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Research Article

Cytoprotective Effects of *Lepidium sativum* Seed Extract on Liver and Pancreas of HFD/STZ Induced Type 2 Diabetic Mice

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ABSTRACT

Type II diabetes mellitus (TIIDM) is the world's largest endocrine disorder. Obesity is one of the leading causes for type II diabetes. In the present study antihyperglycemic and cytoprotective role of Lepidium sativum seed extract (LSE) for obesity associated diabetes in normal and high fat diet (HFD)-streptozotocin induced mice was investigated. Blood glucose, histology of liver and pancreas and body weight in obese diabetic mice was evaluated. Administration of LSE for 28 days significantly lowered blood glucose while increased body weight and recovered degenerative changes in liver and pancreas. These findings suggest that LSE possess antihyperglycemic and cytoprotective action and might be a good candidate for obesity associated diabetes.

Keywords:

INTRODUCTION

Diabetes mellitus (DM) is one of the major health problems in the world. There is increased number of adults with DM in the world, and it will goes to 300 million by the year 2025. The major part of this numerical increase will occur in Asia mainly China and India[1,2]. India continues to be the 'Diabetic Capital' of the world with 50.8 million diabetics. DM is a metabolic disordered characterized by hyperglycemia due to progressive decline in insulin secretion, followed by the inability of β cells to compensate for insulin resistance[3]. Diabetes mellitus especially type 2 diabetes mellitus (T2DM) or non insulin dependent diabetes mellitus (NlDDM) is the most common form of diabetes. More than 90% people are suffering from type 2 diabetes. There are several factors involved in development and progression of T2DM, these are genetic predisposition, aging, obesity and sedentary life style[4] .Various chemicals have been used to induce diabetes in rodents, particularly streptozotocin (STZ), which has been extensively used in diabetes research. The development of hyperglycemia, following STZ injection is primarily due to the direct pancreatic β cell destruction, and resulting insulin deficiency[5,6]. There is a correlation between diabetes and high fat diet (HFD) observed in rodents. In similar lines, mice fed with HFD and injected with STZ, become significantly hyper-glycemic, hyperlipidemic[7,8]. In modern medicine, there is still no satisfactory effective therapy available to cure diabetes without any side effects. Hence in the recent years there is growing interest in herbal medicine all over the world, as they have little or no side effects. Herbs and phytochemicals are known to play a major role in the discovery of new therapeutic agents, and have received much attention as source of biologically active substances including antioxidants, hypoglycemic and hypolipidemic agents[9]. For the present study we have selected seeds *Lepidium sativum* commonly called garden cress is well known traditionally used medicinal herbal plant and possess number of pharmaceutical properties such as hepatoprotective[10], antioxidative[11], anti-inflammatory[12], chemoprotective[13] etc. Keeping such documented in view, the present study has been undertaken to evaluate antihyperglycemic and cytoprotective activity of LSE in liver and pancreas of HFD/STZ induced diabetic mice.

MATERIALS AND METHODS

Preparation of L. sativum seed Extracts

L. sativum seeds were collected from local market of Kolhapur. They were (100 gm) cleaned and ground to fine powder using a grinding machine. Extraction was carried out by soxhlet method. Ethanol was used for extraction for six hrs. The extract was evaporated to dryness under reduced pressure at 60°C by rotary evaporator. Extract was placed in dark bottle and stored at -8°C.

Animals

Three month Swiss albino mice (*Mus musculus*) weighing 30-35gm were used for the present study. Animals were housed in departmental animal house (1825/PO/EReBi/S/15/CPCSEA) in separate cages under proper condition of a 12:12 hr L:D cycle. They had free access to standard rodent pelleted diet (Nutrivet Life Sciences, Pune) and water *ad libitium*.

Experimental design and development of HFD/STZ model of type2 diabetes

Fifteen mice were divided into three groups of five animals each:

Control group

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Table 1: Effect of *Lepidium sativum* seed extract (LSE) on body weight (gm) and blood glucose level (mg/dl) of STZ induced diabetic mice. Values are mean ± S.D. (Numbers in parenthesis denotes number of animals).

Sr No	Treatment (n=5)	Weight of animal (gm)	Statistical significance	Blood glucose (mg/dl)	Statistical significance
1	Control	36.388 ±2.3146	1:2 P<0.01 2:3 P<0.05	101.2 ±2.774	1:2 P<0.01
2	Diabetes	30.178 ±1.5158	1:3 Non significant	276.6 ±40.00	2:3 P<0.01
3	Recovery	34.052 ±2.7375		141± 5.47	1:3 P<0.05

P<0.01=Significant, P>0.05= non significant.

Mice were fed standard diet throughout the experiment and injected with 0.5 ml citrate buffer intraperitoneally (IP), pH 4.5.

Diabetic group (HFD/STZ group)

Mice were fed HFD (40% fat as a percentage of total kcal) for two weeks and then injected with multi low dose of STZ (40mg/kg body wt) intraperitoneally (IP); in citrate buffer; pH 4.5 for five consecutive days[14].

Recovery group (HFD/STZ+LSE group)

Diabetic mice supplemented with LSE (200mg/kg body wt., orally) for 28 days.

The development of hyperglycemia in mice was confirmed by elevated fasting blood glucose (FBG) level after two weeks of STZ injection. The mice having FBG higher than 200mg/dl were considered as diabetic and selected for studies. The LSE treatment was started after diabetes confirmation.

Blood glucose

Fasting blood glucose was measured by collecting a drop of blood from the tail after incision with a sharp blade. The blood glucose level was determined by using a rapid glucose analyzer with a glucose strip inserted in Accuchek blood glucose monitoring glucometer (Roche diagnostics India Pvt. Ltd.). The results were expressed in terms of milligram per deciliter of blood[15].

Statistical Analysis

All values were expressed as mean ±SD. Statistical analysis was carried out by one-way ANOVA, Turkey's HSD test.

Histological examination

After the completion of dose, mice from all groups were sacrificed by cervical dislocation and liver and pancreas were dissected out quickly and fixed in 10% formalin. Tissue were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Sections were cut at 5µ thickness and stained with Hematoxylin-Eosin (HE)[16].

RESULT

Effect of LSE on body weight in HFD/STZ induced diabetic mice

The diabetic mice exhibit a significant loss of body weight (P<0.01) compared to normal group. The administration of LSE showed significant gain in body weight when compared with diabetic group.

Effect of LSE on hyperglycemia in HFD/STZ induced diabetic mice

Fasting blood glucose in control group was within the normal levels and it was significantly increased in diabetic group (P<0.01). After the treatment with LSE for 28 day, there was significant decrease in blood glucose when compared with diabetic group.

Effect of LSE on histological changes in Pancreas of HFD/STZ induced diabetes

Pancreas of control mice (Plate No. I Fig. 1and 2) showed, normal architecture of islets of Langerhans. Diabetic group (Plate No.I Fig 3 and 4), showed shrinkage in size, decrease in number of islets of Langerhans and destruction of cells. Necrotic changes like atrophied and vacuolated cells, loss of cytoplasmic granularity was also observed in Islet cells. After LSE treatment (Plate No. I Fig. 5 and 6) the number of islets was increased and size of each islet of Langerhans was also increased. The islet cells architecture was preserved with minimum pathological changes and showed recovery from necrotic changes.

Effect of LSE on histological changes Liver of HFD/STZ induced diabetes

Control mice liver had normal histology with normal hepatocellular architecture with central vein (Plate No. II Fig 1 and 2). Cytoplasm of hepatocytes stained with pink in color while prominent nuclei appear violet in color. The cells have well defined cell borders, are polygonal and are arranged in sheets. Liver sinusoids were not dilated. In diabetic mice hepatocytes showed (Plate No. II Fig 3 and 4) irregular size, shape and orientation while nucleus was enlarged, displaced and vacuolated. Moderate macrovesicular fatty degeneration of liver with dilated sinusoids was observed. Treatment with LSE restored all these necrotic changes (Plate No .II Fig. 5 and 6) to normal. Hepatocytes had pink eosinophilic cytoplasm without any inclusions and with mostly central single nuclei. These cells, with well defined cell borders, were polygonal and arranged in sheets.

DISCUSSION

Evaluation of plant product in the treatment of DM is become profitable owing to the presence of several bioactive constituents with therapeutic potential. Several researchers are working to study the efficacy of different medicinal plant. Therefore; the present study was aimed to assess the effect of LSE on hyperglycemia and histopathological changes in liver and pancreas of HFD/STZ diabetic mice.

Diabetes mellitus is a complicated group of disorders characterized by hyperglycemia that increase the global

Plate No.1

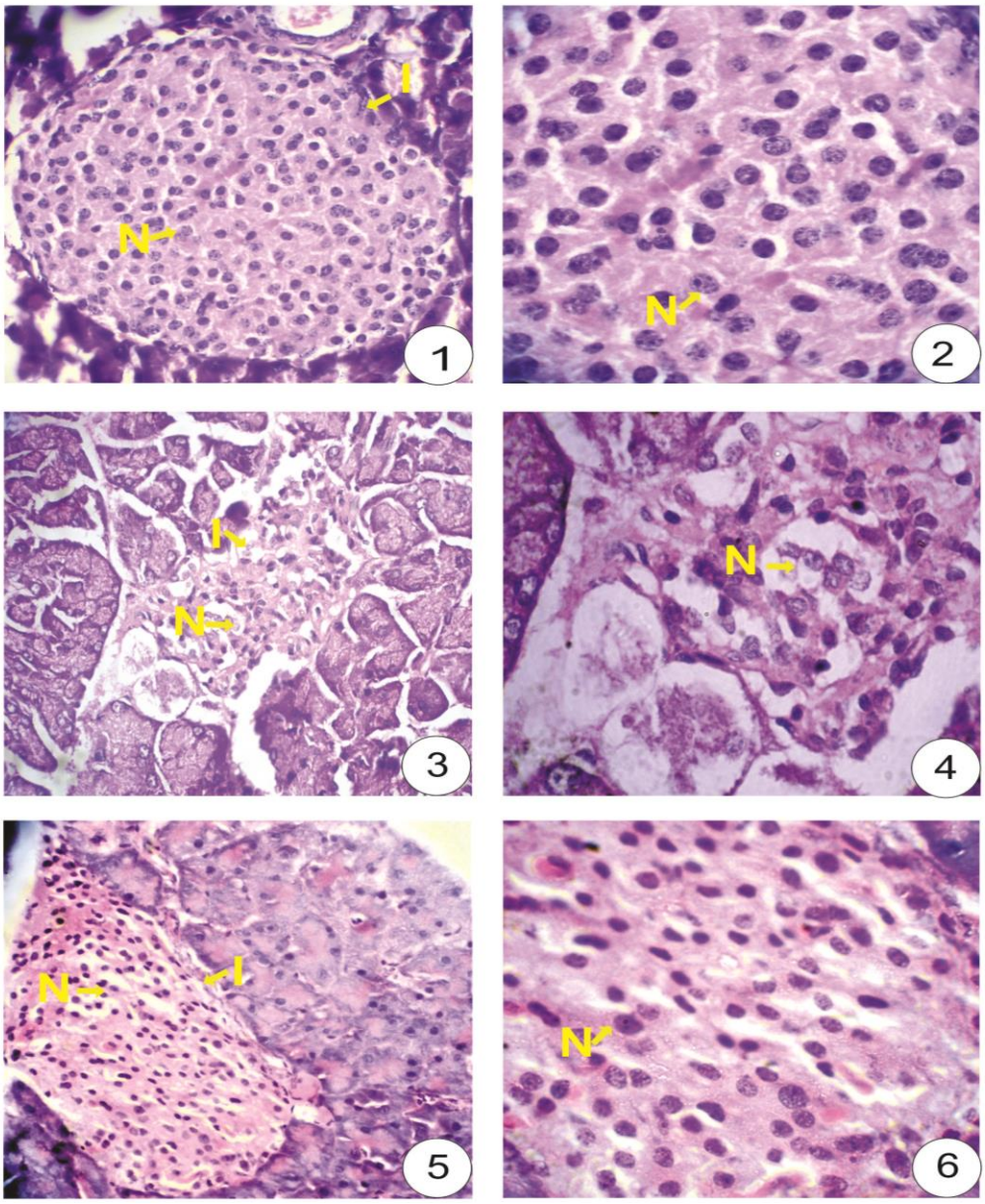

Plate 1: Histopathological changes in pancreas of normal and experimental mice. All the sections stained by HE.
Figure 1 and 2: Control mice pancreas showing normal structure of Islets of Langerhans X 400, X 1000.
Figure 3 and 4: Diabetic mice pancreas showing degenerative and necrotic changes, reduced dimension of Islets of Langerhans X 400, X 1000.
Figure 5 and 6: Diabetic mice treated with LSE showing marked improvement of Islets of Langerhans X 400, X 1000.
Captions: N-Nucleus, I- Islets of Langerhans

prevalence in the present century. HFD-fed mice which are already mildly hyperglycemic, become more susceptible to develop significant hyperglycemia and hyperlipidemic with the diabetogenic effect of STZ[17-18] which are similar to human type 2 diabetes. Oxidative stress is produced under diabetic conditions and possibly causes various forms of tissue damage in patients with diabetes. However, evidences suggest that oxidative stress and free radicals play an important role in the pathogenesis of diabetes mellitus and diabetic complications[19].STZ is a selective β cell cytotoxic agent, enters the cell through glucose transporter causing alkalyation of DNA leading to their necrotic death [20-21]. The STZ diabetic mice exhibited persistent hyperglycemia which is the main diabetogenic factor and contributes to the increase in oxygen free radicals by autoxidation of glucose. Hyperglycemia also generates reactive oxygen species, which in turn, cause lipid peroxidation and membrane damage, also increases oxidative stress in many organs, especially in the liver[22]. Liver is one of the most important organs that maintain blood glucose levels within normal limits. Increase of blood sugar causes imbalance in the oxidation-reduction

Plate No.2

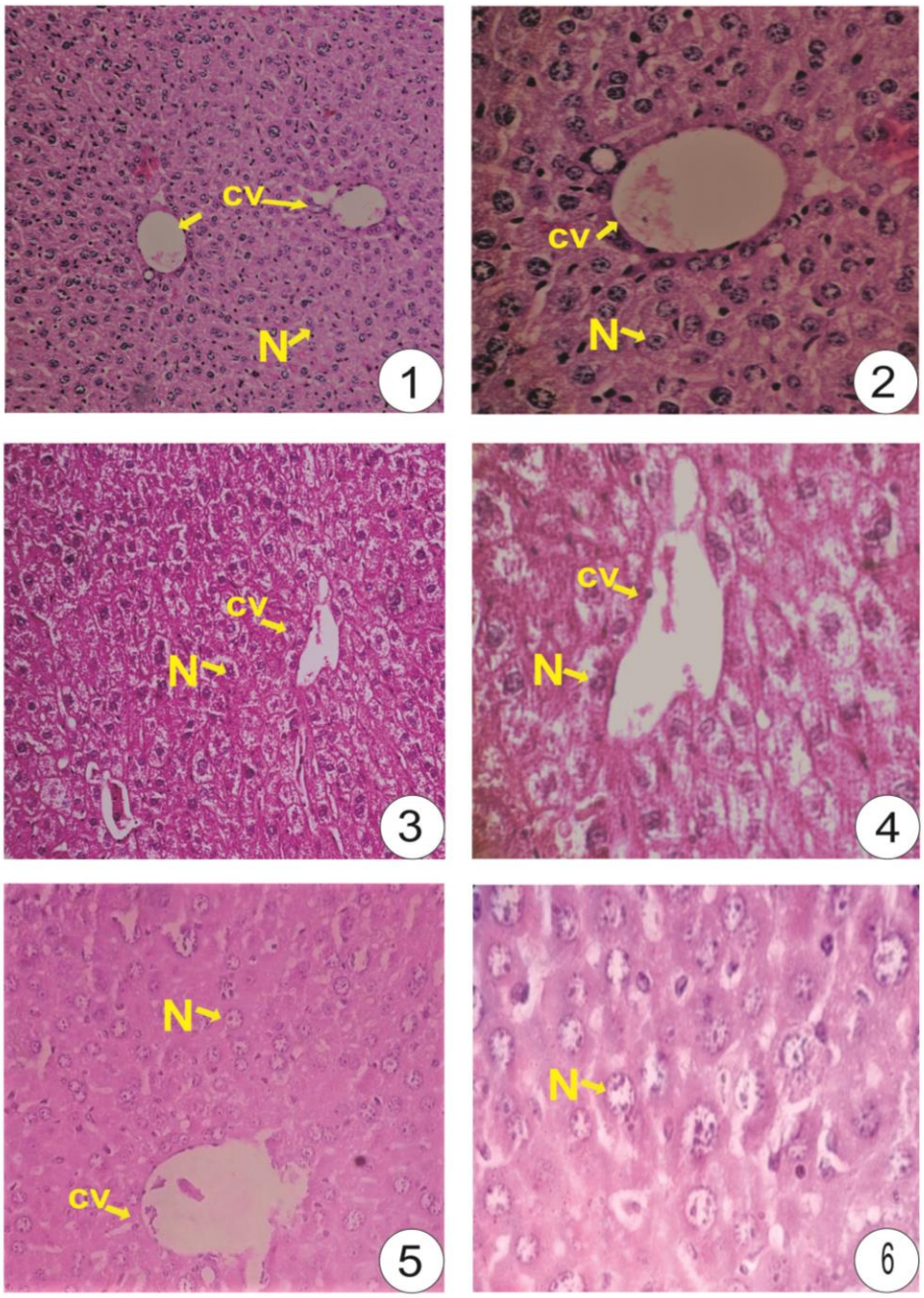

Plate2: Histopathological changes in Liver of normal and experimental mice. All the sections stained by HE.
Figure 1 and 2: Control mice liver shows normal lobular architecture, central vein and normal arrangement of hepatic cords X 400, X 1000.
Figure 3 and 4: Diabetic mice liver shows destroyed architecture, micro and macrovesicular fatty changes, dilation of hepatic sinusoids X 400, X 1000.
Figure 5 and 6: Diabetic mice treated with LSE showing marked improvement in lobular architecture normal blood sinusoids X 400, X 1000.
Captions: CV- Central vein N-Nucleus

reactions in hepatocytes, which leads to increase in AGEs (advanced glycation end products) production and finally increase in free radicals production via disturbance in ROS (reactive oxygen species) production.

Weight loss is a major characteristic of DM. It may be due to protein wasting because of lack of carbohydrate for energy[23]. Treatment with LSE increase body weight in diabetic group, suggesting that LSE may normalize energy metabolism in tissues particularly liver and muscle.

Similarly, oral administration of ethanolic extract of LSE for 28 days showed significant decrease in the blood glucose level. This clearly indicates there may be protection of β cells from damaging effects of free radicals and stimulation of surviving β cells leads into increase in insulin secretions. This was also supported by histopathological examination in pancreas and liver. The histological studies of pancreas showed marked improvement in cellular architecture with increased in size and number of islets after LSE treatment. This regeneration of Islets of recovery group suggests stable cells in the islets with the ability of regeneration. Similarly histological studies of liver of after treatment with LSE significantly reduced hypertrophy of hepatocytes and hepatocellular necrosis showing its hepatoprotective activity. Previously Shukla *et. al*., 2012 studied the antidiabetic activity of LSE in type I diabetic rats. Phytochemicals studies of *Lepidium sativum* is documented to possess alkaloids, Flavonoids, phenols, riboflavin, α-tocopherols, β-carotenes, β-sitosterol, ascorbic, linolenic, oleic, palmitic and stearic acids[25] . Alkaloids, Flavonoids and Phenolic compounds are known for their hypoglycemic and antioxidative properties[26-27]. Thus, the significant antidiabetic and cytoprotective activity of LSE could be due to the presence of more than one active principle and their synergistic properties.

CONCLUSION

In conclusion the antidiabetic activity of LSE may be by sensitizing the insulin receptor or by regenerating beta cells and stimulating the secretion of insulin from it.

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