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

Original Article

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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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Key Words: Diabetes, histopathology, inulin, ovary, uterus.

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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

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with glyosidic 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-inflammation effects[13]. It has been suggested that inulin fructans can maintain the blood 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

progesterone 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[22]. 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 (In*vitro*gen) against rabbit IgG, and 3, 30-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 Ig G 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 distyrene, 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 (Figure1C).

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 if 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 high significant increase (P>0.001) for both CAT and SOD but significant decrease (P<0.001) in MDA level if compared with diabetic group of rats but did not reach to the normal value as in control(Figure 1E).

Histological Observations

The Ovary

In control and inulin feeding groups (Figures 2A,B), the histological structure of the ovary appeared with normal organized pattern of oogenesis including intact Graffian follicles and germinal epithelium. On the other side, the ovarian sections of the STZ-induced diabetic rats showed severe deleterious histological changes including shrunken and atretic follicles, scattered vacuoles and damaged germinal epithelium (Figures 2C,D). Furthermore, in diabetic rats post-supplemented with inulin extract, the ovarian section showed remarkable amelioration in spite of little atritic follicle still found in some area of the section (Figure 2E).

The uterus

In control and inulin supplemented groups, the histological architecture of the uterus appeared normal whereas, the endometrial epithelium consisted of pseudostratified columnar cells and underlying highly cellular connective stroma rich with blood vessels. Additionally, the endometrial glands 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

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).

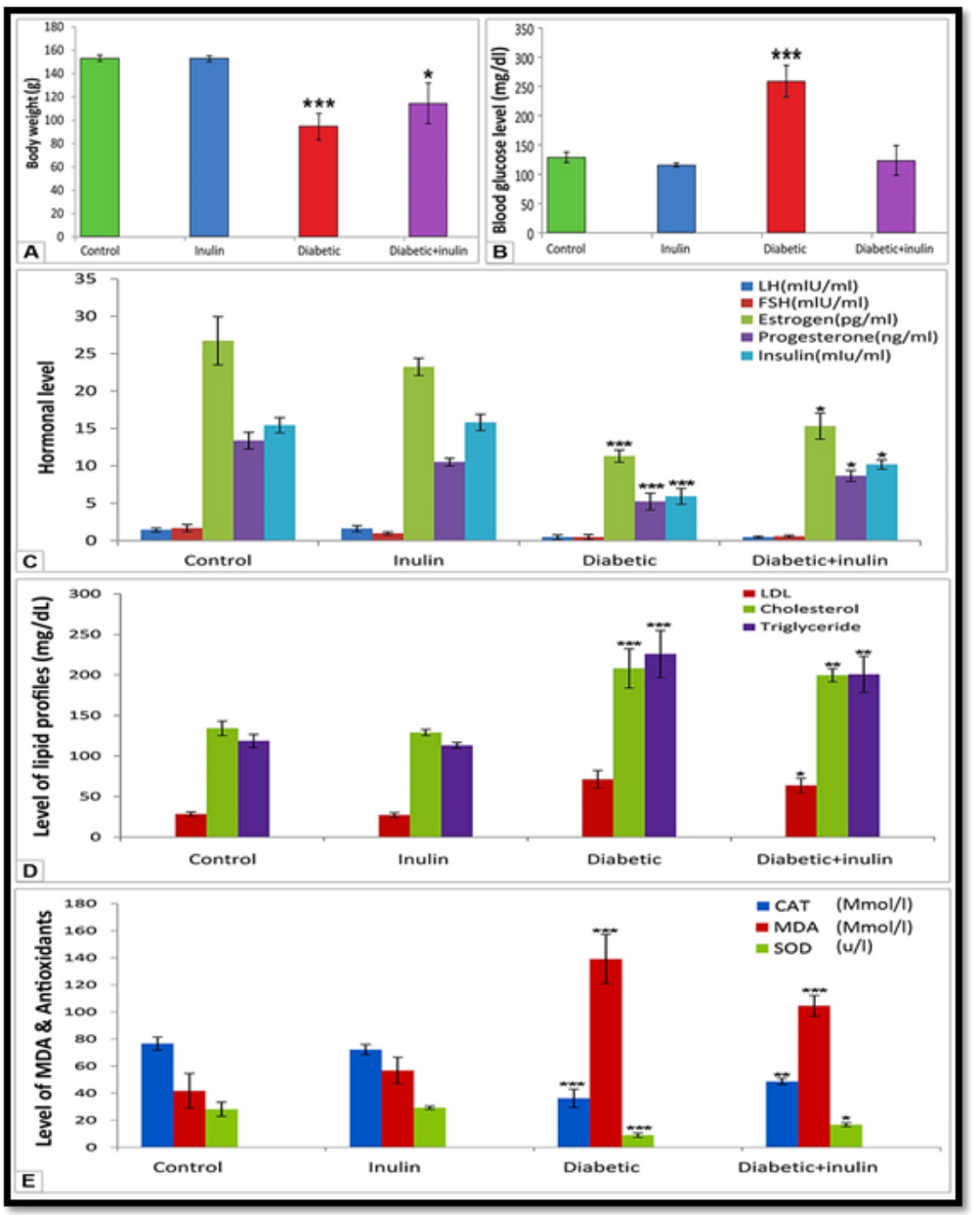

Fig. 1: The body weight changes (A), levels of blood glucose (B), hormones; LH, FSH, Estrogen, progesterone and insulin (C), lipid profiles; LDL, cholesterol and triglyceride (D) and levels of CAT, SOD, and MDA (E) among the different studied groups of rats. Note: a highly significant decrease in the 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.

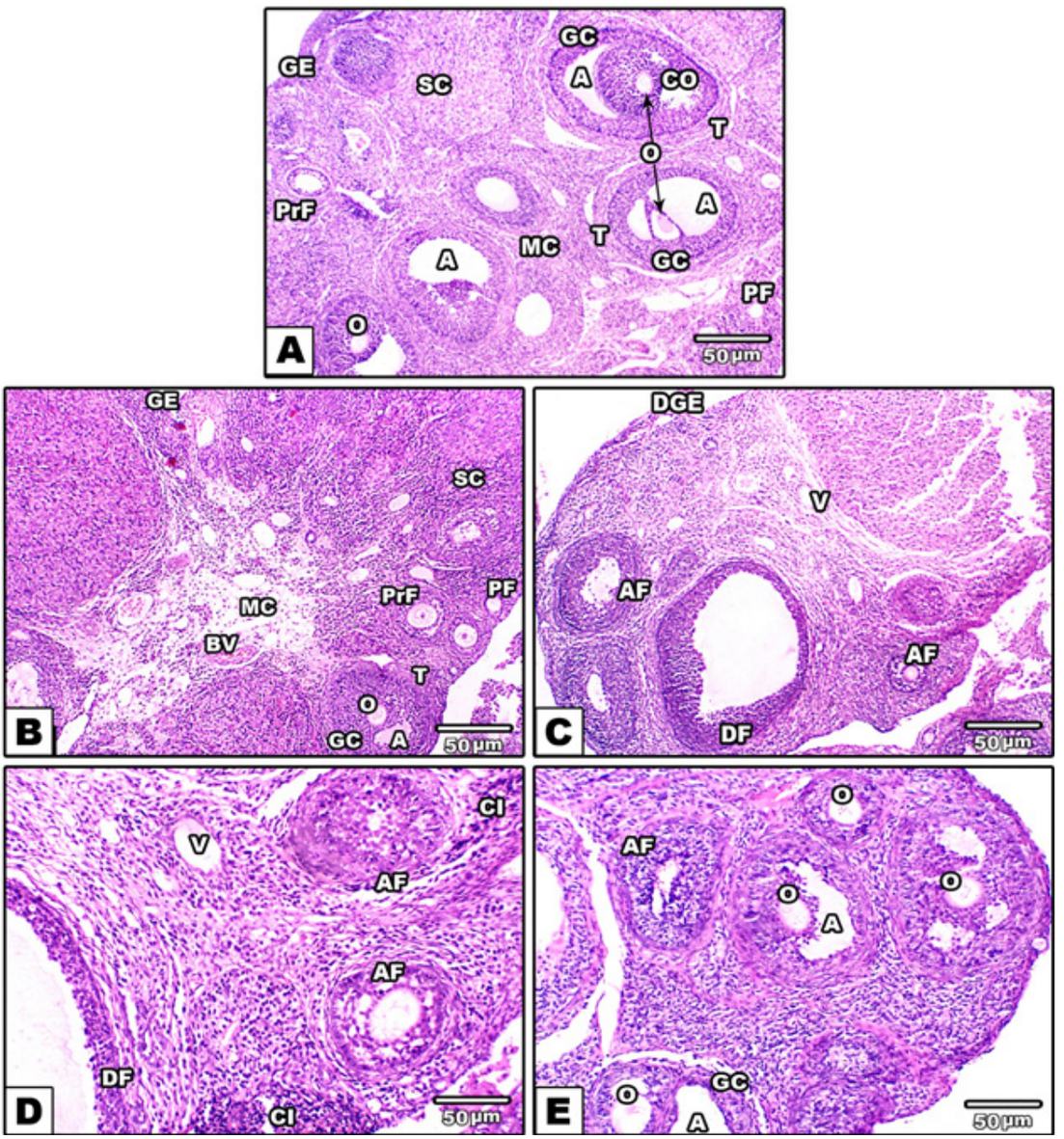

Fig. 2: Photomicrograph of histological sections through the ovaries of the different studied groups.
Note: the normal histological structure of the ovary in control (A) and inulin (B) groups. In diabetic group (C&D), the ovarian sections showing atretic follicles, fragmented germinal epithelium and scattered vacuoles in stromal cells. In diabetic plus inulin group (E), the ovarian section showing remarkable amelioration in its histological architecture [H&E, x250].

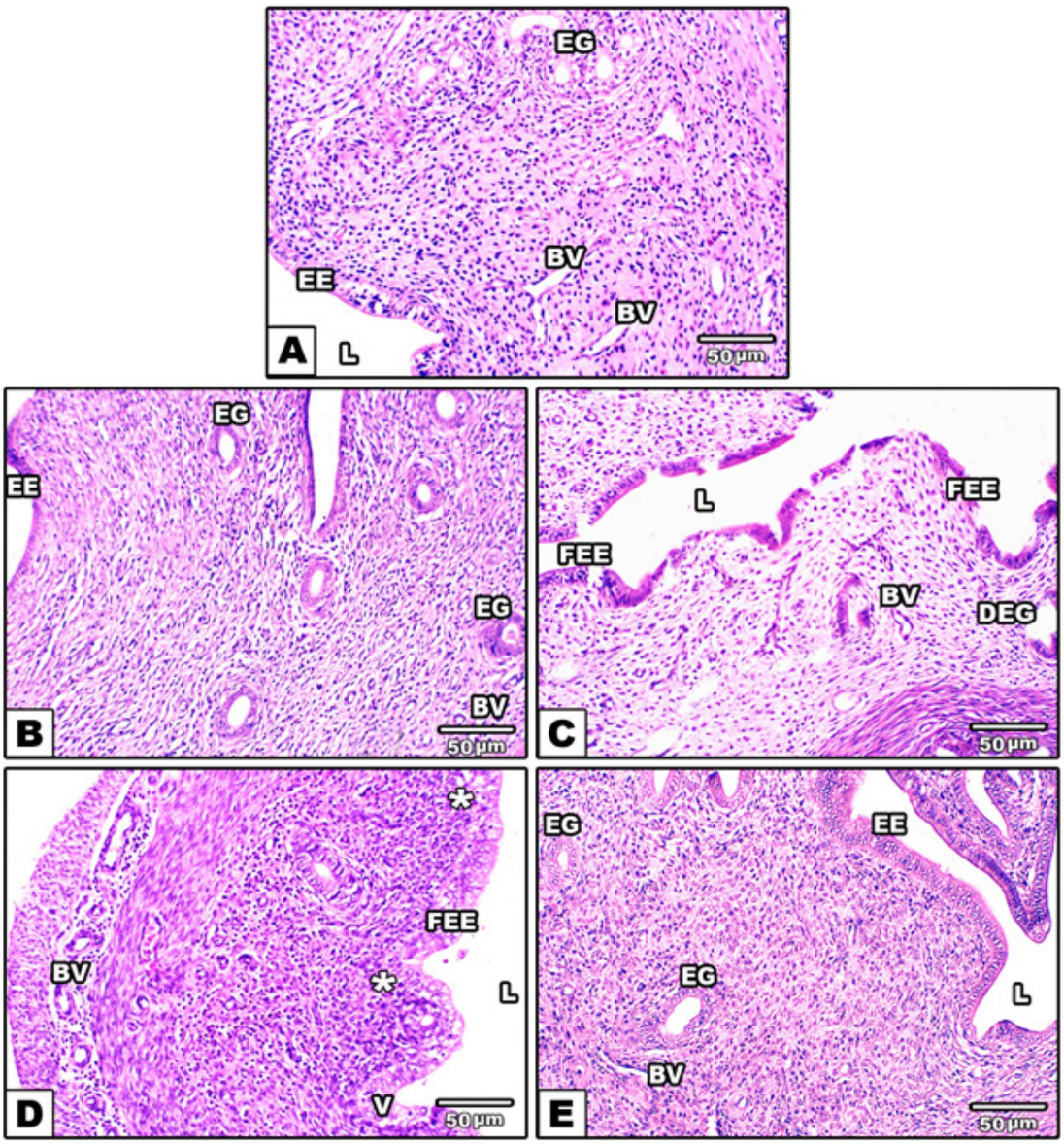

Fig. 3: Photomicrograph of histological sections through the uterus of the different studied groups. Note: The uterine sections of control (A) and inulin (B) supplemented groups appear with normal histological structure. In diabetic group (C&D), the uterine sections showing severe damage including fragmented 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].

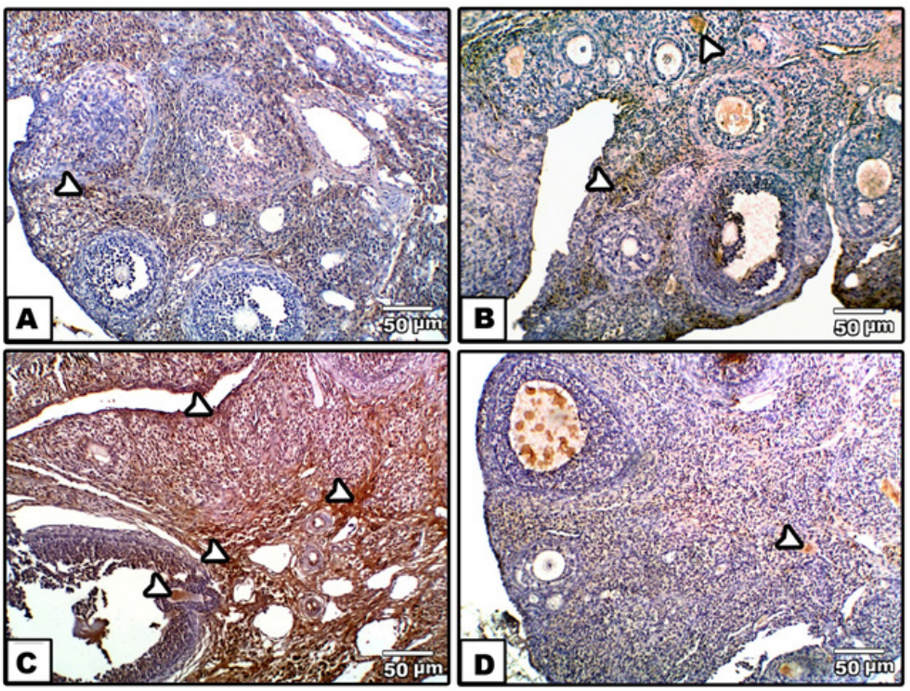

Fig. 4: Photomicrograph of paraffin embedded sections through the ovary stained with NFκB antibody. Note: A weak to moderate immuno-reactivity for NFκB in the ovarian sections of control (A) and inulin (B) supplemented groups; however the ovarian tissues of STZ-treated group (C) display strong positive 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 referes to the immunoreactivity of NFκB

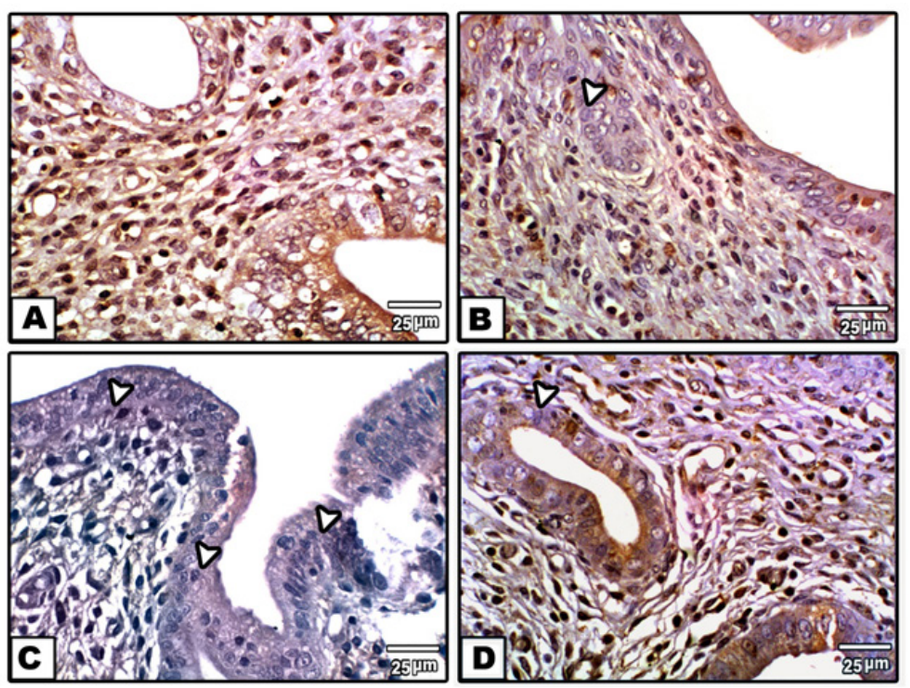

Fig. 5: Photomicrograph of paraffin embedded sections through the uterus stained with BCL-2 antibody.Note: A moderate immune reaction for BCL-2 appears in the uterine sections of control (A) and inulin (B) supplemented groups however, a negative to weak immune expression appears in the uterine sections 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

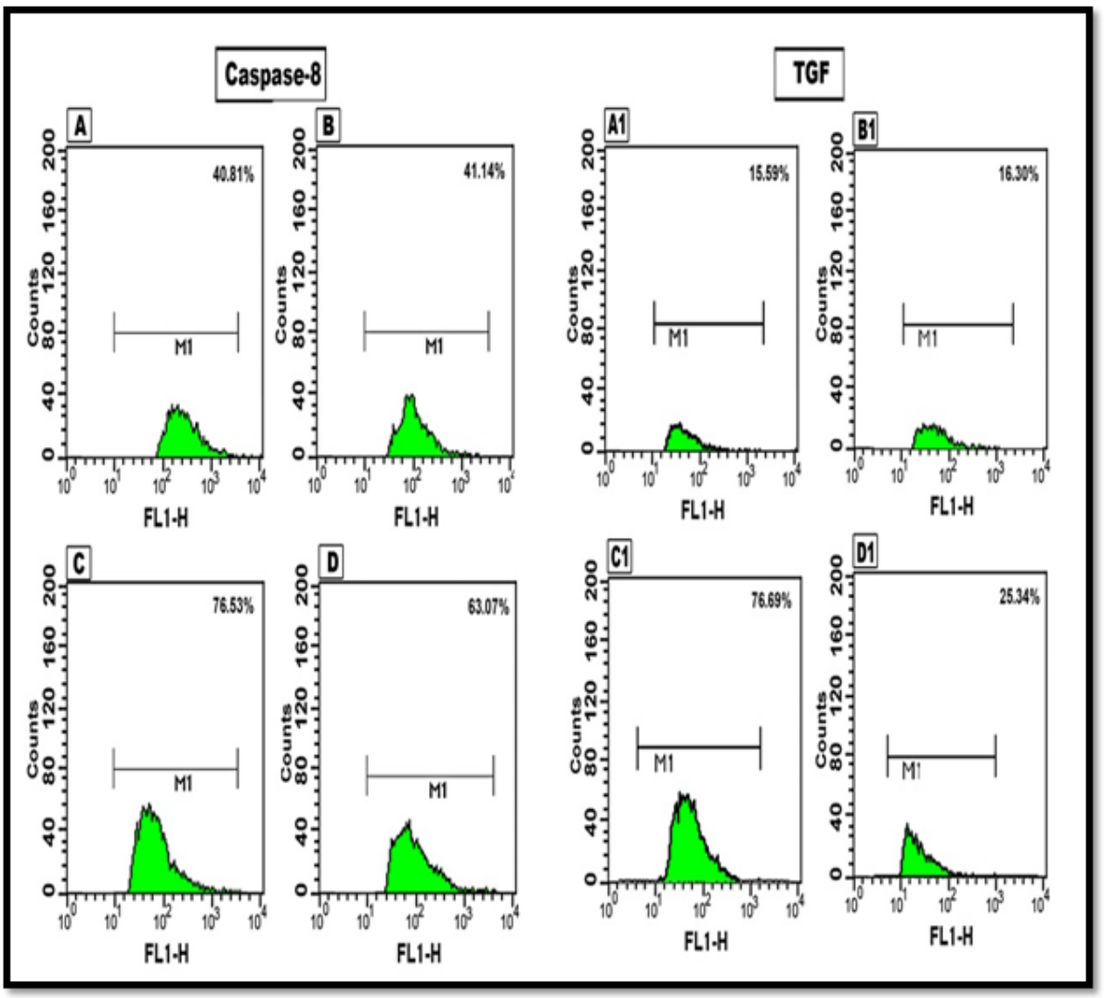

Fig. 6: A flow cytometry chart represents the mean % value of caspase-8 in ovarian tissues (A-D) and TGF- β1 in uterine tissues (A1-D1) for the different studied groups. Note. Highly percentage value of caspase-8 in the ovarian tissues of STZ treated rats (C) if compared with control (A) and inulin (B) supplemented groups. However the ovarian tissues of diabetic rats post-supplemented with inulin (D) reveals 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

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[15]. 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[15]. 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 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[4]. 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[34].

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[45]. It has been suggested that diabetes can induce apoptosis of granulosa cells and inhibition of ovarian angiogenesis[46]. 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 pro-fibrotic 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[35].

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)**, Vcauoles **(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

1. Gupta S, De, A. Diabetes Mellitus and its Herbal Treatment. International Journal of Research in Pharmaceutical and Biomedical Science 2012; 3:708-721.
2. Weinzimer SA, Steil GM, Swan KL, Dziura J, Kurtz N, Tamborlane WV. Fully automated closed-loop insulin delivery versus semiautomated hybrid control in pediatric patients with type 1 diabetes using an artificial pancreas. Diabetes Care 2008; 31:934-939.
3. Arcanjo CL, Piccirillo LJ, Machado IV, Andrade CRM, Clemente EL, Gomes MB. Lipid profile and anthropometrical evaluation in type 1 diabetes. Arquivos Brasileir of Endocrinology & Metabololism 2005; 49:951-8.
4. Lavender T, Platt M J, Tsekiri E, Casson I, Byrom S, Baker L, Walkinshaw S . Women's perceptions of being pregnant and having pregestational diabetes. Midwifery Journal 2010; 26:589-595
5. Radaelli T, Leqercq J, Varastehpour A, Basu S, Catalano PM, Hauguel-de Mouzon S. Differential regulation of genes for fetoplacental lipid pathways in pregnancy with gestational and type 1 diabetes mellitus. Am J Obstet Gynecol 2009; 201:209:1-10
6. Bützow TL, Lehtovirta M, Siegberg R, Hovatta O, Koistinen R, Seppäla M, Apter D.The decrease in luteinizing hormone secretion in response to weight reduction is inversely related to the severity of insulin resistance in overweight women. J Clin Endocrinol Metab. 2000;85(9):3271-5
7. Kim SM, Kwak DH, Kim SM, Jung JU, Lee DH, Lee S, Jung KY, Do SI, Choo YK. Differential expression of gangliosides in the ovary and uterus of streptozotocin-induced and db/db diabetic mice. Archives of Pharmacal Research 2006; 29: 666-76.
8. Bashan N, Kovsan J, Kachko I, Ovadia H, Rudich A. Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. Physiol Rev 2009; 89:27–71.
9. Keane D, Kelly S, Healy NP, McArdle MA, Holohan K, Roche HM. Diet and metabolic syndrome: an overview. Current Vascular Pharmacology 2013; 11: 842-857.
10. Roberfroid M B. Inulin-type fructans: functional food ingredients. The Journal of Nutrition 2007; 137:2493 –2502.
11. Bornet F RJ. Fructo-oligosaccharides and other fructans: chemistry, structureand nutritional effects. Advanced Dietary Fibre Technology, Blackwell Science Ltd. 2008; 480–493.
12. Shoaib M, Shehzad A, Omar M, Rakha A, Raza H, Sharif HR, Shakeel A, Ansari A, Niazi S. Inulin: Properties, health benefits and food applications. Carbohydr Polym. 2016; 147:444-454.
13. Xue J, Li X, Liu P, Li K, Sha L, Yang X, Zhu L, Wang Z, Dong Y, Zhang L, Lei H, Zhang X, Dong X, Wang H. Inulin and metformin ameliorate polycystic ovary syndrome via anti-inflammation and modulating gut microbiota in mice. Endocrine Journal 2019; 66:859-870.
14. Jackson KG, Taylor GRJ, Clohessy AM, Williams CM. The effect of the daily intake of inulin on fasting lipid, insulin and glucose concentrations in middle-aged men and women. British Journal of Nutrition 1999; 82:23–30.

15. Nishimura M, Ohkawara T, Kanayama T, Kitagawa K, Hiroyuki DH, Nishihira J. Effects of the extract from roasted chicory (Cichorium intybus L.) root containing inulin-type fructans on blood glucose, lipid metabolism, and fecal properties. Journal of Traditional and Complementary Medicine 2015; 5:161-167.

16. Azorin O M,Urban C, Ceron JJ, Tecles F, Allende A, Tomas BF, Espin JC. Effect of low inulin doses with different polymerization degree on lipid absorption and intestinal microbiota in rats with fat-metabolism, mineral supplemented diet. Food Chemistry 2009; 113:1058-1065.

17. Gupta AK, Kaur N. Preparation of inulin from chicory roots. Journal of scientific and industrial research 2003; 62:916-920

18. Da masceno DC, Kiss ACI, Sinzato YK. Maternal-fetal outcome, lipid profile and oxidative stress of diabetic rats neonatally exposed to streptozotocin. Experimental and Clinical Endocrinology and Diabetes 2011; 119: 408–413.

19. Draz SN, Abo-zid MM, Ally AF, El-Debas AA. Hypoglycemic and hypolipidemic effect of chicory (Cichorium intybus L.) herb in diabetic rats. Minufiya Journal of Agricultural 2010; 35: 1201 – 1208.

20. Westwood FR. The female rat reproductive cycle: a practical histological guide to staging. Toxicol Pathol 2008; 36: 375 – 384.

21. Lopez-virella MF, Store P, Ellis S, Colwell AJ. Cholestrol determination in high –density lipoprotein separated by three different methods. Clinical Chemistry 1977; 23:882-884.

22. Peluso M, Munnia A, Risso G, Catarzi S, Piro S, Ceppi M. Breast fine-needle aspiration malondialdehyde deoxyguanosine adduct in breast cancer. Free Radical Research 2011; 45:477–82.

23. Sun Y, Oberley, LW, Li YA. Simple method for clinical assay of superoxide dismutase. Clinical Chemistry 1988; 34:497-500.

24. Bancroft JD, Suvarna K, Layton C. Histological Techniques, 8th Edition. Bancroft's theory and practice of histological techniques.2018; 1-672

25. Kautzky-Willer A, Harreiter J, Pacini G. Sex and Gender Differences in Risk, Pathophysiology and Complications of Type 2 Diabetes Mellitus. Endocrine Review2016; 37: 278-316

26. Qamar F, Afroz S, Feroz Z, Siddiqui S, Ara A. Evaluation of Hypoglycaemic Effect of Cassia Italica. Journal of Basic and applied Science 2011; 7 (1):61-64.

27. Andulla B, Varadacharyulu NC. Antioxidant role of mulberry leaves in streptozotocin -diabetic rats. Clinica Chimica Acta Journal 2003; 338:3 -10.

28. Nagarchi K, Ahmed S, Sabus A, Saheb HS. Effect of Streptozotocin on Glucose levels in Albino Wister Rats. Journal of Pharmacy science and Research 7:67-69.

29. Pournaghi, P., Sadrkhanlou, A.R., Hasanzadeh, S. and Foroughi, A. (2012). An investigation on body weights, blood glucose levels and pituitary-gonadal axis hormones in diabetic and metformin-treated diabetic female rats. Veterinary Research Forum 2012; 3: 79 – 84

30. Albajali AA, Nagi AH, Shahzad1 M, Ikram Ullah M, Hussain S. Effect of Allium sativa L. on pancreatic β- cells in comparison to Nigella sativa L. in streptozotocin induced diabetic rats. Journal of Medicinal Plant Research Journal 2011; 5: 5779-5784.

31. Szkudelski T. The mechanism of alloxan and streptozotocin action in β-cells of the rat pancreas. Physiological Research 2001; 50: 536-546.

32. Liber A, Szajewska H. Effects of inulin-type fructans on appetite, energy intake, and body weight in children and adults: systematic review of randomized controlled trials. Annual of Nutrition and Metabolism 2013; 63:42-54.

33. Petlevski R, HadZija M, Slijep C M, Juretiand D , Petrik J. Glutathione S-Transferases and Malondialdehyde in the Liver of NOD Mice on Short-Term Treatment with Plant Mixture Extract P-9801091. Phytotherapy Research 2003; 17: 311–314.

34. Jurgonski A, Juskiewicz J, Zdunczyk Z, Król B. Caffeoylquinic acid-rich extract from chicory seeds improves glycemia, atherogenic index, and antioxidant status in rats. Nutrition 2012; 28:300-6.

35. El-Sayed YS, Lebda MA, Hassinin M, Neoman SA . Chicory (Cichorium intybus L.) root extract regulates the oxidative status and antioxidant gene transcripts in CCl4-induced hepatotoxicity. PLos One 2015; 10: 1-12

36. Ahmed B, Khan S, Masood MH; Siddique AH. Anti-hepatotoxic activity of cichotyboside, a sesquiterpene glycoside from the seeds of Cichorium intybus. Journal of Asian Natural Products Research 2008; 10:223-31.

37. Lante A, Nardi T, Zocca F, Giacomini A, Corich V. Evaluation of red chicory extract as a natural antioxidant by pure lipid oxidation and yeast oxidative stress response as model systems. Journal of Agricultural and Food Chemistry 2011; 59: 5318-24.

38. Ali A, Abdu S. Antioxidant protection against pathological mycotoxins alterations on proximal tubules in rat kidney. Functional Foods in Health and Disease Journal 2011; 4: 118-134.

39. Erbas O Pala GH , Pala EE, Oltulu F, Aktug H, Yavasoglu,A, Taskiran D. Ovarian failure in diabetic rat model: Nuclear factor-kappaB, oxidative stress, and pentraxin-3. Taiwanese Journal of Obstetrics & Gynecology 2011; 53:498:503.

40. Tariq S, Nurulain M S, Rashed H, Lotfy M , Emerald B S, Koturan S, Adeghate EK. Diabetes-induced changes in the morphology and nociceptinergic innervation of the rat uterus. Journal of molecular Histology 2016; 47(1):21-33.

41. Wajant H. Death receptors. Essays in Biochemistry 2003; 39:53–71.

42. Desouky KM, Anwar IR, Algaidi AS. Immunohistochemical Expression of Bcl-2 and Microvessel Density in Uterine Fibroids in Saudi Patients. West Indian Medical journal 2015; 2: 121-126.

43. El-Beltagy BMA, Elghaweet H. Adverse effects of monosodium glutamate on the reproductive organs of adult Female albino rats and the possible ameliorated role of carob (Ceratonia Siliqua). Journal of Bioscience and Applied Research2016; 2:2356-9182.

44. Logs MW, Esselborg S, Scqhulze-Osothoff K. The role of caspases in development, immunity, and apoptotic signal transduction: lessons from knockout mice. Immunity 1999; 10:629–39.

45. Arroba IA, Frago M, Argente SJ, Chowen AJ. Activation of Caspase 8 in the Pituitaries of Streptozotocin-Induced Diabetic Rats: Implication in Increased Apoptosis of Lactotrophs. Endocrinology2005; 146:4417–4424.

46. Wu Y, Li Y, Xinghui- Liao X, Wang Z, Li R, Zou S, Jiang T, Zheng B, Duan P, Xiao J. Diabetes induces abnormal ovarian function via triggering apoptosis of granulosa cells and suppressing ovarian angiogenesis. International Journal of Biological Science 2017; 13: 1297-1308.

47. Eleazu CO, Eleazu KC, Chukwuma S, Essien UN. Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. Journal of Diabetes &Metabolic Disorders2013; 12:1-7

48. Verheule S, Sato T, Everett TT, Engle S.K, Otten D, Rubart-von der Lohe, M., Nakajima HO, Nakajima H, Field LJ, Olgin JE.Increased vulnerability to atrial fibrillation in transgenic mice with selective atrial fibrosis caused by overexpression of TGF-beta1. Circulation Resaerch 2004; 94: 1458–1465.

الملخص العربى

الدور التنظيمى لمستخلص الإنيولين ضد التغيرات الأيضية والتركيبية في المبايض والرحم لدى الجرذان المصابة بالسكري المحدث

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الخلفية: إن الزيادة المفرطة فى سكر الدم تؤدى على المدى الطويل إلى خلل وظيفى وتركيبى فى المناسل الأنثوية خاصة المبايض والرحم مما يؤدى ذلك الى العقم الدى الكثير من الأفراد. يعتبر الأنيولين هو المكون الرئيسى فى أوراق وجذور نبات السريس والذى يستخدم على نطاق واسع فى علاج مرضى السكرى نظرا لما يحتويه هذا المركب على سكر الفركتوز ذو السعرات الحرارية المنخفضة.

الهدف من الدراسة: لقد صممت هذه الدراسة لإيضاح الدور التحسنى لمستخلص الإنيولين على التغيرات الأيضية والتركيبية فى مبايض ورحم إناث الجرذان المستحثة بمادة الأستريبتوذوتوسين والتى تم حقنها بعد الولادة .

المواد والطرق: لقد تم فى هذه الدراسة استخدام أربعه وعشرون من إناث أجنة الجرذان حيث تم تقسيمهم الى اربع مجموعات بالتساوى المجموعة الاولى: الضابطه , المجموعة الثانية: التى تم تغذيتها بمستخلص الإنيولين (١٠مجم/ كجم) وذلك من الأسبوع السادس بعد الولادة ولمدة أسبوعين والمجموعه الثالثة: التى تم حقنها بجرعة واحدة من مادة الاستريبتوذوتوسين (٨٠مجم/ كجم) وذلك فى عمر يومين بعد الولادة وتركت مصابة بالسكرى طوال فترة التجربة (ثمانية أسابيع) والمجموعة الرابعة وهى مجموعه مصابة بالسكرى مثل المجموعه الثالثة ولكن تم معالجتها بالإنيولين وذلك لمدة اسبوعين (من نهاية الأسبوع السادس حتى نهاية الثامن). فى نهاية الاسبوع الثامن تم ذبح جميع المجموعات ,تم تجميع الدم لعمل قياسات مستويات الهرمونات الجنسية والانسيولين ,مضادات الأكسدة, مستويات الدهون, ثم تم فصل المبايض والرحم لعمل الفحص الهستولوجى والصبغات الهستوكيميائية هذا بالاضافة لقياس مستوى موت الخلايا المبرمج من خلال تقنية التدفق الخلوى.

النتائج: لقد أظهرت النتائج أن المجوعه المصابة بالسكرى إذا ما قورنت بالمجموعة الضابطة قد حدث لها نقص نوعى فى مستوى مضادات الأكسدة (سوبر أكسيد ديسميوتيزوالكاتاليز مع زيادة نوعية فى مستوى المالونديالدهيد , وكذلك نقص نوعى فى مستويات الهرمونات الجنسية, زيادة نوعية فى مستويات الدهون (الكوليسترول والدهون الثلاثية), هذا بالاضافة الى تغيرات هستولوجيه مرضية فى أنسجة المبايض والرحم وكذلك زيادة فى عدد الخلايا الايجابية للصبغة الهستوكيميائية المناعية ضد NFκB فى خلايا المبيض ولكن نقص ملحوظ فى عدد الخلايا الإيجابية للصبغة المناعية ضد BCL٢ فى أنسجة البطانة الداخلية للرحم. وقد أوضحت النتائج أيضا أن مركب الأنيولين قد نجح فى تنظيم معظم التغيرات الأيضية والتركيبية التى سببها مرض السكرى إلى مستويات قريبة لحد ما من الطبيعى.

الخلاصة: أستخلصت الدراسة أن مركب الأنيولين له دور حيوى وفعال فى مجابهة الكثير من التغيرات الأيضية وكذلك التركيبية للمبايض والرحم التى احدثها مرض السكرى ولكن يتم التوصية بإجراء المزيد من الأبحاث فى هذا السياق من الدراسة.