyme is glucose-6-phosphatase which regulates the glucose metabolizing enzyme. In STZ induced diabetic rats increased level of glucose-6-phosphatase boost the production of fats from carbohydrates and increased the fats deposition in the liver and kidney.[46] Some investigators claim that increased level of glucose-6-phosphatase enhanced the activity of a gluconeogenetic enzyme.[47] STZ induced diabetic rats treated with Zio had brought back the activity of glucose-6-phosphatase enzyme near to normal control.Glu-6-P-D maintains blood glucose level and reported as the principal source of intracellular reductant. Glu-6-P-D plays an important role in β-cell function and its survival. Hyperglycemia resulted in the decrease of Glu-6-P-D activity which may be one of the reasons for the gradual loss of β-cells.[48] Treatment with Zio increased the activity of Glu-6-P-D in hepatic tissues thereby increasing

glucose utilization through the pentose phosphate pathway. Fructose-1-6-biphosphatase is the vital enzyme of the liver plays an important role in the glycolysis, its convert glucose into the energy.[49] STZ induced diabetic rats increased the level of fructose-1-6-biphosphate. Zio treated rats decreased the level of fructose-1-6- biphosphatase near the normal control rats. In diabetes mellitus, the normal capacities of the liver glycogen synthesis were reported to be impaired due to the inactivation of glycogen synthetase system.[50] The decrease in hepatic glycogen was observed in this study which may be due to lack of insulin. The treatment with Zio for 30 days significantly increased liver glycogen level indicating that it may be due to the enhanced glycogenesis and glycolysis by normalization of insulin secretion.

Histopathological examination of pancreatic islet also showed degenerated and necrotic cells in the pancreatic islets of diabetic rats. While administration of Zio was significantly alleviated these abnormalities in the pancreatic islets of treated diabetic rats. Chronic hyperglycemia causes pancreatic islets destruction, leading to pancreatic dysfunction and development of diabetes.[51-54] Diabetes is also associated with oxidative stress that plays an important role in the development of diabetes complications.[55] and cause a variety of destruction of pancreatic ß-cells.[56] In support of this association, ß-cell death and a reduction in number of islets were observed in pancreas of diabetic rats.[57]

Dietary phytochemicals particularly ginger rhizome has received the attention of scientist due to its non-toxic, safe and potent activity.[58] Interestingly, few recent studies have demonstrated the potential of zingerone in attenuating oxidative and cytogenetic damage induced by radiations. It has been documented that zingerone neutralized radiation-induced free radicals and also showed antioxidant potential in vivo.[59] Zingerone was also found to exert antiapoptotic, anti-genotoxic, and anti-inflammatory activity and free radicals scavenging potential.[60] In general, these results suggested that the Zio would control the pancreatic ß-cell damage and it has hypoglycemic activity.

Scanning electron microscopy based ultrastructural comparison of these 3D ICAs revealed that their surface topography and arrangement between cells is very similar to that of normal pancreatic islets.[61] The distribution of cellular constituents within rat islets is typically represented with α, δ and pancreatic polypeptide (PP) cells peripherally arranged around a central core of β cells.[62] Our observations showed a normal pancreatic islets are found in control rats, more serious damage in pancreatic islets on diabetic (STZ) induced rats, and

Diabetic with Zio treated groups also showed a developing and improvement of pancreatic islets.

In conclusion, the result of the present study indicate that the administration of Zingerone to diabetic rats gives good control over tissue glycogen content by enhancing the peripheral utilization of glucose and correcting the impaired liver glycolysis and by limiting its gluconeogenic formation similar to insulin. This suggests the efficacy of Zingerone in the maintenance of glucose homeostasis and may be used as a therapeutic agent in the management of diabetes mellitus. There is a first report for the Zingerone has managed diabetes mellitus and product the pancreas, liver and other organs.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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