Modulatory Role of Inulin Extract against Metabolic and Structural Changes in the Ovaries and Uterus of Induced Diabetic albino Rats

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ABSTRACT

Background: Chronic hyperglycemia is associated with long-term damage in the structure and functions of female gonads, especially the ovaries and uterus. Inulin is the major component of Chicory (Cichorium intybus L.) roots and leaves. Inulin is used to prepare appropriate low caloric foods for diabetic patients to maintain blood sugar levels. Aim: This work was mainly designed to evaluate the ameliorative role of inulin extract on the metabolic and structural changes in the ovaries and uterus of female rats neonatally induced by streptozotocin. Material and Methods: Twenty-four offsprings of female rats were used in the present work and separated into 4 groups (n=6). Group 1: control group, group 2: inulin supplemented group (10mg /kgbw), group 3: the streptozotocin-induced group that subjected to a single intraperitoneal dose of streptozotocin (80 mg/Kgbw), and group 4: Streptozotocin plus inulin extract. Body weight, levels of female sex hormones, antioxidants, and lipid profiles were measured. Also, the ovarian and uterine specimens were also processed for histological and immunohistochemical investigation. Results: The obtained results revealed a remarkable significant decrease in the levels of serum antioxidants (superoxide dismutase and catalase) and female sex hormones as well as a significant increase in serum lipid profiles in the diabetic group. Moreover, several histopathological signs appeared in the ovarian and uterine sections of the diabetic group including, atretic follicles, degenerated germinal epithelium, stromal hemorrhage, fragmented endometrial glands, and cellular hypertrophy. Furthermore, the ovarian sections of the diabetic group displayed strong positive expression for NFκB and the uterine sections showed weak expression for B-cell lymphoma-2 if compared with the control. Conclusion: Inulin has a potential role in the amelioration of disrupted ovarian and uterine structure of diabetic rats through exerting hypoglycemic, hypolipidemic, antioxidant, anti-apoptotic effects and modulation of female sex hormones.

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INTRODUCTION

Diabetes mellitus (DM) is a category of metabolic disturbance manifested by hyperglycemia, altered metabolism of carbohydrates, protein, and lipids resulting from insulin deficiency, or action, or both[1]. There are two major types of DM; type1 DM (insulin dependent DM) and type2 (non-insulin-dependent DM). Type 1 DM may appear under 40 years old as a result of inadequate secretion of insulin by the β-cells in the pancreas or disturbance of carbohydrate metabolism[2]. The declined insulin caused lipid metabolism, give rise to elevated levels of triglyceride, free fatty acids and HDL[3]. Type 2 DM, in which the β-cells do not produce adequate insulin or faulty utilization of this hormone. DM can also induce female infertility by causing amenorrhea, faulty ovulation and tubal disease that intervenes with oocyte pickup, and disturbance in gamete transport and implantation[4]. Radaelli et al, reported that, the hypothalamic-pituitary-gonadal axis is disrupted in diabetic females[5]. The levels of serum glucose have been correlated with the levels of luteinizing hormone (LH) reply to gonadotropin releasing hormone (GnRH)[6]. Another study on mice declared that DM is implicated in abnormal ovarian development and uterine structure[7]. Also, DM can disrupt the antioxidants through induction of free radical liberation which leads to oxidative damage to cell components[8].

It is well documented that utilization of efficacious foods and their vital components can be used to ameliorate and prevent lifestyle-associated diseases like obesity, dyslipidemia, hypertension, and diabetes mellitus[9]. In fact, some of the medicinal plants can have a bigger impact on blood sugar control and lifespan than usual hypoglycemic drugs. Chicory (Cichorium intybus L.) is a widespread plant especially in the winter among the fields of clover and wheat in Egypt. The major component of chicory roots and leaves is the inulin which is a polymer of fructose...
with glycosidic linkages. Inulin is also found naturally in onion, dahlia tubers, leek, banana, wheat, asparagus and garlic[10,11]. Inulin is used to prepare appropriate low caloric foods for diabetic patients to maintain the blood sugar levels because it give only 25–35% of energy if compared to usual digestible carbohydrates[12]. Moreover, inulin can alleviate polycystic ovary syndrome (PCOS) through anti-inflammatory effects[13]. It has been suggested that inulin fructans can maintain the blood glucose levels of lipid profiles and insulin[14] as well as can improve the glucose tolerance[15]. Other studies confirmed that feeding on chicory roots decreased the levels of plasma glucose, cholesterol, triglyceride, and total lipids of streptozotocin-diabetic rats[16].

Accordingly, the present study was designed to evaluate the potential ameliorative role of inulin extract on the structural changes of the ovaries and uterus of STZ-induced rats that exposed to diabetes induction at 2days old.

MATERIAL AND METHODS

1. Chemicals: Streptozotocin (STZ) was purchased from Sigma Company in Cairo, Egypt.

2. Inulin (Cichorium intybus L) extract was obtained from the National Center of Agriculture Research, Cairo, Egypt. Inulin is prepared from the aqueous extract of sun-dried chicory roots using differential precipitation by ascending series of ethanol (20, 40 and 60 %). For yielding of better inulin, chicory roots should be extracted with water at pH 7[17].

3. Experimental design

Experimental animals

Twenty adult albino rats (fifteen virgin females and five healthy males) weighing 120-130 g were obtained from breeding stock in the laboratory animal department, faculty of pharmacy, Mansoura University, Egypt. The animals received standard diet and allowed free excess to water ad libitum throughout the period of the experiment. After two weeks of acclimatization; adult virgin females were kept with healthy adult male overnight for mating. After ensuring of pregnancy through the observation of vaginal plugs, pregnant rats were separated and preserved in their cages until parturition. All procedures were performed in accordance with the guidelines of the bioethics committee of Mansoura University.

Induction of diabetes

After parturition, twenty-five 2 days old newborn of rats were injected intraperitoneally by a single dose of STZ (80 mg/kg)[18]. After weaning (21 days postnatal), female offspring were isolated from their mothers. The blood sample was collected from the tail vein to measure blood glucose level. Hyperglycemic offspring above 150 mg/dl was isolated and considered as a diabetic individual and represented by 12 offspring while, the thirteen non-diabetic individuals were excluded. This model of diabetic neonatal female rats is used specifically to elucidate the complications of diabetes on the changes of their uterus and ovaries after two months from diabetes induction.

Experimental groups

Twenty-four offspring of female albino rats were used in the present work.

Group I (control): included 6 offspring without treatment.

Group II (Inulin): included 6 offspring that received a daily oral dose 10 mg / kgbw of inulin powder from the 6-week age for two weeks using a very fine gastric tube to avoid any stress[19]

Group III (Diabetic): included 12 female offspring that were injected with intraperitoneal dose of streptozotocin at 2nd day postnatal. After six weeks from the start of experiment offspring were divided into two groups (n=6) as follows:

- Group A (Diabetic): include 6 rats that kept as diabetic for two months post-natal.
- Group B (Diabetic and inulin): included six diabetic rats that received a daily oral dose of 10 mg / kg body weight from the 6-week age for two weeks.

Sample collection and tissue preparation

At the end of the experimental (two months) period, the fasted rats were weighed and sacrificed. Blood samples were collected in clean dry nonheparinized tubes then centrifuged at 3000 rpm for 15 minutes and kept frozen at -20oC for further biochemical analysis. Rats were then dissected; the two ovaries and uterus were removed. The ovary and uterus from each rat were processed for histological and immunohistochemical study.

Investigated Parameters

Estimation of body weight

The animals of each experimental group of rats were weighed weekly to record the body weight changes among the different studied groups.

Serum Analysis

The blood serum samples were collected weekly to estimate the following parameters.

i. Determination of serum glucose

The serum glucose levels of the different experimental animals were estimated using SPINREACT diagnostics kit, Spain. The intensity of the color formed was proportional to the glucose concentration in the sample.

ii. Measurement of sex hormones levels

The levels of LH and follicular stimulating hormone (FSH) were determined using ELISA specific diagnostic kits (DRG Instruments GmbH, Germany). Estrogen and
progestrone was measured using their specific diagnostic kits (Diaplus Inc. USA). To get accurate results from hormonal tests, each group is divided into two sub-groups: in the follicular stage and in the luteal stage of the ovary depending on the sexual period of each rat[20].

iii. Measurement of insulin level

Insulin was measured by enzyme linked immunosorbant assay (ELISA) Kit purchased from Boehringer Mannheim, Germany, using Boehringer analyzer ES300. This method is depending on the oligoclonal technique in which diversified Insulin was measured by enzyme-linked immunosorbent assay (ELISA) Kit purchased monoclonal antibodies aimed against specific epitopes of insulin.

iv. Measurement of serum lipid profiles concentration

Serum blood cholesterol, triglycerides, and low density lipoprotein (LDL) were measured with phosphotung state and magnesium[21].

v. Determination of serum malondialdehyde and antioxidants (catalase and superoxide dismutase)

Malondialdehyde (MDA) and catalytic action of enzyme catalase (CAT) were estimated spectrophotometrically. MDA was assessed by thiobarbituric acid (TBA) assay based on the liberation of color complex due to reaction TBA with MDA. CAT activity was evaluated by the assay based on the rate of a hydrogen peroxide/ammonium molybdate complex formation[23]. Measurement of serum superoxide dismutase (SOD) was done according to the method of Sun et al.[23].

Light microscopic studies

The ovaries and uterus of all groups were removed and fixed in 10% neutral formalin solution for 3 days then prepared for.

i. Hematoxylin and Eosin (H&E) stain

The 5-6-micron thick sections of ovaries and uterus were obtained, stained with H&E stain to evaluate their histological structures in all studied groups[24].

ii. Nuclear Factor Kappa B (NFκB) and b-cell lymphoma-2(BCL-2) Immunohistochemistry

For immunohistochemical demonstration of NFκB, the ovarian embedded paraffin sections were incubated in primary antibodies for NFκB (Santa Cruz Biotechnology, Santa Cruz, CA; 1/1000) for 24 hrs at 4 °C. Antibody detection was performed with the Histostain-Plus Bulk kit (Invitrogen) against rabbit IgG, and 3, 3′-diaminobenzidine was used to visualize the final product.

For BCL-2, the uterine sections were blocked using goat serum for 15 minutes at 37 °C to inhibit non-specific antibody adherence and then kept separately in mouse-anti-human BCL-2 (primary antibody) at 4 °C overnight. After that the sections were washed in PBS (three times), and then incubated with the biotin-assorted goat anti-mouse IgG for 30 minutes at 37 °C. The sections were washed again in PBS, and then incubated in streptavidin-peroxidase at 37 °C for 30 minutes. Staining was displayed with 3, 3′-diaminobenzidine at room temperature for 10 minutes.

Finally, the prepared ovarian and uterine sections were counterstained by hematoxylin solution and placed in xylene for 5 min and mounted with a mixture of diestrene, a plasticizer and xylene (DPX) to preserve stain. The immune-histochemical prepared slides were examined, microphotographed using an Axioscop 2 plus microscope (Zeiss, Germany) with a Leica camera (DFC 320 digital, Germany).

Flow cytometry study

i. Caspase-8 assay in the ovary

The ovarian cell suspensions were prepared using buffered PBS/BSA, incubated with antibody (FITC Rabbit Anti- Active Anti-Caspase-8), shake well and preserved at room temperature for 30min. The cells were immersed in BD Perm, centrifuged at 400g for 10 min and the supernatant was removed. Finally, the cells were re-suspended in BD Perm/wash and resolved by flow cytometry technique.

ii. Assay of TGF-β1 in the uterus

The number of cells was determined in uterine suspension of PBS. The energetic TGF-β1 percentage in 200 mL of suspension from PTCs was calculated using an enzyme-associated immunosorbent test (ELISA; R&D Systems, Minneapolis, MN, USA). The total protein content of lyzeduterine cells was determined, and TGF- β1 was concerned by the percentage of control.

Statistical Analysis

Data are expressed as means ± Standard error. Means in the same row with different superscript letters are significantly different (P<0.05) (one-way ANOVA). When P<0.05, *Significant at P-value ≤ 0.05** Significant at P-value ≤ 0.01 and *** Significant at P-value ≤ 0.001.

RESULTS

Body weight changes

The result of the present work revealed non-significant change in the body weight of the inulin feeding group however, the mean body weight of STZ treated group of rats was significantly decreased (P<0.001) if compared with control. On the other side, the mean body weight in the STZ induced diabetic rats post-supplemented with inulin extract appeared significantly higher if compared with STZ treated rats but did not reach to the normal body weight as control (Figure 1A).

Blood glucose level

In both control and inulin feeding groups, the blood glucose level was appeared in the normal range however, the blood glucose level of STZ-induced rats appeared significantly higher (P>0.001) than that of control. Moreover, in STZ treated group post-supplemented with
inulin extract, the blood glucose level was significantly declined to the normal value as control (Figure 1B).

The changes in hormones

In STZ induced diabetic rats, the serum levels of LH, FSH, estrogen, progesterone and insulin appeared significantly lower (P<0.001) than control group. In the diabetic group post-supplemented with inulin extract, the levels of serum LH, FSH appeared with non-significant change with control while the levels estrogen, and progesterone as well as insulin still significantly lower (P<0.001) than control but significantly higher (P<0.001) than STZ-treated group (Figure 1C).

The changes in lipid profile

In inulin supplemented rats, the levels of LDL, cholesterol, and triglycerides appeared with non-significant change if compared with control. In STZ-induced group, the serum levels of LDL, cholesterol, and triglycerides appeared significantly higher (P<0.001) than those of control. Moreover, in diabetic rats post-treated with inulin extract, the serum levels of LDL, cholesterol, and triglycerides showed high significant decrease (P<0.001) if compared with diabetic rats but still significantly higher compared with control (Figure 1D).

Changes in MDA and Antioxidants

The serum levels of MDA, CAT and SOD appeared in the normal range either for control or inulin feeding group. In the STZ treated group, the serum levels of CAT and SOD showed remarkable significant decrease however the MDA level appeared significantly higher (P<0.001) if compared with control. On the other side, the diabetic group post-supplemented with inulin extract showed remarkable amelioration in spite of little atritic follicle still found in some area of the section (Figures 2C,D). Furthermore, in diabetic rats post-treated with inulin extract, the levels of serum MDA, CAT and SOD showed remarkable significant decrease however if compared with diabetic group post-supplemented with inulin showed remarkable amelioration of the uterine section that represented by rebuilding of endometrial lining cells, intact stromal connective tissue and glands (Figure 3E).

Histological Observations

The Ovary

The histological architecture of the ovary appeared normal whereas, the endometrial epithelium consisted of pseudostratified columnar cells and underlying highly cellular connective stroma rich with blood vessels. Additionally, the endometrial stroma appeared regular and lined with simple columnar epithelial cells (Figures 3A,B). In STZ-induced diabetic rats, the uterine sections displayed severe histopathological signs including fragmented endometrial endothelial lining with scattered necrotic cells as well as pronounced damaged endometrial glands were appeared in the sections (Figures 3C,D). On the other side, the uterine section of the diabetic rats post-supplemented with inulin showed remarkable amelioration of the uterine section that represented by rebuilding of endometrial lining cells, intact stromal connective tissue and glands (Figure 3E).

Immunohistochemical Observations

NFκB in the ovary

In control and inulin supplemented groups, the nuclei of ovarian stroma cells and little cells of follicles exhibited weak immune expression for the NFκB protein (Figures 4A,B). However, the ovarian section of STZ-induced diabetic rats showed strong positive immune reaction for NFκB. Such immune expression apparently confined to nuclei of ovarian stroma cells as well as granulosa cells of Graffian follicles (Figure 4C). On the other side, the ovarian section of the diabetic group post-supplemented with inulin extract displayed weak immune reaction for NFκB that more confined to the nuclei of granulosa cells of Graffian follicles and the cells of ovarian stroma (Figure 4D).

BCl-2 in the uterus

The immunohistochemical expression of BCL-2 protein appeared moderate to strong in the uterine sections of control and inulin supplemented groups (Figure 5A,B). Such reaction was more localized in the cytoplasm of endometrial cells. In contrast, the uterine section of the STZ-induced diabetic rats revealed very weak immune expression for BCL-2 protein (Figure 5C). In the diabetic group post-supplemented with inulin extract, the uterine sections revealed moderate immune BCL-2 reaction (Figure 5D).

Flow cytometric analysis

Caspase-8 in the ovary

The obtained flow cytometric data indicated that, the mean percentage value of caspase-8 activity in the processed ovarian cells appeared higher (76.53%) in STZ-induced diabetic rats if compared with control (40.81%) and inulin (41.14%) supplemented group. On the other side, in diabetic group post-supplemented with inulin extract, the mean percentage values of caspase-8 activity was declined (63.07%) if compared with diabetic rats but still higher than control (Figures 6A-D).

TGF-β1 in the uterus

The flow cytometric results revealed that the mean
El-Beltagy et al., percentage value of TGF-β1 in the uterine tissues of diabetic rats appeared higher (76.69%) than control (15.59%) and inulin (16.30%) supplemented group. In the diabetic rats post-supplemented with inulin extract, the mean percentage values of TGF-β1 was decreased (25.34%) than diabetic rats but still higher than control (Figure 6 A1-D1).

Fig. 1: The body weight changes (A), levels of blood glucose (B), hormones; LH, FSH, Estrogen, progesterone and insulin (C), lipid profiles; LDL, cholesterol and triglyceride (D) and levels of CAT, SOD, and MDA (E) among the different studied groups of rats. Note: a highly significant decrease in the body weight, highly significant increased levels of serum glucose, lipid profiles, and highly significant decreased levels of female sex hormones and antioxidants with highly significant increased level of MD in STZ treated rats if compared with control.
Fig. 2: Photomicrograph of histological sections through the ovaries of the different studied groups. Note: the normal histological structure of the ovary in control (A) and inulin (B) groups. In diabetic group (C&D), the ovarian sections showing atretic follicles, fragmented germinal epithelium and scattered vacuoles in stromal cells. In diabetic plus inulin group (E), the ovarian section showing remarkable amelioration in its histological architecture [H&E, x250].
Fig. 3: Photomicrograph of histological sections through the uterus of the different studied groups. Note: The uterine sections of control (A) and inulin (B) supplemented groups appear with normal histological structure. In diabetic group (C&D), the uterine sections showing severe damage including fragmented uterine endothelium as well as damaged endometrial glands and pronounced necrotic cells. In diabetic & inulin group (E) the uterine section showing remarkable amelioration in its histological architecture [H&E, x250].
Fig. 4: Photomicrograph of paraffin embedded sections through the ovary stained with NFκB antibody. Note: A weak to moderate immuno-reactivity for NFκB in the ovarian sections of control (A) and inulin (B) supplemented groups; however the ovarian tissues of STZ-treated group (C) display strong positive reaction for NFκB. The ovarian sections of diabetic rats post-supplemented with inulin (D) showing a weak reaction [Anti- NFκB immunohistochemical stain, x250]. Arrows heads refers to the immunoreactivity of NFκB

Fig. 5: Photomicrograph of paraffin embedded sections through the uterus stained with BCL-2 antibody. Note: A moderate immune reaction for BCL-2 appears in the uterine sections of control (A) and inulin (B) supplemented groups however, a negative to weak immune expression appears in the uterine sections of diabetic group (C). In diabetic and inulin group (D), the uterine sections appears with a moderate to strong reaction for BCL-2 [Anti-BCL2 immunohistochemical stain, x250] Arrows heads refers to the immunoreactivity of BCL-2
Figure 6: A flow cytometry chart represents the mean % value of caspase-8 in ovarian tissues (A-D) and TGF-β1 in uterine tissues (A1-D1) for the different studied groups. Note: Highly percentage value of caspase-8 in the ovarian tissues of STZ treated rats (C) if compared with control (A) and inulin (B) supplemented groups. However, the ovarian tissues of diabetic rats post-supplemented with inulin (D) shows low % value of caspase-8 if compared with diabetic group but still highly increased if compared with control. The % value of TGF-β1 in uterine tissues appears highly elevated in STZ treated rats (C1) if compared with control (A1) and inulin (B1) supplemented groups. However, the uterine tissues of diabetic rats post-supplemented with inulin (D1) reveals low % value of TGF-β1 if compared with diabetic group but still highly increased if compared with control.

**DISCUSSION**

Chronic hyperglycemia is associated with long-term damage in the structures and functions of different body organs, especially the sexual organs, kidneys, nerves, and cardiovascular system[25]. Medicinal plants have been used since long times for the treatment of diabetes and continue to be currently accepted as an alternative therapy[26]. Previous researches have been showed that inulin from chicory has a potential role in modulation of plasma glucose level and lipid profiles in diabetic rats[12,16]. Accordingly, this work was carried out to evaluate the ameliorative role of inulin extract from Chicory (Cichorium intybus L.) against adverse effects of diabetes on the structure and function of the ovarian and uterine tissues of rats neonatally induced by streptozotocin.

The obtained data revealed that the mean body weight of STZ-induced rats was significantly lower than the other studied groups. Previous studies had been found that STZ could decrease body weight through the induction of hyperglycemia and decreased glucose uptake by the cells and stimulation of lipolysis. Additionally, the reduction in the body weight of the diabetic individuals could be attributed to the breakdown of tissue proteins[27]. On the other side, supplementation of inulin extract to STZ-treated group revealed marked recovery in their body weights. It has been suggested that the inulin-type fructans maintain the body weight through the regulation of lipid metabolism and insulin level[15].

In the current work, the blood glucose level in STZ-induced rats was significantly higher than that of the other studied groups. It had been reported that streptozotocin could inhibit the secretory power of β-cells of pancreas via the negative feedback mechanism and also decrease glucose uptake by the cells[28]. Other studies revealed that
INULIN EXTRACT ATTENUATES GONADAL DYSFUNCTION

STZ causes the destruction of pancreatic β-cells, leading to insufficient production of insulin\(^{[29]}\). Flavonoids and polyphenols in inulin extract could activate glucose metabolism through activation of β-cells and increase glucose uptake by the cells\(^{[33,34]}\). In this study supplementation of inulin extracts to diabetic group showed significant restoration of serum glucose level.

In the current work, the serum levels of FSH, LH, estrogen, progesterone, and insulin were significantly lowered in diabetic rats, however, the levels of these hormones were markedly ameliorated in the diabetic group post-supplemented with inulin extract. The elevated hormones in diabetic rats in this study agree with previous reports\(^{[29]}\). It had been reported that the decreased serum FSH and LH levels in diabetic neonatal rats are related to the direct inhibitory effect on the secretory gonadal axis pituitary. Moreover, the diminished level of serum estrogen and progesterone is a feedback mechanism to the consequential decreased level of secreted FSH and LH\(^{[29]}\). Furthermore, the significant decreased level of insulin is attributed to the direct destructive effect of STZ on the β-cells of the pancreas\(^{[30]}\). Another study postulated that STZ could resist the action of insulin in the cells and subsequently inhibits the secretory capacity of β-cells through the negative feedback mechanism\(^{[31]}\). A previous study reported that fructans in inulin extract may stimulate the secretory power of the pituitary gland to release gonadotropins\(^{[32]}\). Such finding goes parallel with our obtained result.

In the STZ induced diabetic rats, the data concerning lipid profiles showed significant increase in the levels of serum LDL, cholesterol and triglycerides, however, post-supplementation of inulin extract to diabetic rats, the levels of lipid profiles were markedly restored near to the normal values as in control. The elevated serum lipid profiles in diabetic condition are attributed to induction of adipose tissue lipolysis as a result of insulin decrease and consequently, mobilization of free fatty acids from the peripheral depots since insulin inhibits the activity of lipase hormone\(^{[33]}\). The decreased levels of lipid profiles post-supplementation of inulin extract to diabetic rats may be attributed to the hypolipidemic effect of polyphenolic compounds of inulin extract.

Previous reports declared that diabetic hyperglycemia is a causative factor for the progress of oxidative stress which is followed by elevated lipid peroxidation end product (malondialdehyde) and decreased levels of SOD and CAT\(^{[33,34]}\). Such finding is in line with our obtained results. In the current work significant increase in the two enzymatic antioxidants; CAT and SOD but significant decrease in the MDA was recorded in the ameliorated group with inulin extract if compared with the non-treated diabetic group. It had reported that inulin extract is the medicinally active constituents in chicory and has been shown to have anti-diabetic and antioxidant effects\(^{[35]}\), anti-inflammatory and antihepatotoxic activities\(^{[36]}\). Inulin had been assessed for its potential role in the synthesis of antioxidants in the body\(^{[37]}\), regulation of glucose metabolism and antioxidant status in diabetic individuals\(^{[38]}\).

In the current work, several histopathological observations were appeared in the ovarian sections of STZ induced diabetic rats, including, degenerated germinal epithelium, atretic follicles, decreased number of oocytes, hemolytic stroma and scattered vacuoles. The obtained results go parallel with the findings of previous studies\(^{[39]}\). Lavender et al reported that deficiency in insulin secretion or its action had a direct deleterious effect on the ovarian structure through inhibition of FSH and LH secretions\(^{[40]}\). In STZ induced rats, the uterine sections showed severe damage of endometrial endothelium, obvious necrotic and vacuolated cells as well as damaged endometrial glands. As mentioned above, the decreased secretion of estrogen may be considered the main factor for the destruction of the uterine endometrium\(^{[40]}\). In the current work, supplementation of inulin extract to the diabetic group showed ameliorative effect on the histological structure of ovary and uterus. The ameliorative capacity of inulin extract may be attributed to its enhancing role in modulation of FSH and estrogen secretion.

NFκB protein is associated with the extrinsic cell death pathway, which can be activated by pro-apoptotic signals, including activation of death receptors\(^{[41]}\). The immunohistochemical study of the present work revealed that, NFκB protein was highly expressed in the ovarian follicles and stroma of diabetic rats. The obtained result goes parallel with the finding of Erbas et al, who reported that overexpression of NFκB protein in tissue is sign for follicular inflammatory pathway induced by oxidative stress of diabetes\(^{[39]}\).

BCL-2 is an oncoprotein, which inhibits the rate of apoptosis\(^{[42]}\). The obtained results indicated that, the uterine tissues of STZ-induced diabetic rats appeared weakly stained with BCL-2 protein. This result indicates that, the cellular apoptosis is enhanced in cases of diabetes leading to weight loss\(^{[43]}\). In the diabetic rats supplemented with inulin extract, the immune-reactivity for BCL-2 was moderately expressed. This result may be attributed to the anti-inflammatory and anti-oxidant capacities induced by the nutritional ingredients of inulin extract to maintain apoptosis\(^{[40]}\).

Flow cytometry can be applied to evaluate chromatin condensation, cytoplasmic dehydration, and shrinkage during apoptosis. The obtained results of flow cytometry analysis revealed that the mean percentage values of caspase-8 in the ovarian tissues and TGF-β in the uterine tissues of STZ-induced diabetic rats were appeared higher than control. Caspases are critical mediators of programmed cell death and serve as markers of apoptosis\(^{[44]}\). Activated caspase 8, perform as progenitor caspase, which split cell death or directly stimulates apoptosis\(^{[44]}\). It has been suggested that diabetes can induce apoptosis of granulosa cells and inhibition of ovarian angiogenesis\(^{[45]}\). Other hypothesis reported that STZ administration
stimulates cellular apoptosis through down-regulation of BCL-2 protein as this compound inhibits apoptosis by sequestering preforms of death-driving cysteine proteases or caspases[47]. It had been confirmed that over-expression of TGF-β protein in various tissues is a good key marker for fibrosis[48]. Currently, supplementation of inulin extract following STZ exposure significantly reduced the level of apoptotic markers; caspase-8 in the ovarian tissues and profibrotic protein; TGF-β in the uterine tissues although such markers did not reach statistical significance. El-Sayed et al., reported that inulin extract from Cichorium intybus plays a significant role in the inhibition of apoptosis and tissue fibrosis through modulation of cellular division[49].

CONCLUSION

Inulin extract from Cichorium intybus has a potential role in amelioration of disrupted ovarian and uterine structure of diabetic rats through exerting hypoglycemic, hypolipidemic, antioxidant, anti-apoptotic effects and modulation of female sex hormones. However, more investigations are needed to further confirm the related pathways and mechanisms.

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ABBREVIATIONS

Atretic follicles (AF), Granulosa cells (GC), Antrum (A), Stroma cells (SC), Medullary cords (MC), Blood vessel (BV), Primordial follicles (PrF), Primary follicles (PF), Corpus luteum (CL), Oocyte (O), Germinal Epithelium (GE), Degenerated germinal epithelium (DGE), Degenerated follicles (DF), Vcaules (V), Secondary follicles (ScF), Theca externa (T), Cellular hyperplasia (star), Endometrial epithelium (EE), Endometrial glands (EG), Fragmented endometrial epithelium (FEE), Damage endometrial glands (DEG) and Lumen (L).

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES


الملخص العربي

الدور التنظيمي لمستخلص الأنيولين ضد التغيرات الأيضية والتركيبية في المبايض والرحم لدى الجرذان المصابة بالسكري المحدث

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الخلفية: إن الزيادة المفرطة في سكر الدم تؤدي على المدى الطويل إلى خلل وظيفي وتركيبي في المناسل الأثنوية خاصة المبايض والرحم مما يؤدي ذلك إلى الفتق الذي كثير من الأفراد يعانون منه. يعتبر الأنيولين هو المكون الرئيسي في أوراق وذروت نبات السريس والذي يستخدم على نطاق واسع في علاج مرضى السكري نظرًا لما يحتويه هذا المركب على سكر الفركتوز ذو السعرات الحرارية المنخفضة.

هدف الدراسة: لقد صممت هذه الدراسة لإيضاح الدور التحسيني لمستخلص الأنيولين في التغييرات الأيضية والتركيبية في مبايض ورحم الإناث الجرذان المستحثة بمادة الاستريبتوذوتوسين والتي تم حقنها بعد الولادة.

المواد والطريقة: لقد تم في هذه الدراسة استخدام أربع عينات من إناث أجنة الجرذان حيث تم تقسيمهم إلى اربع مجموعات بالتساوي المجموعة الأولى: الضابطة، المجموعة الثانية: تغذية المستخلص الأنيولين، المجموعة الثالثة: التي تم حقنها بجرعة واحدة من مادة الاستريبتوذوتوسين (40 مجم/ كجم) وذلك من الأسبوع السادس بعد الولادة ولمدة أسبوعين والمجموعة الرابعة: التي تم حقنها بجرعة واحدة من مادة الاستريبتوذوتوسين (80 مجم/ كجم) وذلك في عمر يومين بعد الولادة وتكرر مسحة السكري في طوال فترة التجربة (ثمانية أسابيع) والمجموعة الرابعة هي مجموعه مشابهة بالسكرى مثل المجموعة الثالثة ولكن تم معالجتها بالأنيولين وكذلك لمدة أسبوعين (من نهاية الأسبوع السادس حتى نهاية الثامن). في نهاية الأسبوع الثامن تم نزح جميع المجموعات ثم تجميع الدم لعمل قياسات مستويات الهرمونات الجنسية، مضادات الأكسدة، مستويات الدهون، ثم تم فصل المبايض والرحم لعمل الفحص الهستولوجي والصبغات الهستوكيميائية بالإضافة إلى قياس مستوى موت الخلايا المبرمج من خلال تقنية التدفق الخلوى.

النتائج: لقد أظهرت النتائج أن المجموعة المصابة بالسكرى إذا ما قورنت بالمجموعة الضابطة حدث لها نقص نوعي في مستوى مضادات الأكسدة (سوبر أكسيد ديسميوتيزوالكاتاليز مع زيادة نوعية في مستوى الولونديدهيد). وكذلك نقص نوعي في مستويات الهرمونات الجنسية. زيادة نوعية في مستويات الدهون (الكوليسترول والدهون الثلاثية). هذا بالإضافة إلى تغيرات هستوبيولوجية مرضية في أنسجة المبايض والرحم وكذلك زيادة في عدد الخلايا الإيجابية للصبغة في خلايا المبيض ولكن نقص ملحوظ في عدد الخلايا الإيجابية الصبغة المناعية NFκB و BCL2 ضد التغيرات الأيضية والتركيبية التي سببها مرض السكري إلى مستويات قريبة لحد ما من الطبيعية.

الخصائص: استخلصت الدراسة أن مركب الأنيولين له دور حيوي وفعال في مكافحة الكثير من التغيرات الأيضية وكذلك التركيبية للمبايض والرحم التي أحدثها مرض السكري ولكن يتم التوصية بإجراء المزيد من الأبحاث في هذا السياق من الدراسة.