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Therapeutic Effects of Canagliflozin and Zinc Sulphate Alone and in Combination on Pancreatic Histology in Type-2 Diabetic Rat Model

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ABSTRACT

Introduction: Diabetes Mellitus is a common metabolic syndrome characterized by persistently elevated blood glucose levels. Canagliflozin is an SGLT-2 inhibitor that controls hyperglycemia by reducing the reabsorption of filtered glucose and excreting it in the urine. Zinc sulphate exhibits some beneficial role in diabetes mellitus but has not been compared to canagliflozin individually and in combination. **Aims & Objectives**: To observe the effects of treatment with canagliflozin and zinc sulphate on pancreatic histology in streptozotocin induced type-2 diabetic rat model. **Place and duration of study:** The study was conducted in the Department of Pharmacology, KEMU and PGMI, Lahore for the period of two months. **Material & Methods:** It was an animal experimental study of eight weeks duration in which 48 adult healthy albino rats of male gender were divided into six groups and were provided the high fat diet throughout the study period. Groups A and B were maintained as healthy and diseased controls respectively. Groups B, C, D, E and F were administered single I/P dose streptozotocin (35mg/kg) at day 22 for inducing diabetes. Upon confirmation of diabetes after a week the rats were further treated as per group designation orally for 4 weeks , individually or in combination with full or half doses of canagliflozin (10 mg /kg/day, 5mg/kg/day) and zinc sulphate (30mg/kg/day, 15mg/kg/day) . All animals were euthanized at the completion of study duration. The pancreatic tissue was taken out and examined for the histopathological changes (size and number of pancreatic islets, karyolysis and ballooning degeneration). **Results:** There was a marked improvement in the size and number of islets as well as the inflammatory changes in the combined treatment group (with canagliflozin in full as well as half dose of zinc sulphate) as compared to the groups given zinc sulphate and canagliflozin separately. **Conclusion:** Combined treatment with canagliflozin and zinc sulphate has a better protective effect on the pancreatic tissue in diabetes than either of them used alone.

Key words: Diabetes mellitus, canagliflozin, zinc sulphate, histopathological, streptozotocin

INTRODUCTION

There has been an alarming increase in the number of individuals having diabetes during the last two decades, which poses a threat to increase the health expenditure for diabetes worldwide and specifically in the developing countries, where the rate of rise is more.[1,2] The current treatment options provide control of hyperglycemia but the associated side effects worsen the patient's compliance, so there is a dire need of a therapy which can provide a good glycemic control with minimal side effects.[3] In this regard, canagliflozin, which belongs to the class of SGLT-2 inhibitors seems to be a good option since it is well-tolerated in the body and has additional benefits of lowering blood pressure and body weight. It promotes urinary excretion of glucose via decreasing its reabsorption at the proximal tubule and thus causing a reduction in the renal glucose threshold.[4] Zinc regulates the functions of many tissues and has the potential to normalize the hyperglycemia.[5] Limited studies have shown the efficacy of zinc combined with oral hypoglycemic agents in combating diabetes mellitus.[6] The high-fat diet/streptozotocin (HFD/STZ) rat model is an example of the experimentally induced type 2 diabetes model. It is prepared by combining a fat-rich diet to produce insulin resistance and then treating with STZ (a toxin of the beta cells), that leads to greater decrease in the functional mass of the pancreatic beta cells.[7]

The present study is designed to observe the role of zinc and canagliflozin alone and in combination on pancreatic histology in type-2 diabetes mellitus and to hypothesize that the combination therapy has a better effect than each given separately.

MATERIAL AND METHODS

Study Design: Animal experimental study.
Setting: The study was conducted in the Department of Pharmacology, KEMU and PGMI, Lahore.
Sampling Technique: Simple random sampling.
Sample Size: Forty eight rats were divided into six groups by lottery method.
Inclusion Criteria: Male Sprague-Dawley rats, weighing 120g to 180g.
Exclusion Criteria: Rats showing signs of any disease.
48 adult healthy albino rats of male gender were purchased from UVAS (University of Veterinary and Animal Sciences) and kept in the animal house of PGMI (Post-Graduate Medical Institute), Lahore. Animals were divided randomly into 6 equal groups having 8 rats in each group. The rats were exposed to natural day and night cycles at room temperature of 22 ± 2 °C with 50 ± 5 % humidity throughout the experiment. They had a free access to rat chow and water ad libitum. An interval of seven days was given to them to get acclimatized before the start of the experiment.
Preparation of Doses & Sampling: The calculated dose for individual rat, i.e. 10mg/kg/day of canagliflozin[8] and 30mg/kg/day of zinc sulphate[9] were weighed and dissolved in 1ml of distilled water. High fat diet was prepared by combining 1.5g of cholesterol, 1g of sodium deoxycholate and 8ml of coconut oil in every 100g of normal rat chow.[10,11] Hyperlipidemic rats were then injected intraperitoneally with streptozotocin (35mg/kg) at day 22, dissolved in 0.1M sodium citrate buffer at pH 4.5 (prepared by adding 46.5ml of citric acid to 3.5ml of sodium citrate solution and making it up to 100ml with distilled water).[12] After a week from injection of STZ, blood samples obtained from the lateral tail vein were used in determining the blood glucose value using glucometer and those having blood glucose > 180 mg/dl were labeled as diabetic.
Grouping of animals: Forty eight rats were divided into six groups randomly by lottery method, with 8 rats in each group. These groups were labelled as A,B,C,D,E and F. Each of the group was kept in a separate iron cage. Rats in **group A (normal control group)** were fed with standard rat diet (normal rat chow), throughout the study period of 8 weeks. Rats in all the other groups (B, C, D, E and F) were given high fat diet throughout 8 weeks and streptozotocin after 3 weeks for the induction of type-2 diabetes. Rats in **group B (disease control group)** were only given streptozotocin to induce diabetes. Rats in **group C** were administered zinc sulphate orally from 4^{th} to 8^{th} week after the induction of diabetes. Rats in **group D** were administered canagliflozin orally from 4^{th} – 8^{th} week after the induction of diabetes. Rats in **group E** were administered full doses orally of canagliflozin (i.e. 10mg/kg/day) and zinc sulphate (30mg/kg/day) from 4^{th} – 8^{th} week after the induction of diabetes. Rats in **group F** were administered half doses orally canagliflozin (i.e. 5mg/kg/day) and zinc sulphate (15mg/kg/day) from 4^{th} – 8^{th} week after the induction of diabetes. **Euthanization:** Twenty four hours after last dose administered the rats were sacrificed at the end of week 8. Pancreas of each rat was identified and dissected out en-bloc and preserved in formalin separately.
Histological Examination: After removing the pancreas, it was stretched on filter paper and fixed in 10% buffered formalin (pH 7.4) at room temperature. The fixed specimens were sliced, processed and embedded into paraffin blocks. The blocks were then cut into 4µm paraffin sections by a rotator microtome. Clean glass slides were put in hot air oven for 24 hours and were properly numbered and 4µm thick representative sections of tissues were mounted. De-waxing of tissues was done by dipping them in xylene. Slides were hydrated by passing through decreasing concentrations of alcohol and were dipped in hematoxylin. Afterwards, these were washed in the running water. Then, these slides were placed in 1% acid alcohol, again washed under tap water and put in 1% ammonia solution followed by water wash. Then, the slides were put in eosin for 5 minutes and washed under tap water. The stained sections were mounted with DPX and examined under microscope to evaluate for the presence or absence of the structural changes like signs of inflammation (karyolysis and ballooning) and atrophy (changes in the size and number of islet of Langerhans).[13] The size and number of pancreatic islets were measured with the help of a deca-head microscope (Nikon Imaging Software package-D) and mean was taken for further calculations.

Statistical analysis:
All the data was entered in SPSS version 23 for qualitative data and graph pad prism version 8 for quantitative data. Quantitative data (size and number of islets) was expressed as Mean ± S. D.

Mean plots were used for graphical presentation to see the changes in the parameters. The data was evaluated by one way analysis of variance followed by Tukey's multiple comparison tests. Qualitative data (karyolysis and ballooning degeneration) were scored as numbers and expressed as percentage of changes in groups. The significance of differences was measured through Kruskal-Wallis ANOVA and Mann-Whitney U test. p-value of less than 0.05 was considered significant.

RESULTS

1) Size of Islets of Langerhans (µm):

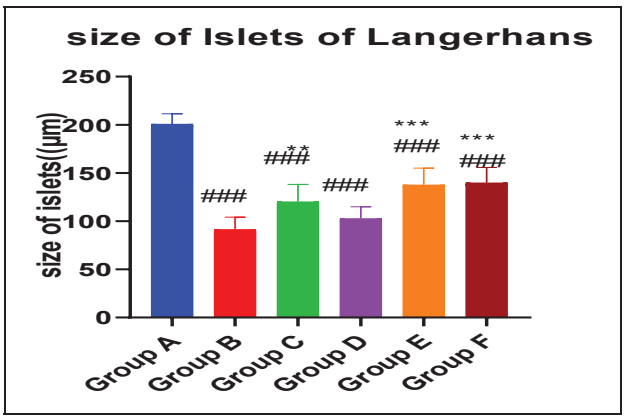

Fig-1: Comparison of mean size of islets of Langerhans of groups A, B, C, D, E and F

indicates p-value is <0.001 as compared to group A.
*** shows p-value is <0.001
** means p value <0.01, when compared to group B.

Group A=normal control, Group B=disease control, Group C=Zn treated, Group D =Cana treated, Group E = Zn+Cana (full dose), Group F = Zn+Cana (half dose).

2) Number of Islets of Langerhans:

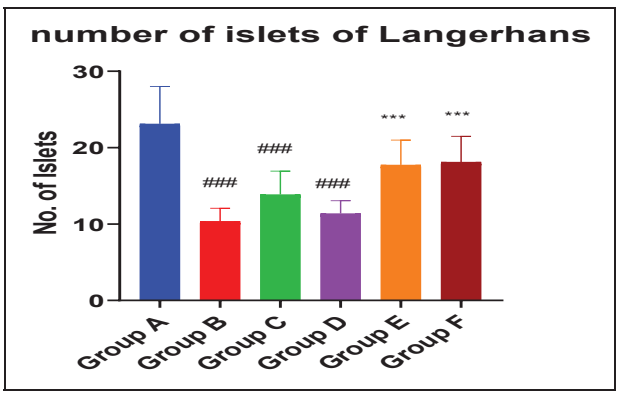

Fig-2: Comparison of mean number of islets of Langerhans among groups A,B,C,D,E and F

shows p-value is <0.001 when compared to group A.
*** means p-value is <0.001 as compared to group B.

Group A=normal control, Group B=disease control, Group C=Zn treated, Group D =Cana treated, Group E = Zn+Cana (full dose), Group F=Zn+Cana (half dose).

3) Karyolysis:

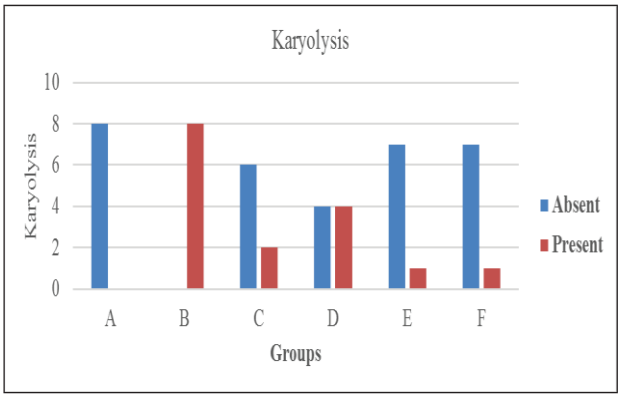

Fig-3: Comparison of karyolysis among groups A,B,C,D,E and F.

Group A=normal control, Group B=disease control, Group C=Zn treated, Group D =Cana treated, Group E=Zn+Cana (full dose), Group F=Zn+Cana (half dose).

4) Ballooning degeneration:

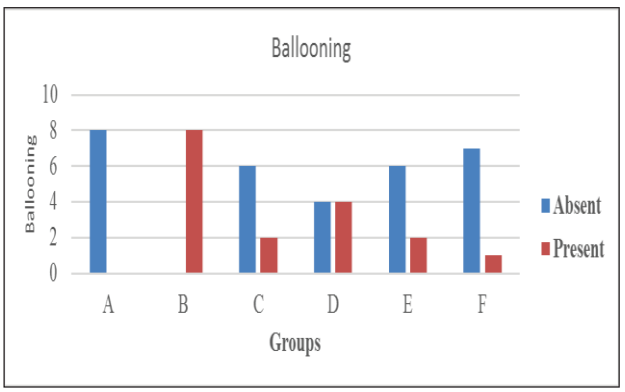

Fig-4: Comparison of ballooning degeneration among groups A, B, C, D, E and F.

Group A=normal control, Group B=disease control, Group C=Zn treated, Group D =Cana treated, Group E = Zn+Cana (full dose), Group F=Zn+Cana (half dose).

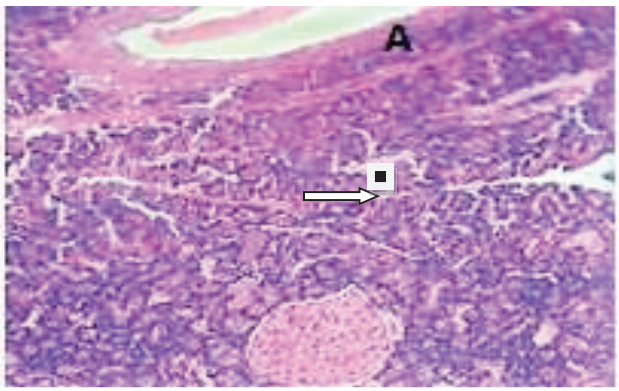

Fig-5: Group A (normal control) Histology of pancreas showing ■ normal islets of Langerhans (40 x; H&E)

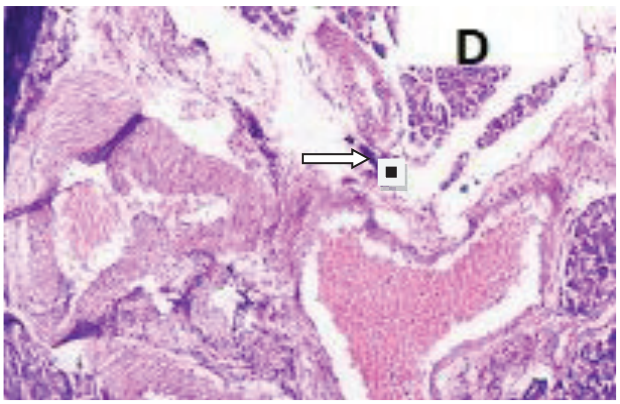

Fig-8: Group D (HFD+STZ+Canagliflozin)
■ Some restoration in size and number of islets, ballooning and karyolysis still present after treatment with cana alone (40x; H&E)

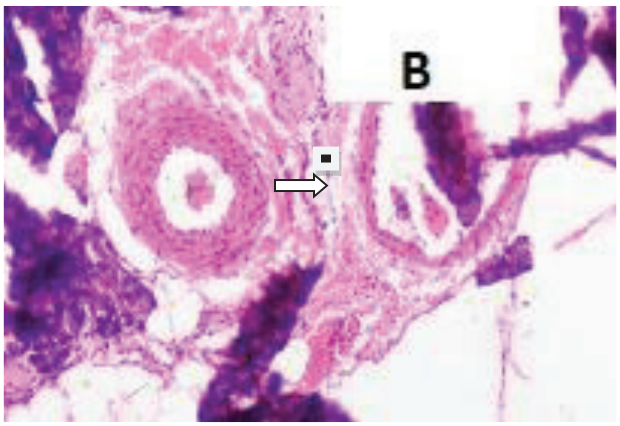

Fig-6: Group B (positive control)
■ Severe reduction in the size and number of islets in STZ-induced diabetic rat (40 x; H&E)

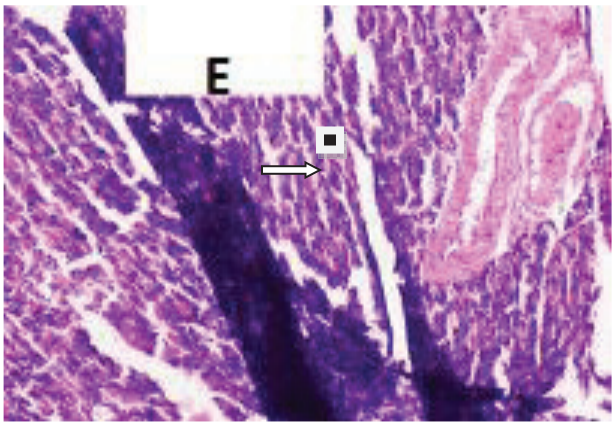

Fig-9: Group E (HFD+STZ+Zinc+full Cana)
■ Restoration of size and number of islets with mild karyolysis and ballooning (40x; H&E)

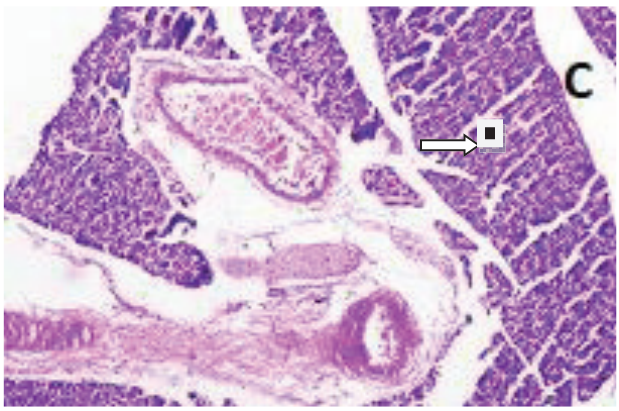

Fig-7: Group C (HFD+STZ+Zinc)
■ Some restoration in size and number of islets with moderate karyolysis and ballooning (40 x; H&E) after treatment with zinc alone

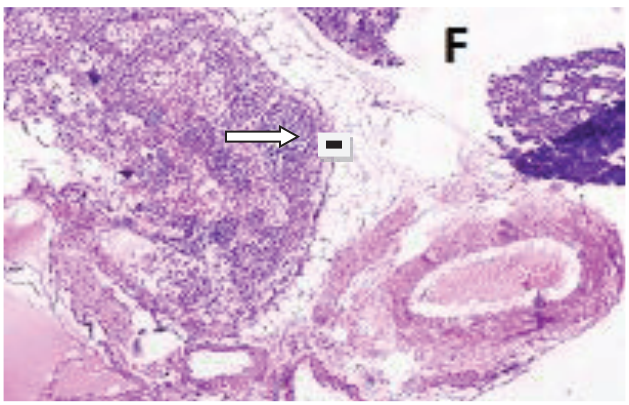

Fig-10: Group F (HFD+STZ+Zinc+halfCana)
■ Restoration of the size and number of islets with mild karyolysis and ballooning (40x; H&E)

DISCUSSION

Histopathological study with the light and deca-head microscopy revealed some gross changes in the size, number and structure of the islets of Langerhans. STZ administration led to the shrinkage and

disruption of the structure of islets and the degenerative changes were pronounced, like karyolysis of the nuclei and ballooning degeneration, causing the cells to have a highly vacuolated cytoplasm. Upon injection with STZ, atrophy of islets was prominent causing a visible reduction in the size and number of the islets of Langerhans. Similar findings were reported in the recent works using the rat model of STZ induced diabetes.[13,14] After the administration of zinc for four weeks, there was some reversal of these histopathological changes in the pancreatic sections of the rats of group C. A significant restoration of the size of islets as well as the reversal of karyolysis and ballooning degeneration was observed on histopathological examination, whereas no significant difference was observed in the number of islets with zinc treatment. These results resemble those of another study in which zinc supplementation given to STZ-induced diabetic rats was associated with significant rejuvenation of the islets histology.[5] Zinc preserves the pancreatic architecture probably through its anti-oxidant property.[15] Canagliflozin was also found to exert a beneficial effect in restoring the histological morphology of pancreas (karyolysis and ballooning degeneration) but to a lesser extent than zinc. This observation is in accordance with a study done earlier in which the treatment of ZDF (Zucker Diabetic Fatty) rats with canagliflozin minimized the degenerative changes in diabetic induced islets.[16] Another study indicated that dapagliflozin (closely related to canagliflozin and both belong to SGLT-2 inhibitors) has a role in the preservation of pancreatic islets morphology of HFD fed diabetic rats[17]. In diabetes, increase in demand of insulin secretion occurs which can cause stress of the endoplasmic reticulum, leading to increased apoptosis of the beta-cell. By decreasing hyperglycemia through a non–insulin-dependent procedure, SGLT-2 inhibitors reduces the demand on the beta cells to produce insulin and thus prevents excessive apoptosis of these cells as seen in diabetes.[8,18] Even better results were seen in the combination groups E and F, where the addition of zinc to canagliflozin further improved the morphological changes in the islets. The degenerative signs (karyolysis and ballooning) almost disappeared and the number and size of the pancreatic islets were comparable with the negative control group. No study to date has suggested this potentiating effect of zinc with canagliflozin.

CONCLUSION

This study has demonstrated that the combined administration of zinc and canagliflozin has exerted a stronger effect on restoring pancreatic histology back to normal in type-2 diabetic rat model as compared to both of these drugs given alone.

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