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THE THERAPEUTIC ROLE OF *CINNAMOMUM CASSIA* EXTRACTS IN DIABETIC MALE ALBINO RATS MODEL INDUCED BY STZ

Fikry Ali Abushofa[1]*, Ahmed Basher Abdulhamed[2], and Sara Bashir Ali EL-Hengary[3]
[1,3]Department of Zoology, Faculty of Science, University of Zawia, Libya
[2]Department of Biology, Faculty of Education, University of Zawia, Libya
**Author for Correspondence: f.abushofa@zu.edu.l*

ABSTRACT
Background: Diabetes mellitus is a syndrome with a disorder of carbohydrate, fat, and protein metabolism characterized by high blood glucose levels (hyperglycaemia). It is caused by either a lack of insulin secretion (type 1 diabetes) or decreased sensitivity of tissues to insulin (type 2 diabetes). Chronic hyperglycaemia leads to the production of multiple biochemical sequels and chronic disorders such as cardiovascular disease, as well as retinopathy which is a leading cause of adult blindness. Other long-term complications include nephropathy and kidney failure, neuropathy and peripheral nerve damage, and impaired peripheral circulation which leads to gangrene. Cinnamon is a strong anti-oxidant for its ability to reduce oxidative stress in the long-term treatment of streptozotocin (STZ)-induced diabetes in animal models. many performed studies on rodents have shown that some antioxidants could decrease blood glucose in diabetes *Objectives***:** The present study aims to design a model of diabetes in albino rats simulating diabetes using STZ to verify the potential therapeutic role of cinnamon extract on pancreatic tissues in male rats. *Materials and Methods:* In the current study, 3 groups of 8 male rats each were used; The negative control was given normal nutrition, 2nd group was a positive control injected intraperitoneally (60 mg\kg) of Streptozotocin, and 3rd group was injected with Streptozotocin (60mg/kg) intraperitoneally single dose and then gavage with Cinnamon for 21 days. On the last day of the experiment the animals were killed then blood samples and the whole pancreas with parts of the gut were collected. The histopathological investigation was conducted for the pancreas tissues of all groups. *Results:* Histological and biochemical results indicate a significant improvement. Where treatment of male rats with cinnamon showed evidence of cellular regeneration among the islets of Langerhans, which display a structure similar to that of normal islets. Atrophic change in the acinar cells is also less severe and the borders between exocrine and endocrine portions have become more distinct**.** *Conclusion:* It can be concluded that the current study has succeeded to obtain a rat model that mimics diabetes using STZ. The study also showed that cinnamon has a positive effect on beta cells whereas decreasing the level of glucose in the blood and reducing its symptoms. Therefore, diabetic patients are advised to take cinnamon extract in limited quantities during the week, due to its excellent ability to lower blood sugar levels and improve pancreatic beta cells**.**

Keywords*: Diabetes mellitus, cinnamon aqueous extract, STZ, hyperglycaemia, and pancreatic beta cells pathophysiological change*

INTRODUCTION
Diabetes mellitus is a syndrome with a disorder of carbohydrate, fat, and protein metabolism characterized by high blood glucose levels (hyperglycaemia). It is caused by either a lack of insulin secretion (type 1 diabetes) or decreased sensitivity of tissues to insulin (type 2 diabetes). In addition, chronic hyperglycaemia leads to the production of multiple biochemical sequels and chronic disorders such as cardiovascular disease, as well as retinopathy which is a leading cause of adult blindness. Other long-term complications include nephropathy and kidney failure, neuropathy and peripheral nerve damage, and impaired peripheral circulation which leads to gangrene (Ormazabal *et al.*, 2018)

Metabolic disorders occurring in diabetic patients include high blood sugar, either because the pancreas does not produce enough insulin or because cells do not respond to the insulin that is produced. Somehow, the pancreatic function is disturbed, resulting in impaired blood sugar levels.

Glucose is a vital component for various cells in the body, especially in the brain, since glucose is the only source of energy available to brain cells, and also in the muscles and most other tissues. The occurrence of disorders in blood sugar levels as a result of diabetes can lead to many health problems. The blood sugar level in a normal healthy person range between 70 and 100 mg/dl during fasting and before breakfast, while it increases in people with diabetes to reach more than 126 to 140 mg/dl during the first hour or so after a meal. The feedback systems for the control of blood glucose then return the glucose concentration rapidly back to the control level, usually within 2 hours after the last absorption of carbohydrates.

Conversely, in starvation, the gluconeogenesis function of the liver provides the glucose that is required to maintain the fasting blood glucose level (Laird and McFarland, 1996), (Mutel *et al.*, 2011).

Insulin is a hormone made by the pancreas that acts like a key that allows glucose from food to pass from the bloodstream into cells in the body in order to produce energy. All carbohydrate foods are broken down into glucose in the blood, and insulin helps the glucose get into the cells. A diabetic patient has classic symptoms that are commonly experienced, including the need to urinate frequently and feeling thirsty as a result of blood sugar levels that are higher than normal.

It is important here to consider oxidative stress, which is related to many processes that occur in the human body. It is a state caused by an imbalance between the production and accumulation of reactive oxygen species (ROS) in cells and tissues and the capability of a biological system to detoxify these reactive products (Pizzino *et al.*, 2017), (EL-Hengary et al., 2023). Diabetes-induced oxidative stress could play a role in the symptoms and progression of the disease. ROS may result in the overproduction of oxygen-free-radical precursors and decreased efficiency of the antioxidant system. The generation of oxygen free radicals is associated with the auto-oxidation of glucose, impaired glutathione metabolism, alterations in the antioxidant enzymes, and the formation of lipid peroxides (Gupta *et al.*, 2007).

Cinnamon is strong anti-oxidants and has been shown to reduce oxidative stress in the long-term treatment of streptozotocin (STZ)-induced diabetes in animal models. A study by Khaki et al. (2014) investigated the anti-oxidant effects of ginger and cinnamon on diabetic rats (Khaki *et al.*, 2014). Cinnamon extract to control hyperglycaemia in hyperlipidaemic and diabetic rats affected all of the groups, and especially the diabetic rats, which the authors attributed to the enhancement of insulin secretion in the beta cells of the pancreas. Cinnamon extract lowered the levels of total cholesterol, TG, VLDL, and LDL and improved the level of HDL in hyperlipidaemic and diabetic rats. In the diabetic rats treated with cinnamon, lower levels of RBCs and platelets count were significantly improved (Mahmood *et al.*, 2015).

MATERIALS AND METHODS

Animals

Twenty-four male albino rats with an average age (14-15 weeks) and weight at the beginning of the experiment (weeks and g) were used in the study. Rats were housed in plastic cages 8 animals in each group in the Animal House, Al-Zawiya Centre for Medical Research, for 7 days before the start of the experiment to acclimate. All rats were grouped into three groups; the first one is negative control; which was not subjected to any treatment. The remaining sixteen rats were induced to be diabetic through intraperitoneal injection of a single dose (60 mg/kg) of streptozotocin (STZ)(Al-Hariri, 2012). These diabetic rats were divided into two groups; rats of the diabetic group served as a positive control with no treatment; rats of the diabetic Cinnamon group were administered 30 mg/kg/day of the oral aqueous extract day for 21 constitutive days.

Chemicals:

Streptozotocin (C8H15N3O7) was purchased from Bouattour Chemical Equipment and Services Company, supplied by Sigma-Aldrich (Catalog no. S0130 SIGMA, Germany) Tunisia office. 30 mg of STZ was weighed and put into a 2.5 ml Eppendorf tube with aluminum foil coverage, the citrate buffer was prepared

in advance immediately prior to injection, and STZ dissolved in the 50 mM sodium citrate buffer (pH 4.5) to a final concentration of 30 mg/ml. Using a 1-ml syringe for (i.p) injection.

Preparation of Plant extracts:

Cinnamon; was purchased from the local market, then washed with distilled water and left to dry at room temperature, then ground in an electric mixer and then weighed 50 mg of powder and mixed with 150 ml of distilled water, and heated at 88 ºC, before filtering leave it to cool. The animals received 20 ml/kg/day of the oral aqueous extract by dosage up to 21 days.

Measurement of Serum Biochemical Parameters

The serum activities of ALT, AST were measured according to the methods described by Reitman and Frankel, (1957). Serum ALP activity was determined according to Kind et al., (1980). Serum glucose was determined using Trinder method (1969).

Methods:

Experimental Design

I. Control groups:

Group 1: - Negative control (NC), this group was given a normal diet for 21 days.

Group 2: positive control (DM), which is injected intraperitoneally (i.p) with signal dose (60 mg\kg) of Streptozotocin.

II. Treated groups:

Group 3: (CDM); rats were injected with Streptozotocin (60mg/kg) intraperitoneally single dose. Then starting from the third day of the injection the extract of cinnamon was given daily for 21 days orally.

RESULTS

Histopathological result:

Pancreatic tissues samples were studied of the three groups and a comparison was conducted between normal pancreatic Langerhans islets and those of STZ-induced diabetic rats and groups treated with cinnamon extracts. The comparisons showed various histopathological patterns of pancreatic tissue, including both endocrine and exocrine components, in terms of tissue degeneration, signs of inflammation, and atrophy. On the other hand, it was possible to demonstrate histological improvements in the pancreatic tissues of treated rats.

Effect of single STZ injection (60 Mg/kg, IP) on blood glucose:

The results show that the glucose level in the negative control (NC) group was normal during the experimental period (21 days), while the positive control group has shown a significant increase in glucose levels during the experimental period 570±27 as shown in figure 1.1.

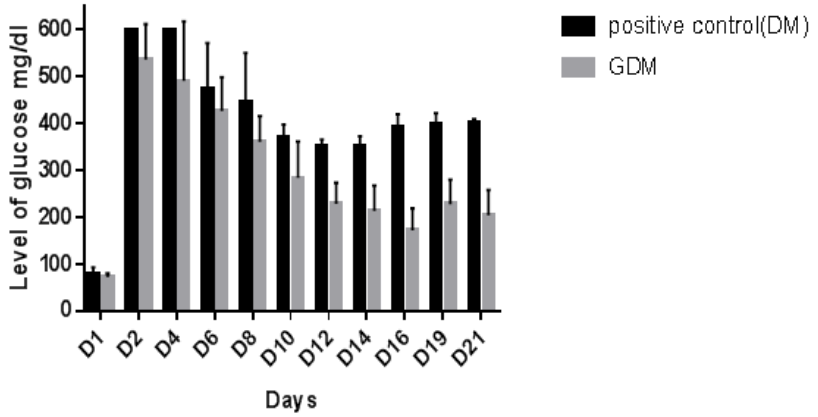

Figure 1.1 Showing the effect of cinnamon on glucose levels for treating diabetic rats.

The group treated with cinnamon showed an improvement and a continuous decrease in blood sugar levels, especially after the tenth day of cinnamon gavage.

Liver function in diabetic rat

The results show a clear and significant increase in the levels of liver enzymes (ALP, AST, and ALT) in the diabetic group ($p<0.0001$). In the group treated with cinnamon a significant decrease was found in the levels of the ALP and AST enzymes ($p<0.0001$), while that for the enzyme ALT remained high.

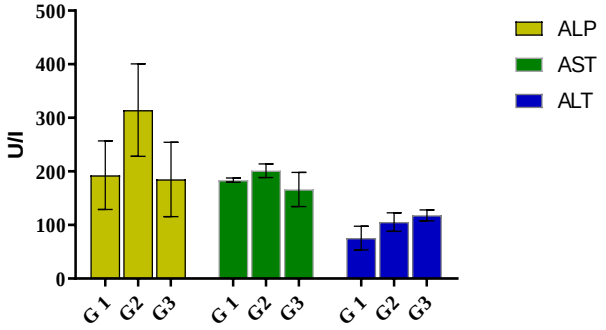

Figure 1.2: Changes in liver enzymes in treated and non-treated diabetic rats

Histological alterations in STZ-induced diabetic rats (DM) compared with normal rats (NC):

The histopathology investigation of pancreas sections in Figure 1.3, of the negative control group of rats revealed the normal architecture of the pancreas. A great number of Langerhans islets emerged normal rounded proportions were found all around the pancreatic acini. Prominent nuclei are seen arranged and the pancreatic lobules were surrounding islet cells. The islets appeared more lightly stained than the surrounding acinar cells. The islet cells are seen to be interspersed between the acinar cells. The exocrine component forms of the pancreas are closely packed by acinar cells and arranged in small lobules. Pancreatic lobules are separated by intact intralobular and interlobular connective tissue septa. The acinar cells are formed of pyramidal cells with basal nuclei and apical acidophilic cytoplasm. The main and interlobular ducts were lined with single layers of cuboidal epithelium (Fig. 1.3- A). However, the rats which received STZ (DM) exhibited pathological changes in both exocrine and endocrine components. Cellular damage to the pancreatic acini and islets showed pancreatic β-cell damage and degeneration, with the islet β-cells almost entirely lost. The acinar cells were swollen and small asymmetrical vacuoles were observed in almost all of these cells (Fig 1.3-B).

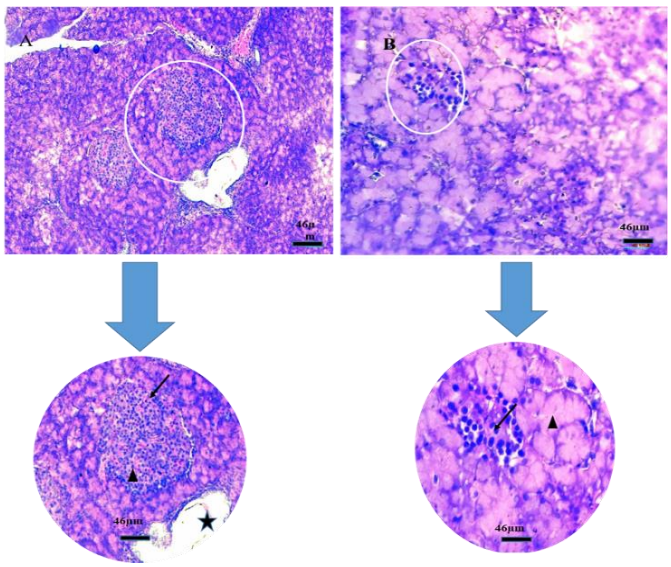

Figure 1.3: Photomicrographs sections of the pancreas stained by H&E

Photomicrograph A; Negative group showing granulated cytoplasm of islet cells with small, dark nuclei on the periphery (alpha-cells) (arrow) but with light and large nuclei (β cells) (arrowhead), and the lumen in the main pancreatic duct is lined by a single layer of cuboidal duct cells (star); photomicrograph B; pancreas section of a diabetic rat showing cytoplasmic degenerative changes in most islet cells, especially in the centre of the islet (arrow). The acinar cells were swollen, with small vacuoles in almost all acinar cells (arrowhead).

Comparison of histological alterations in cinnamon-treated rats (CDM) and diabetic rats (DM)

Figure 1.4 shows the pancreatic tissue of diabetic rats treated with cinnamon extract (CDM), which may indicate that both endocrine and exocrine components improve. The photomicrograph of a rat in the cinnamon-treated group shows evidence of cellular regeneration among the islets of Langerhans, which display a structure similar to that of normal islets. Atrophic change in the acinar cells is also less severe and the borders between exocrine and endocrine portions have become more distinct.

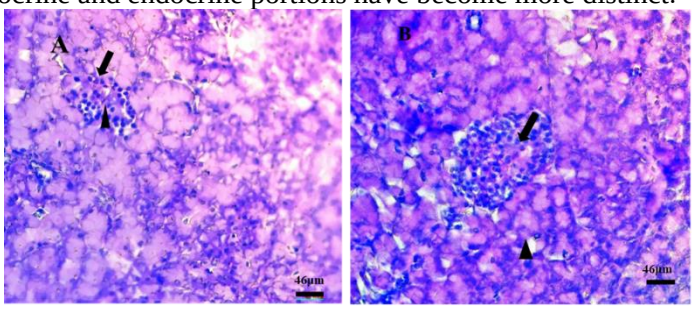

Figure 1.4 Photomicrographs of sections of the pancreas stained by H&E

A, section from pancreas of a diabetic rat showing degenerative cytoplasmic changes in most islet cells (arrow) and especially in centre of the islet beta-cells (arrow head); B, Pancreatic islet of Langerhans showing improvements in increasing beta cells and less atrophy (arrow), while atrophic change in the acinar cells is less severe and the borders between exocrine and endocrine portions are more distinct (arrowhead).

DISCUSSION

Streptozotocin is an antibiotic that destroys pancreatic islet β-cells and is widely used experimentally to produce a model of type 1 diabetes mellitus (T1DM). The current study succeeded in designing a rat model that simulates type I diabetes. The animals were injected with Streptozotocin solution intraperitoneally (IP) with a single dose (60 mg/kg body weight). It is important to note that previous studies vary in the use of STZ concentrations in obtaining a diabetic animal model. It is advisable to find a model that mimics diabetes at the lowest possible dose to avoid the suffering of rodents as required according to animal rights ethical guidelines. Sihota *et al*., (2020) succeeded in preparing a model using female rats which were stimulated with STZ at a concentration of 35 mg/kg IP to develop T2D in female Sprague-Dawley rats (Sihota *et al.*, 2020). (Annadurai *et al.*, 2013) induced diabetes in overnight-fasted Wistar rats (150–180 g) by intraperitoneal injection with STZ (50 mg/kg body weight)(Annadurai, Thomas and Geraldine, 2013). The dose prepared at this concentration was sufficient to obtain a rat model that mimics diabetes. Meanwhile, a study by (Hmza *et al.*, 2013) obtained a rat model by the intraperitoneal injection of a single dose of STZ (65 mg/kg), where T1DM was induced in overnight-fasted male Sprague- Dawley rats (Adewole and Ojewole, 2007) used STZ on Wistar rats at a concentration of 75 mg/kg of body weight in a single dose. All animals treated with STZ displayed glycosuria, ketonuria, hyperglycaemia, hypo-insulinemia, and moderate loss of body weight. The histological analysis confirmed the diabetic model in finding pancreatic tissue degeneration with necrotic changes and shrinkage of the islets of Langerhans Obtaining such a model was the starting point for the purpose of the experiment with various aqueous extracts and determining their potentially positive effects in the treatment of hyperglycemia. STZ-induced hyperglycaemia has been described as a useful experimental model in the study of the activity of antidiabetic agents with or without insulin (Szkudelski, 2012), (Singh and Pathak, 2015). Animals are employed in assessments of the pathological consequences of diabetes and for the screening of potential therapies for the treatment of this condition (Furman, 2015). In the current

study, the blood sugar level increased in the positive control group 10 hours after giving the dose to more than 570 ± 27mg/dl, then it decreased after 24 hours to reach more than 350mg/dl in all animals of the affected group. During the 21 days of the experiment, it did not fall below 325mg/dl. This finding is consistent with Yanlin who reported that the positive group showed a glucose level of 526 ± 12 mg/dl (TG) in diabetic rats (n = 15), He explained that due to inflammatory cell infiltration into the pancreatic islets are strong evidence of inflammation in the STZ-induced diabetic model (Wang-Fischer and Garyantes, 2018).

The current study emphasized that STZ led to pancreas tissue pathological changes in both endocrine and endocrine components, including cellular damage to acinar and pancreatic islets that showed cell damage and degeneration. An almost complete loss of islet cells was observed, along with swelling and small asymmetric vacuoles in almost all acinar cells. In order to evaluate the therapeutic role of antioxidants such as cinnamon on the effects of diabetes, the present study includes an evaluation of histological changes. It has been reported that cinnamon is possess a variety of medicinal properties, including hypoglycaemic, hypocholesterolaemic, and hypolipidaemic activity (Herman *et al.*, 1995). The current study focused on the extent of changes in the Islets of Langerhans, especially beta cells, as well as studying the biochemical changes in terms of measuring the level of glucose in the serum throughout the duration of the experiment, depending on most of the studies indicated an improvement in pancreatic tissue by measuring the level of insulin in the blood, and an increase in its level is clear evidence of an improvement in beta cells and the return of insulin secretion.

Evaluating the level of liver enzymes (ALT, AST, and ALP), following up on weight measurements of animals, and evaluating changes in body weight in all groups.

Our findings show that cinnamon has a positive effect on blood glucose, where decrease in glucose levels was observed in the group treated with cinnamon extract, which is consistent with the conclusions of previous research by Shalaby and Saifan (2014) who studied the effect of cinnamon. They assessed some of the pharmacological effects of aqueous extracts of cinnamon and ginger on obese diabetic Sprague-Dawley rats which were subcutaneously injected with alloxan, cinnamon and ginger aqueous extracts. The findings included significantly reduced body weight and body fat mass, normalized serum levels of liver enzymes, an improved lipid profile, reduced blood glucose and leptin and increased insulin serum levels in the obese diabetic rats. Both extracts also increased the activity of kidney antioxidant enzymes (Shalaby and Saifan, 2014).

A study by Li *et al.*, (2013) was designed to investigate the effects of 14 days of intragastric ally-administered cinnamon polyphenols in treating mice with diabetes induced by the intraperitoneal injection of streptozotocin (150 mg kg) and fed on a high-sugar and high-fat diet (Li *et al.*, 2013) . The diabetic mouse model was successfully established through a fasting blood glucose test to determine the level of sugar, and the treatments resulted in the significant down-regulation of blood glucose and insulin levels in serum, while the levels of oxidative stress markers were markedly lowered according to the ELISA assay. To conclude, it seems that cinnamon elicits the regulation of glucose metabolism in tissues by insulin-mimetic effect and enzyme activity improvement, and many in vivo studies confirmed that.

Conclusion

It can be concluded that the current study has succeeded to obtain a rat model that mimics diabetes using STZ. The study also showed that cinnamon has a positive effect on beta cells whereas decreasing the level of glucose in the blood and reducing its symptoms. Therefore, diabetic patients are advised to take cinnamon extract in limited quantities during the week, due to its excellent ability to lower blood sugar levels and improve pancreatic beta cells (Silva *et al.*, 2022)**.**

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