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HISTOPATHOLOGICAL AND ULTRASTRUCTURAL STUDIES OF THE EFFECT OF FENUGREEK SEED EXTRACT ON PANCREAS OF ALLOXAN INDUCED DIABETIC MICE

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ASTRACRT: To evaluate the protective effect of fenugreek (*Trigonella foenum graecum* L.) on alloxan induced diabetic mice, 15 male albino mice weighing 26-30 gm were used. They were divided into 3 groups. Control group received subcutaneous injection of 0.15 M acetate buffer for 15 days. Alloxan induced group received subcutaneous injection of alloxan 150 mg/ kg body weight to induce diabetes. Recovery group received subcutaneous injection of fenugreek seed extract 15 mg/kg body weight per day for 15 days. At the end of experiment histological and ultrathin sections of pancreas prepared. In diabetic mice disturbances in pancreatic arrangement is observed. While in electron microscopic study pyknotic nuclei, vacuolation and morphological changes in mitochondria, endoplasm reticulum and degranulation of β cells were observed. After treatment with fenugreek repair in pancreatic arrangement especially in β cells was clearly observed. In conclusion fenugreek seed extract could normalize the diabetes and provides protection to pancreatic tissues from damaging effect of diabetes.

INTRODUCTION: Diabetes mellitus (DM) is considered as one of the five leading causes of death in the world. It has been estimated that number of diabetes in India is expected to increase 57.2 million by the year 2025[1]. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia altered metabolism of lipids, carbohydrates and protein and increased risk of complication from various diseases[2].

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Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications. Enhanced oxidative stress and changes in antioxidant capacity, observed in both clinical and experimental diabetes mellitus though to be the etiology of chronic diabetic complications[3].

Herbal medicine is one of the therapeutic strategies in the treatment of diabetes mellitus. It is considered to be less toxic and have fewer side effects than synthetic ones[4]. One of them is *Trigonella foenum graecum* L. that is fenugreek. Fenugreek is a spice rich in dietary fibers and has a traditional history of medicinal use in the management of diabetes mellitus, in Egypt, Southern Europe, India, Northern Asia, Northern

Africa etc.[5] Fenugreek seeds have shown potential as a dietary supplement and cause a marked decrease in the symptoms of diabetes mellitus. Fenugreek seeds mainly contain 4-hydroxyisoleucine, trigonelline, flavonoids, coumarins, proteins, saponins and lipids[6-7]. Out of these content 4-hydroxyleucine, a novel amino acid from fenugreek seeds increased glucose stimulated insulin release by islet cells in both rats and humans[8].

The present investigation was undertaken to evaluate the protective effect of fenugreek seed extract on the Islets of langerhans especially β cells in alloxan induced diabetic mice.

MATERIAL AND METHODS:

Preparation of fenugreek seed extract[9]:

Fenugreek seeds were collected from the local market of Kolhapur. They were (10g) were cleaned and ground into a fine powder using a grinding machine. Ethanol was used for extraction by soxhelt extraction method. The extract was evaporated to dryness under reduced pressure at 60^0C by rotary evaporator. Extract was placed in dark bottle and stored at -8^0C.

Animals:

Male albino mice (*Mus musculus* L) were used for present study. They were bred and reared in departmental animal house (CPCSEA/233) in separate cages under proper conditions of light, temperature and humidity. They were supplied with Amrut mice feed (Pranav Agro Industries) and water *ad libitum*.

Experimental design:

Mice were divided in to 3 groups:

Control Group: Three months male mice were given subcutaneous injection of 0.15M acetate buffer pH 5.4 for 15 days.

Diabetic Group: Three months male mice were given subcutaneous injection of alloxan 150 mg/kg body weight for15 days[10-11.]

Recovery group: Three months male mice were given subcutaneous injection of fenugreek seed extract at a dose of 15mg/ kg body weight to diabetic mice for 15 days[12].

Histopathological study[13]:

After the completion of dose, mice from all groups were killed by cervical dislocation and pancreas were dissected out quickly and fixed in 2% CAF fixative. Tissue were processed and embedded in paraffin wax. Sections were cut at 5µ thickness and stained with HE and examined under light microscopy.

Electron microscopic study:

The small pieces of splenic lobes of pancreas were fixed with 3% glutaraldehyde in 0.1M phosphate buffer pH 7.2 for 24 hrs and post fixed in osmium tetroxide, dehydrated in ethanol and in epoxy resin. Ultrathin sections cut with LEICA EM UC6 ultra microtome. The prepared ultra thin sections were placed on copper grids, doubly stained with uranyl acetate followed by lead citrate[14] and examined with Techani G^2 at 60KV accelerating voltage and photographed for observation.

RESULTS:

Histopathological study: The light micrograph of sections of pancreas of control mice showed exocrine pancreas and islets of langerhans.

Acinar cells:

The exocrine pancreas consists of closely packed acini with very little connective tissue in between. The acinar cells were pyramidal in shape, having very small acinar lumen. They have rounded basally located nuclei with dispersed and prominent nucleoli. **(Plate No. I Fig. 1, 2).**

In diabetic group histologically, sections of the diabetic pancreas showed disturbance in the acinar pattern structure and reduction in diameter of islets of langerhans as compared to control group. The acinar cells showed vesicular nuclei and the cytoplasm of most acinar cells showed many small vacuoles. Thick septae was observed between these acini **(Plate No. I Fig. 3, 4).**

In recovery group the light micrographs revealed that the pancreatic acini had retained their pyramidal- shaped appearance as compared to diabetic group. Numerous zymogen granules were observed in the apical regions of cytoplasm of most acinar cells. Many sections revealed the presence of islets with their rounded outline as in the normal control. **(Plate No. I Fig. 5, 6).**

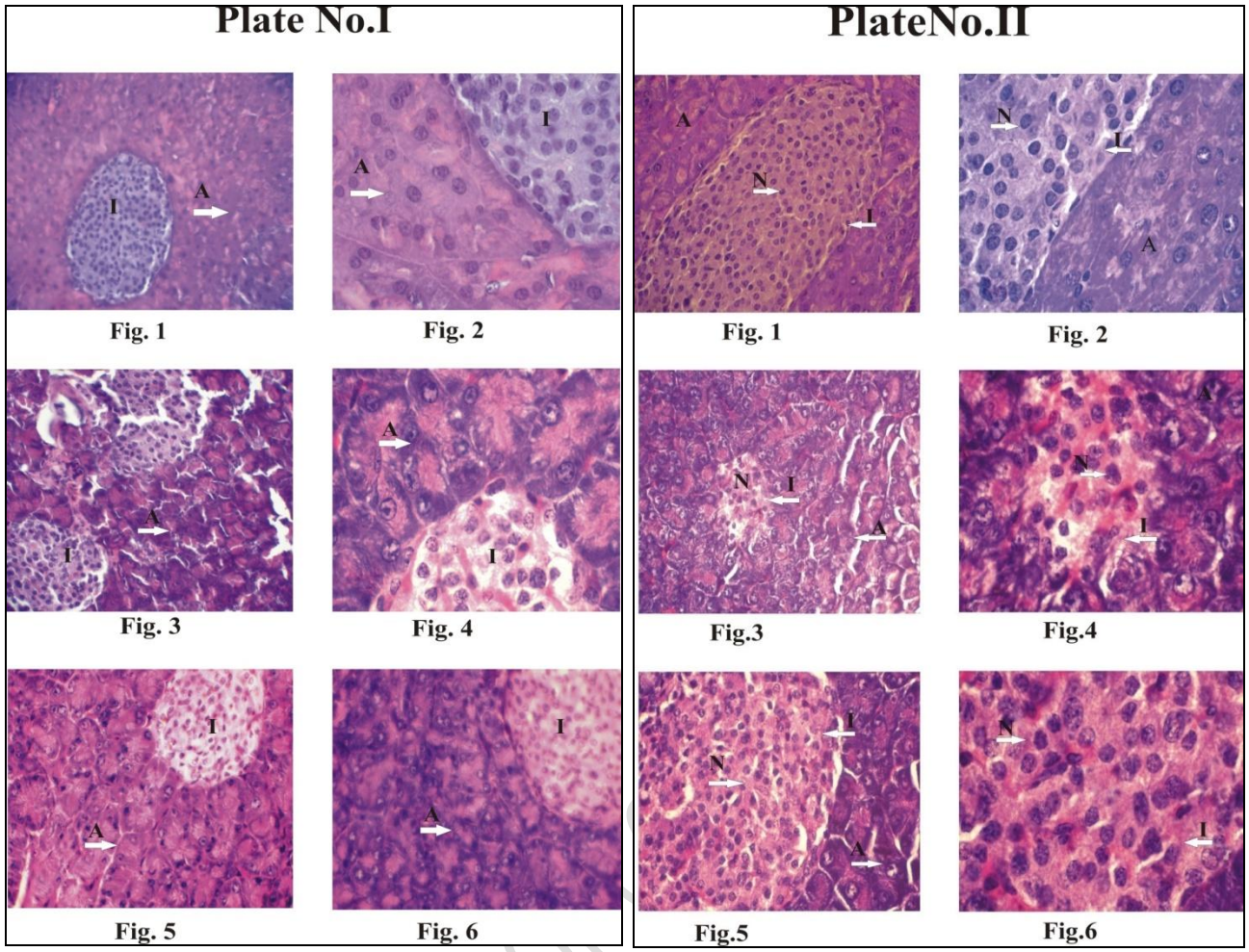

PLATE NO. I: ACINAR CELLS OF PANCREAS STAINED BY HE.

Fig No.1 and 2 Control mice pancreas showing normal structure of pancreatic acini X400, X1000.

Fig No.3 and 4 Diabetic mice pancreas showing distribution in the acinar pattern structure. X400, X1000.

Fig No.5 and 6 Diabetic mice treated with fenugreek seed extract showing pancreatic acini of recovery group retained their pyramidal shaped appearance. X400 X1000.

Captions: A-Acinar cells, **N**-Nucleus, **I-** Islets of langerhans

Plate No. II: Endocrine cells i.e. islets of langerhans of pancreas stained by HE.

Fig No.1 and 2 Control mice pancreas showing normal structure of Islets of langerhans X400, X1000.

Fig No.3 and 4 Diabetic mice pancreas showing degenerative and necrotic changes, reduced dimension of islets of langerhans. X400, X1000.

Fig No.5 and 6 Diabetic mice treated with fenugreek seed extract showing marked improvement of islets of langerhans. X400, X1000.

a) Endocrine cells:

In control group under the light microscope examination of the islets of langerhans showed alpha and beta cells. They have large rounded and basophilic nuclei and prominent nucleoli **(Plate No. II, Fig No.1, 2).**

In diabetic group there were certain degenerative features in most β cells of these islets. Their nuclei became packed together in groups and the cytoplasm had lost their granules as well as it also shows reduction in diameter of islets of langerhans as compared to control group. **(Plate No. II, Fig No**.3, 4).

In recovery group there was marked improvement of islets of langerhans and regeneration of β cells may be due to preventing the death of β cells as

compared to diabetic group. (Plate No. II, Fig No.5, 6).

Electron microscopic study:

Acinar cells:

In control group ultra microscopically the acinar cells showed basally located rounded nuclei relatively with large nucleolus. Dense zymogen granules were scattered in the apical cytoplasm in the basal cytoplasm was crammed with lamellar profiles of rough endoplasm reticulum within which are scattered rounded and oval shaped mitochondria were seen. Supranuclear Golgi area was found located near zymogen granules. **(Plate No.III, Fig No.1, 2).**

In diabetic group the electron micrographs revealed certain irregularities of the nuclear outline of these acinar cells. The zymogen granules had decreased in number in cytoplasm of most of these cells. The mitochondria were increased in number and size, they were enveloped in the lamellar rER, most of them had lost their cristae, some were highly vacuolized and the autophagic vacuoles appeared in its interior **(Plate No.III, Fig No.3, 4).**

In recovery group the electron micrographs of the nuclei of these cells still showed slight irregularities in their nuclear outline. The moderate increase in the lowered secretory vesicles with granules and also slight destruction with loss of cristae within the mitochondria of β cells, which elucidates the efficacy of fenugreek seed extract in prevention of β cell damage by alloxan when compared to control. **(Plate No.III, Fig No.5, 6).**

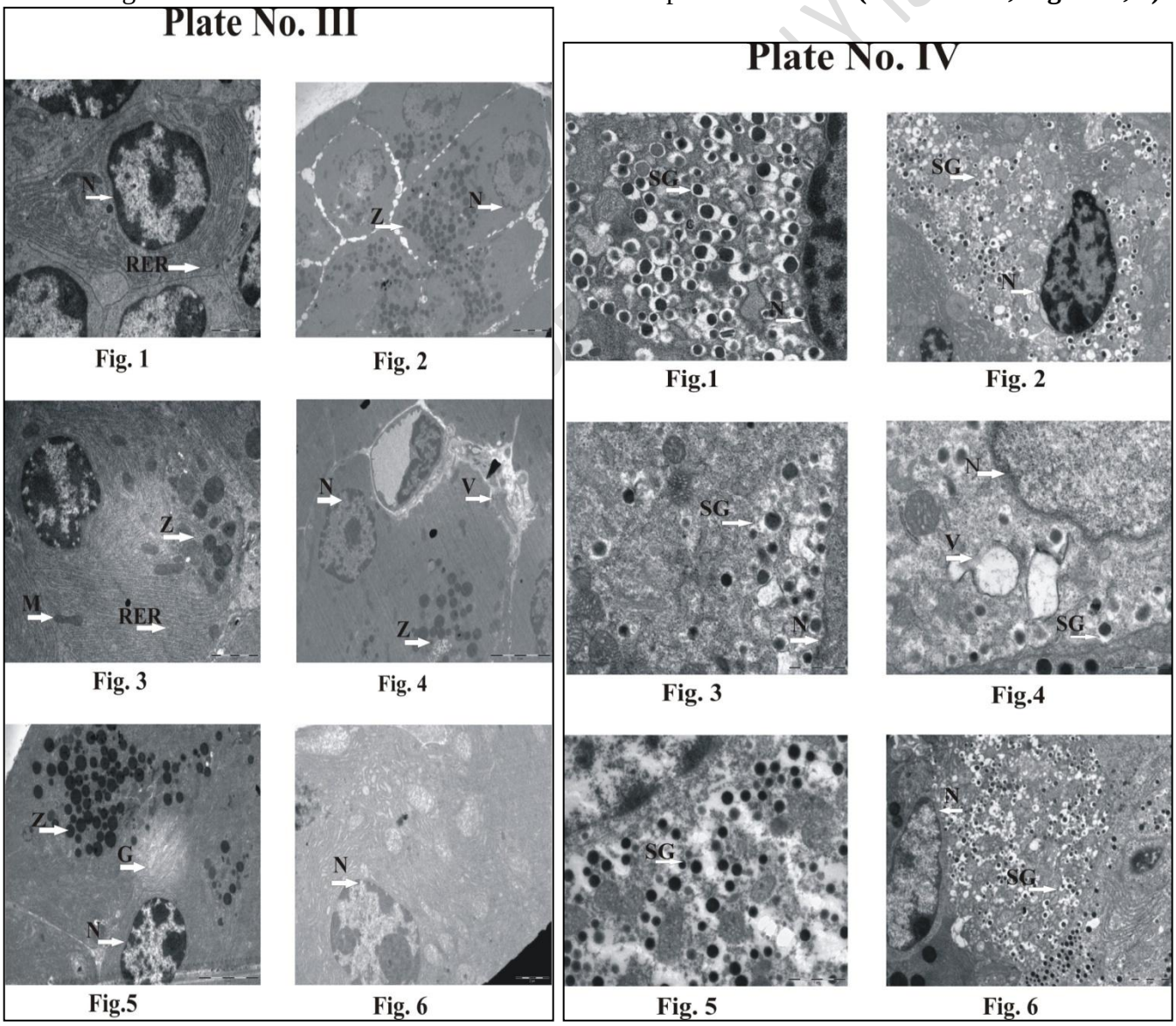

PLATE NO.III: ULTRATHIN SECTIONS OF ACINAR CELLS OF PANCREAS.

Fig No.1 and 2 Control mice pancreas showing normal structure acinar cells, mitochondria, RER X7500.

Fig No.3 and 4 Diabetic mice pancreas showing vacuolized mitochondria decreased secretory granules X7500.

Fig No.5 and 6 Diabetic mice treated with fenugreek seed extract showing normal mitochondria and increased secretory granules. X7500

Captions: N-Nucleus, **M-**Mitochondria **RER-** Rough endoplasmic reticulum
Z-Zymogen granules, **V-**Vacuoles
G - Golgi complex

Plate No.IV: Ultrathin sections of β cells of pancreas.

Fig No.1 and 2 Control mice pancreas showing normal structure β cells, large number insulin secretory granules. X18,500, X6800.

Fig No.3 and 4 Diabetic mice pancreas showing degenerated mitochondria, few insulin secretory granules 2X18,500, 1X18,500**.**

Fig No.5 and 6 Diabetic mice treated with fenugreek seed extract showing less number of β cells with vacuoles, increased insulin secretory granules. 1X18, 500, 1X6800.

Endocrine cells:

In control group there were no obvious differences between alpha and beta cells in light microscope, while they were clearly identified and distinguished morphologically by the electron microscope. The β cells had rounded or slightly oval nuclei of regular contour. The cytoplasm contains many secretory granules with an electron dense core surrounded by an electron lucent halo. The mitochondria are scattered throughout the cytoplasm, they were fine and appeared as rounded or plump filamentous, having dense matrix. The Golgi elements and endoplasmic reticulum are observed in many cells among the β-granules. **(Plate No. IV Fig No.1, 2).**

In diabetic group ultrastructurally, the nuclei of these cells showed variable changes, some appeared pyknotic and others were vesicular. A decrease in the secretory granules of B cells of the alloxan induced diabetic group was observed in comparison to control group. The mitochondria were vacuolized and characterized by an extreme loss of matrix, density, some were ruptured so that their cristae were not clearly observed. Obvious dilated and extended Golgi elements were noticed in cytoplasm of many of these β cells. **(Plate No. IV Fig No.3, 4).**

In recovery group there was an increase in amount of β granules in most β cells, they were of normal structure as the control, having dense core surrounded by lucent halo space. Most of the mitochondria were still have certain vacuolation and dissolution in their cristae with an extreme loss of matrix density. **(Plate No. IV Fig No.5, 6).**

DISCUSSION: Alloxan is well known for its selective pancreatic Islet β cells cytotoxicity and have been extensively used to induce type–I diabetes in experimental mice model. It interferes with cellular metabolic oxidative metabolisms[15]. Increasing evidences in experimental and clinical studies suggests that oxidative stress plays a major role in the development and progression of diabetes. Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and subsequent oxidative degeneration of glycation proteins. Diabetes is usually accompanied by impaired antioxidant defenses.

In the present study the diabetes induced by single dose of alloxan showed significant structural damage in pancreatic tissue like disturbance in acinar pattern structure, shrinkage, pyknotic and vesicular nuclei of most acinar cells. The nuclei of β cells showed aggregation of the heterochromatin along their nuclear envelops. It was suggested that these changes may be due to condensation and shrinkage of the nuclear material. The shrinkage of the nuclei to be as the result of accumulation of secretory granules in the acinar cells that pushed these nuclei to the periphery[16-18]. Other authors had reported that alloxan at low doses induces apoptosis and at high doses causes necrosis in murine pancreatic B cells[19-20]. Degranulation of β cells in the present study is most probably due to the decrease in insulin synthesis[21].

It was of an important in this study to note that administration of fenugreek seed extract to these diabetic mice had decreased many of these light and microscopic observations. The electron micrograph revealed that B cells had retained their normal structures with its normal β granules.

CONCLUSION: These findings suggest that fenugreek seed extract treatment has a therapeutic protective effect against diabetes by decreasing oxidative stress and preserving pancreatic β cell integrity. Consequently fenugreek may be clinically useful for protecting β cell against oxidative stress.

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Reviewer's recommendations:

1. Specify designation and current full address of corresponding author.
3. References are out of format. See Instructions to Authors.
4. Revise manuscript as per New Instructions to Authors. You can refer our latest issue formatting.