

12-1-2017

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Recommended Citation

Al-Ani IM, Al-Ani GA, Azzubaidi MS, Al-Ahmed BI. The Effect of Flaxseed Ethanolic Extract on the Structure of the Kidney and the Endocrine Pancreas in Streptozotocin Induced Diabetic Rats. Makara J Health Res. 2017;21.

The Effect of Flaxseed Ethanolic Extract on the Structure of the Kidney and the Endocrine Pancreas in Streptozotocin Induced Diabetic Rats

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Abstract

Background: The present investigation has been designed to study the possible protective effect of flaxseed extract on the structures of the endocrine pancreas and kidneys of streptozotocin (STZ) induced diabetic rats for 30 days. **Methods:** Forty male Sprague-Dawley rats were randomized into five groups (n=8). Normal control group (NC); received distilled water orally, normal flaxseed group (NF); treated orally with (400 mg/kg) extract of flaxseed, diabetic control group (DC); treated with single intraperitoneal dose of STZ (60 mg/kg), diabetic flaxseed group (DF); diabetic rats treated with extract of flaxseed (400 mg/kg), diabetic glibenclamide group (DG); diabetic rats treated with (0.6 mg/kg) glibenclamide. **Results:** Histological observation of sections in pancreas in DC group revealed shrunken islets of Langerhans with degenerated and degranulated β -cells, vacuolations and congested capillaries while sections of kidneys showed shrinkage of some glomeruli and degeneration of others with wide urinary space and hydropic degeneration in some tubular epithelial cells, dilated tubules and cell debris scattered in tubular lumina. These pathological changes were ameliorated in the flaxseed extract and glibenclamide treated rats. **Conclusions:** It is concluded that flaxseed extract may represent a good alternative treatment for management of diabetes and its related complications such as diabetic nephropathy.

Keywords: diabetes mellitus, endocrine, pancreas, kidney, flaxseed streptozotocin

Introduction

Diabetes mellitus (DM) is a complex group of disorders that affect the metabolism of carbohydrates, protein and fat in the body. It results from the reduction or absence of insulin secretion, or reduction in the sensitivity of the tissue to insulin.¹ It is a global disease which affects humans regardless of their socio-economic status and geographic distributions.² Many drugs like biguanides and sulfonylureas which are used to decrease hyperglycemia in (DM) are currently obtainable, but these drugs have specific side effects like hypoglycemia at higher doses, gastrointestinal disturbances, weight gain and liver toxicity. Thus to control these side-effects; it is fundamental to search for newer compounds. Herbal drugs are proposed for the treatment of diabetes because they are non-toxic, effective and with no or less adverse effects.³ Flaxseed is one of these medicinal plants which have been reported to possess antioxidant and antidiabetic action through its main component lignans.⁴ The results of clinical trials,

epidemiological investigations and experimental studies reveal that ingestion of flaxseed oil has shown to have a positive impact on diabetics as well as pre diabetics.⁵ The major source of α -linolenic acid and the richest source of lignans is flaxseed, the health advantages of both substances have been documented.⁶ The extent of diabetes in STZ-induced diabetic rats is minimized by the action of seco-isolariciresinol diglucoside (SDG) which is the main lignans contents of flaxseed.⁷ The preventive role of lignans against some cancers (breast, prostate, lung and colon) due to its strong anti-proliferative, antioxidant, anti-oestrogenic and/or anti-angiogenic activity have been shown in animal and human studies.⁴ Serum total and low-density lipoprotein cholesterol concentrations and postprandial glucose absorption is reduced by lignans action in flaxseed.⁸

Streptozotocin (STZ) is a glucosamine-nitrosourea compound (N-nitroso-n-methylurea derivative of 2-deoxy-d-glucose) a wide-spectrum antibiotic originates

from *Streptomyces achromogenes* which is used in the management of pancreatic β cell carcinoma. Chronic diabetic state resembling human diabetes mellitus has been induced in animal's model using STZ by destruction of β cells of the pancreatic islets,⁹ recently, flaxseed extract has been demonstrated to improve blood glucose levels, and histopathological induced changes in the liver of STZ-induced diabetic rats.¹⁰ The objectives of this study were to investigate the effect of flaxseed ethanolic extract on the structure of the kidney and the endocrine pancreas in STZ- induced diabetic rats.

Methods

Flaxseed. Flaxseeds were obtained from the Faculty of Science, Philadelphia University, Jordan, and the ethanolic extraction was prepared in the Natural Product Laboratory, Department of Biotechnology, Kulliyah (Faculty) of Science, International Islamic University Malaysia and flaxseed powder was defatted by mixing with hexane and 200 grams of the defatted powder was mixed with 1.2 L of 70% ethanol solvent for 24 hours at 30 °C. The extract was then filtered by a sand core funnel and then further concentrated at 40 °C using rotary evaporator at 90 rpm, followed by freeze drying for 4 days at -80 °C. The end product was the crude extract; the crude extract then diluted with distilled water before giving to the rats at a dose of 400 mg/kg.¹¹

Animals and experimental design. Forty Sprague Dawley healthy adult male rats aged from 10 to 12 weeks old (weighing 200-250g were used). The animals were housed in polypropylene cages (two rats per cage) under standard laboratory conditions (adequate cross ventilation; temperature: 24 ± 2 °C; 12:12 hrs light: dark cycle; relative humidity: 46-79%), and were allowed two weeks period to acclimatization. The rats were fed with standard commercial dry pellet diet and given water *ad libitum*. Diabetes was induced in the experimental rats with a single 60 mg/kg body weight intraperitoneal STZ injection dose. Prior to injection, STZ was melted in freshly prepared 0.1 M citrate buffer solution with a PH of 4.5 and immediately injected into the rats to avoid decay. Rats were confirmed to be diabetic on the fifth day after STZ administration, only when the level of fasting blood glucose was equal to or more than 14 mmol/l, and these rats were then selected for further study.

Rats were randomly divided into 5 groups of 8 rats in each. Normal control group (NC); healthy rats received distilled water orally. Normal flaxseed group (NF); healthy rats treated orally with (400 mg/kg) extract of flaxseed. Diabetic control group (DC); rats treated with single intraperitoneal dose of STZ (60 mg/kg). Diabetic flaxseed group (DF); diabetic rats treated with extract of flaxseed (400 mg/kg). Diabetic glibenclamide group (DG);

diabetic rats treated with (0.6 mg/kg) glibenclamide. Oral treatment was given on the sixth day after STZ injection and was considered as the first day of treatment. The oral gavage treatment was performed daily and continued for thirty days. Fasting blood glucose (FBG) level was done on day 1, 5, 10, 20 and 30 of the study by using the Accu-Chek Performa glucometer. Rats were handled according to the standards and regulations for the Care and Use of Laboratory Animals of the National Institutes of the Health and to the guidelines of IIUM animal ethical committee under the reference number: IIUM/519/14/4/IACUC.

Histological Studies. On day 30, the rats were sacrificed using Ketamine as an aesthetic. Pancreas and both kidneys from each rat were fixed in 10% formal saline for 72 hrs, dehydrated by ethanol, cleared in xylene and embedded in paraffin wax. Sections of 5 μ m thickness were cut using rotary microtome and stained by Haematoxylin and Eosin (H and E). Images that showed considerable histological differences from the NC group were captured and confirmed by two experienced histologists who were blind to study groups.

Statistical analysis. Data analysis was performed using statistic software IBM SPSS Statistics 19 (IBM Corporation, NY, USA). Data was expressed as mean \pm standard deviation (SD). The results were considered as significant at *p*-value less than 0.05. Repeated measure ANOVA was used to analyze fasting blood glucose data between the experimental groups, followed by, Scheffe's post hoc test for multiple group's comparison.

Results

Flaxseed and fasting blood glucose level. The effect of flaxseed extract on FBG levels in all rats groups were observed throughout the whole period of the study (Figure 1). The mean FBG level of the NC group, was maintained throughout the study and ended with (5.62 ± 0.67 mmol/L) in day 30. In NF group, the mean FBG levels were almost the same throughout the whole period of the study. Statistically there was not-significant difference (*p* > 0.05) in FBG level between NF and NC groups.

The mean FBG level of the DC group, was (20.78 ± 3.05 mmol/L) in the first day and increasing progressively reaching (27.31 ± 3.29 mmol/L) in day 30 of the study. Statistically there was significant differences (*p* < 0.05) in FBG levels between DC group and both NC and NF groups. In DF group, the mean FBG level was (21.30 ± 3.35 mmol/L) in day one of the study, then gradually elevated till the tenth day after that the level declined slightly in the day 20, and then elevated again reaching (23.05 ± 3.99 mmol/L) in the last day of the study. The changes in FBG level in DF

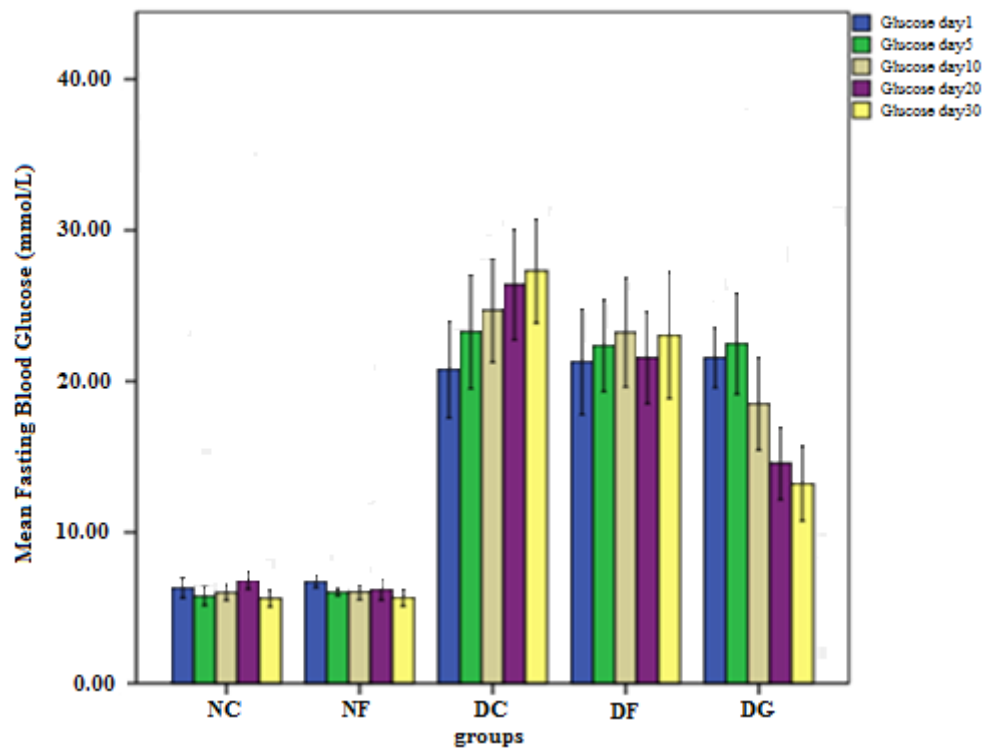


Figure 1. Blood Glucose Concentrations of All Groups during Different Days. Values are the (mean \pm SD)

group was statistically not-significant in comparison with DC group ($p > 0.05$). In DG group, the mean FBG level was (21.56 ± 1.89 mmol/L) in day one then slightly raised in the next few days, then slightly declined on day 10 and then decreased gradually (13.23 ± 2.36 mmol/L) till the last day of the study. The lowering in the FBG level in DG group was statistically significant ($p < 0.05$) in comparison with both DC and DF groups.

Flaxseed and the endocrine pancreas. The pancreas of normal control (NC) group and flaxseed treated (NF) group showed normal histological architecture of Islets of Langerhans (Figure 2A). The pancreas of diabetic (DC) group exhibited pathological changes characterized by shrunken islet of Langerhans with degranulated and degenerated β -cells, vacuolations, hyaline deposition and congested capillaries (Figure 2B). Treatment with ethanolic extract of flaxseed and glibenclamide minimized the extent of these changes; the endocrine pancreas of DF group exhibited less pathological changes and mild improvement in their structure (Figure 2C). In the DG group there was restoration of the structure of islets of Langerhans to the normal architecture (Figure 2D).

Flaxseed and the kidney. The kidneys of normal control (NC) and flaxseed treated (NF) groups showed normal histological structure of glomeruli and renal tubules (Figure 3A). The kidneys of diabetic control group showed many pathological changes characterized by shrinkage of

some glomeruli and degeneration of others with wide urinary space and hydropic degeneration in some tubular epithelial cells, dilated tubules and cell debris scattered in tubular lumina (Figure 3B). Treatment with ethanolic extract of flaxseed and glibenclamide minimized the extent of these changes. Sections of the kidneys of DF group exhibited less pathological changes and somewhat improved kidney architecture; however some renal tubules were still vacuolated (Figure 3C). Sections of the kidneys of DG group also revealed restoration of the normal kidney structure with dilated tubules and congested blood vessels (Figure 3D).

Discussion

Diabetes mellitus is considered as a leading cause of death due to its high morbidity and mortality.¹² Diabetic nephropathy (DNP) is a major long-term complication of diabetes mellitus.¹³ It is believed that oxidative stress is the mechanisms that plays pivotal role in the pathogenesis of diabetic nephropathy.¹⁴ Flaxseed is of particular importance in the dietary treatment for diabetes; adding flaxseed to the food may reduce insulin resistance in pre-diabetics and help decrease the danger of developing type-2 diabetes.⁵ The antioxidant activity of flaxseed is responsible for its beneficial effects on human health. The antidiabetic and antioxidant properties of flaxseed lignans have been confirmed in human and experimental animals.^{15,16}

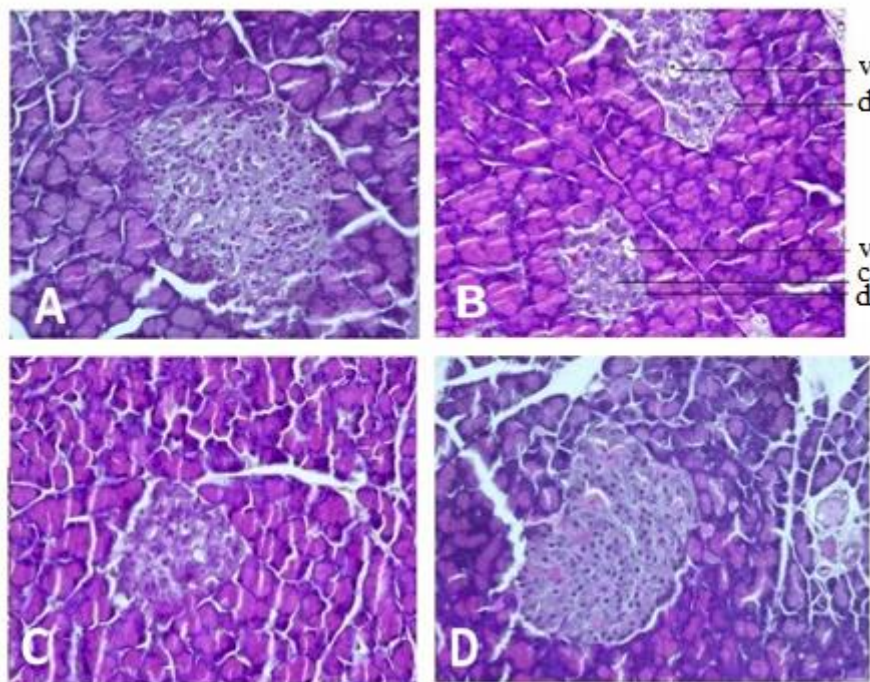


Figure 2. Photomicrographs of Pancreas of (A); Normal Control Group Showing Normal Histological Structure of Islets of Langerhans. (B); STZ Treated Group Showing Shrunken Islet with Degranulated and Degenerated β -cells “d”, Vacuolation “v”, and Congested Capillaries “c”. (C); STZ and Flaxseed Treated Group Showing Mild Improvement in the Structure of Islets of Langerhans. (D); STZ and Glibenclamide Treated Group Showing Restoration of the Structure of Islets of Langerhans to the Normal Architecture. H&E, x400.

In this study a significant increase in fasting blood glucose level was observed in the diabetic untreated rat group which reinforces the previous records on the capability of STZ to induce diabetes in experimental rats.^{17,18} The oral administration of ethanolic extract of flaxseed resulted in some decrease in the level of fasting blood glucose in diabetic rats; this is in agreement with previous records of antidiabetic activity of ethanolic extract of flaxseed through its main component secoisolariciresinol diglucoside (SDG), by its antioxidant activity and suppression of phosphoenolpyruvate carboxykinase (PEPCK) gene expression which inhibit hepatic gluconeogenesis.¹⁹⁻²¹

Glibenclamide is a second-generation sulfonylurea that reduces blood glucose by increasing insulin secretion from pancreatic beta cells, it has been extensively prescribed in treatment of type 2 diabetic patients for decades.²² In the present study, treatment of diabetic rats with oral glibenclamide has reduced the fasting blood glucose level, however, administration of glibenclamide was found to be more effective than flaxseed on FBG.

This is coincide with previous study that found glibenclamide is more effective than powdered stem bark of *Albizzia Lebbeck Benth* in STZ diabetic rats.²³

Assessment of sections of pancreas in rats exposed to STZ induced diabetes showed many histopathological changes such as shrunken islet of Langerhans with degranulated and degenerated β -cells, vacuolations, and congested capillaries. These finding are in agreement with earlier studies on the action of STZ on endocrine pancreas.²⁴⁻²⁶ However mild improvement with restoration in the structure of islets of Langerhans was observed in flaxseed ethanolic extract treated diabetic rats, these changes are in agreement with previous studies that observed regeneration of islet cells in STZ diabetic rats treated with flaxseed,^{27,28} and other plants extract such as cinnamon extract,¹⁸ olive leave extracts,²⁴ cinnamaldehyde from the bark of cinnamon,²⁹ and curry leave extract.³⁰

The kidney tissue of rats exposed to STZ- induced diabetes showed the development of many histopathological changes such as shrinkage and degeneration glomeruli and hydropic degeneration in tubular epithelial cells, dilated tubules and cell debris scattered in tubular lumina. These observations are in agreement with previous reports on the action of STZ on kidney.^{13,23,26} Treatment of STZ diabetic rats in this study with flaxseed extract moderately improved the kidney architecture and these changes are in agreement with previous reports on the effects of flaxseed extract

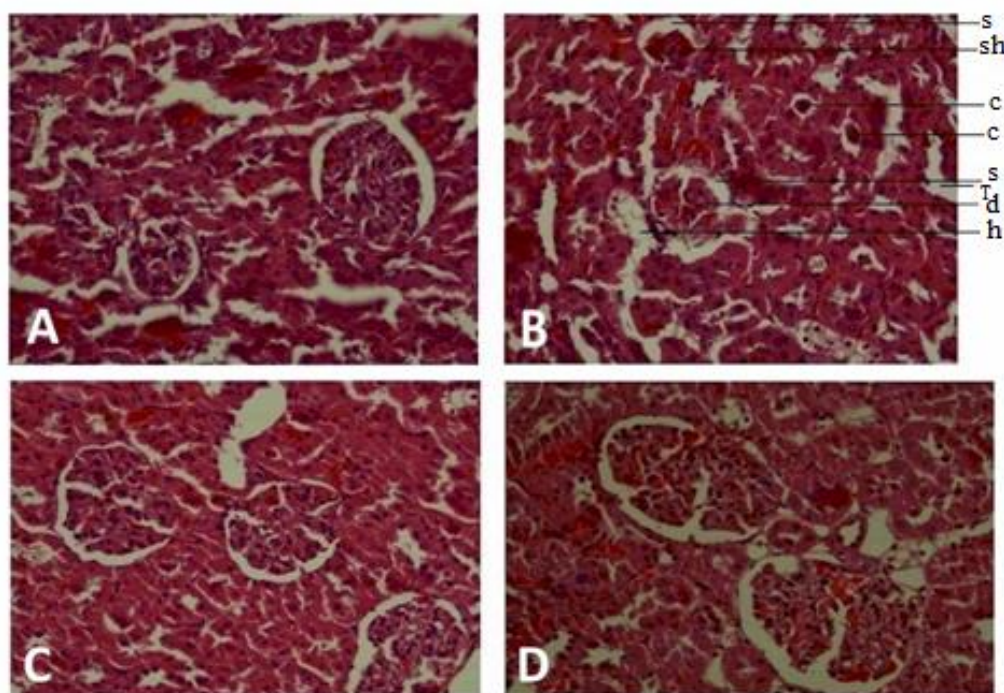


Figure 3. Photomicrographs of Kidneys of (A); Normal Control Group Showing Normal Histological Structure of Glomeruli and Proximal Convoluted Tubules. (B); STZ Treated Group Showing Shrinkage of Some Glomeruli (sh) and Degeneration “d” of Others with Wide Urinary Space “s” and Hydropic Degeneration in some Tubular Epithelial Cells “h”, Dilated Tubules “T” and Cell Debris Scattered in Tubular Lumina “c”. (C); STZ and Flaxseed Treated Group Showing Improvement of the Kidney Architecture. (D); STZ and Glibenclamide Treated Group Showing Restoration of the Normal Kidney Structure. H&E, x400.

treatment on the structures of the kidney in obese spontaneously hypertensive/NIH-corpulent (SHR/N-cp) rat,³¹ and STZ rats treated with other plants extract such as curry leave extract,³⁰ *Zingiber officinale* extracts²⁶ and zerumbone tropical ginger sesquiterpene.³² It is found that flaxseed has protective effects against renal damage caused by diabetes but it was less than that of glibenclamide. Lignans which is the main constituent of flaxseed, by its anti-hyperglycemic and antioxidant activities may explain the mechanism associated with the nephroprotective activity against diabetic nephropathy.¹⁵

Conclusions

This study showed that intraperitoneal STZ injection of 60 mg/kg body weight induced diabetes in Sprague Dawley rats manifested in structural derangements in the pancreas. Moreover, oral treatment of diabetic rats with ethanolic extract of flaxseed restores the histopathological changes of pancreas and kidneys. The preventive effect of ethanolic extract of flaxseed is less than that of glibenclamide. However, ethanolic extract of flaxseed is considered as a good alternative treatment for management of DM and its complications such as diabetic nephropathy.

Conflicts of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgement

This study was supported by the grant program from RMC, International Islamic University Malaysia; Research Endowment Fund Grant No. (EDW B 14-214-1099).

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