



The role of date palm pollen against adverse effects of Diabetic Neonatal Albino rats on infertility in their adult life

Reham A. Ghanem¹, Amoura M. Abou-El-Naga², Abd El-Fattah B.M. El-Beltagy³, Mervat A. Omran² and Yasmin M. Tag^{1*}

¹Oral Biology Department, Faculty of oral and dental medicine, Delta University for Science and technology, Gamasa, **Egypt**

²Zoology Department, Faculty of Science, Mansoura University, Mansoura, **Egypt**

³Zoology Department, Faculty of Science, Damanhour University, Damanhour, **Egypt**

*Correspondence: YasminTag85@yahoo.com, Yasmine.tag@deltauniv.edu.eg Received 21-05-2022, Revised: 26-06-2022, Accepted: 03-07-2022
e-Published: 09-07-2022

Diabetes mellitus (DM) is a metabolic disorder associated with a high level of blood glucose. In addition to disorders in carbohydrates metabolism, protein, and lipids which involved in the pathogenesis of female fertility due to the accumulation of free radicals. Date palm pollens are documented as antidiabetic and antioxidant as it used to enhance reproductive function and fertility in female humans and also in laboratory animals. The present work-study the role of DPP against the metabolic and structural changes in female rat ovaries and uteri of neonatally induced by streptozotocin. Twenty-four offsprings of female rats were used and separated into 4 groups (n=6). Group 1: control group, group 2: DPP group (1mg/kgbw), group 3: the streptozotocin-induced group that subjected to a single intraperitoneal dose of streptozotocin (80 mg/Kgbw), and group 4: Streptozotocin plus DPP. Bodyweight, levels of female sex hormones, antioxidants, and lipid profiles were measured. In addition, histological and immunohistochemical examination of the ovarian and uterine specimens. Results displayed a significant depletion in the serum level of superoxide dismutase, catalase, and female sex hormone while a remarkable increase in serum lipid profile in the diabetic group. Furthermore, histopathological investigations showed the presence of atretic follicles, degenerated germinal epithelium, stromal hemorrhage, fragmented endometrial glands, and cellular hypertrophy in the ovaries and uterus in the diabetic group. Also, the ovarian section of the diabetic group displayed positive expression for NFκB and the uterine sections showed weak expression for B-cell lymphoma-2 if compared with the control. Conclusion: DPP has an ameliorative role in damaged ovaries and uteri by exhibiting hypoglycemic, hypolipidemic, antioxidant, anti-apoptotic adjustment, and female sex hormone modulation.

Keywords: Diabetes, Immunohistochemistry, Flow cytometry, DPP, Ovary, Uterus, Rats.

INTRODUCTION

Diabetes mellitus is considered a worldwide health problem. It is a metabolic disease associated with hyperglycemia that results from insulin deficiency, or because the body's cells do not respond properly to insulin, or both (Bhaskarachary and Joshi, 2018). Blood glucose is the main nutrient that supplies power to all body cells. It is absorbed by microvilli in the small intestine directly into the bloodstream to the body cells. However, glucose cannot enter the cells alone, it needs insulin to facilitate its transport into the cells (Petersen and Shulman 2018). In normal conditions; the blood glucose level is tightly controlled by insulin that is secreted from β-cells of the pancreas. Dysfunction of the pancreatic b-cells with consequent insulin deficiency to abnormalities causes resistance to insulin action which leads to the development of diabetes. The severity of diabetes is increasing day by day; as may cause many complications

like peripheral neuropathy, cardiomyopathy (Zatalia and Sanusi, 2013). As well, the National institute of diabetes and digestive and kidney diseases reported that diabetic males and female humans can develop sexual problems due to vascular and nerves damage which are essential for the normal function of the genital organs (NIDDK, 2008). Also, malformations were found in fetuses of diabetic pregnant women (Bayoumy et al. 2020). In addition, it can induce female infertility by causing amenorrhea until the betterment with insulin therapy took place. These complications are linked to poor glycemic control or improper management of this pathology (Uniuofin and Lebelo, 2020). Here, it is necessary to control the prevalence of this disease and focus on natural resources for treatment and prevention. In the medical system, diabetes is too difficult to handle without any side effects. So, our study aimed to use Date palm pollen; a famous medicinal plant as antidiabetic to support women's

fertility (Shehzad et al., 2021).

Date palm pollen shows important effects to activate pancreatic b-cells as it increases the production of insulin and decreases glucose absorption by the intestine. Dates contain many active ingredients like, phenol, flavonoids, steroids that act as anti-diabetic agents. These components inhibit realizing the free radical in diabetic conditions and play a specific role in improving the biochemical parameters in diabetic rats (Michael et al., 2016).

MATERIALS AND METHODS

Chemicals

1.1. Streptozotocin was purchased from Sigma Aldrich Company dissolved in a 0.01 M citrate buffer solution (PH 4.5).

1.2. Date palm pollen (DPP): Fresh pollen of date palm has been assembled from the garden of Mansoura University, Egypt, it was verified and authenticated by the Department of Botany, Faculty of Science, Mansoura University. DPP was separated and kept refrigerated at 4°C until use. The aqueous suspension of DPP was freshly prepared daily by adding 500mg of DPP to 5ml dist. water for oral gavage administration by gastric tube in a dose of 1mg/kg/b.wt (Mia et al. 2020).

2. Experimental animals

Twenty adult albino rats (fifteen virgin females and five healthy males) weighing 120-130 gm were obtained from breeding stock in the Laboratory of Animal Department, Faculty of Science, Mansoura University, Egypt. Standard diet and water ad libitum were allowed to the animals during the experiment. One week later, after acclimatization, adult virgin females have been mated with adult healthy males overnight (3:1). The morning when sperm was found in the vaginal smear was designated as day zero of pregnancy. Pregnant rats have been separated and preserved in their cages until parturition. The females that failed to become pregnant during this period were considered infertile and excluded from this study. All experimental procedures were performed following the guidelines of the Bioethics Committee of Mansoura University.

2.1. Induction of neonatal diabetes mellitus

Induction of diabetes to female neonates (Cheng et al. 2019). After parturition, 25 female newborns at 2 days old were injected with a single dose of 80 mg/kg streptozotocin intraperitoneally. All neonates remained with their mothers until they reached day 21. Female offspring were separated from their mothers next to the weaning period (21 days postnatal). To measure blood glucose levels, blood samples were collected from the vein of the rats' tails. About twenty-four hyperglycemic offspring above 150 mg/dl were isolated and considered as diabetic animals.

2.2. Experimental groups

Twenty-four rats at six-week age were used in the present work (12 diabetic offspring and 12 non-diabetics represented as; control 6 animals, and Date palm pollen 6 animals).

Group I (control) n=6: Animals received saline solution with no treatment.

Group II (date palm pollen) n=6: Animals received DPP oral dose daily from the 6th week till the 8th week age [9].

Group III (Diabetic) n=6: Animals kept as diabetic female rats.

Group IV (Diabetic supplemented with DPP) n=6: diabetic female rats supplemented daily oral doses of 1mg/kg body weight of date palm pollen by gastric tube from 6th week old till 8th weeks old for 2weeks.

2.3. Sample collection and tissue preparation:

The fasted female rats of control and other groups were weighed and sacrificed. Blood samples have been collected and serum was used for biochemical analysis. Moreover, dissection of the two ovaries and uteri were processed for histopathological & immunohistochemical investigations and also for flow cytometric analysis.

3. Investigated parameters

3.1. Estimation of body weight

The animals of each group were weighed weekly to record body weight changes.

3.2 Biochemical Parameters

3.1. Estimation of serum glucose and insulin hormone levels:

Serum blood glucose and insulin hormone were estimated based on the methods of Pushparaj et al. (2000); Andulla and Varadacharyulu, (2003) respectively.

3.2. Estimation of serum lipid profile level

3.2.1. Total cholesterol (CHO), Triglyceride level (TG):

The level of total serum cholesterol was evaluated based on the work of Mohamed et al. (2018), while triglycerides levels were estimated following the method of Shi et al. (2013).

3.2.2. Estimation of High –density lipoprotein cholesterol (HDL- C), Low-density lipoprotein cholesterol (LDL-C), and Very Low-density lipoprotein cholesterol (VLDL-C) levels.

Serum HDL-c, LDL-c, and VLDL-c were measured and calculated dependent on the methods of Zhang et al. (2013); Nagarchi et al. (2015); Abdel-Shaheed et al. (2021) respectively.

3.3. Measurement of female sex hormones levels in serum:

The levels of follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) were determined using ELISA specific diagnostic kits (DRG Instruments GmbH, Germany). Estrogen and progesterone was measured using their specific diagnostic kits (Diaplus Inc. USA).

3.4. Determination of serum antioxidant markers (Catalase, Superoxide dismutase, and Malondialdehyde):

The CAT activity measured obeying the Marsh (2011) method. On the other hand, the level of SOD has been determined by following the Beylot (2005) method. While the level of MDA was estimated by using Stefanska et al. (2019).

3.5. Flow cytometric study

3.5.1. Assay of caspase-8 and propidium iodide staining (Pi) in the ovary

Fresh ovarian tissue was placed in a pre-chilled phosphate-buffered saline (PBS) and followed the steps of the Goodarzi et al. (2011) method. Then, the ovarian cell suspensions were prepared with a PBS/BSA buffer and incubated with antibody (FITC Rabbit Anti- Active Anti-Caspase-8) then mixed and incubated for 30min at room temperature. Washing the cells with BD Perm/Wash (BD Bioscience) was done, centrifuged at 400 rpm for 5min and the supernatant was discarded. The cells finally were re-suspended in BD Perm/wash and analyzed using flow cytometry.

Sections of ovary for studied groups were taken, and cell suspension was prepared with Tris-EDTA buffer (pH7.4) (Sigma-Aldrich Co.). Then was fixed in ice-cold 96-100 % ethanol at 4 °C overnight, centrifuged at 1,500 rpm for 10 min, and then resuspended in PBS containing 50 µg/mL propidium iodide (PI) (Sigma-Aldrich Co.).

3.5.2. Assay of Transform Growth Factor-β1(TGF-β1) in the uterus

Uterine cells were re-suspended in PBS and the number of the cells were counted. The active TGF-β1 concentration in 200 mL of media collected from PTCs was measured using an enzyme-linked immune-sorbent assay. The total protein content of lysed uterine cells was measured and TGF-β1 was expressed by percentage of control.

4. Light Microscopic Investigations

4.1. Histopathological examination of ovary and uterus

The ovaries and uteri specimens of all the experimental animals were histologically examined according to the method of Mellembakken (2011).

5. Immunohistochemical Investigations

5.1 Nuclear factor kappa-B (NF-Kapa-B) in the ovary

For an immunohistochemical demonstration of NF-kappa-B, Incubation for 24 hours of the ovarian sections in primary antibodies (NF-kB, Santa Cruz Biotechnology, Santa Cruz, CA; 1/1000) at 4 °C. Detection of the antibody completed with the Histostain-Plus Bulk kit (Invitrogen) against rabbit IgG, and for concluding the final product, 3,30-diaminobenzidine (DAB) has been used. Photographed with an Olympus C-5050 was processed after washing sections in PBS. The ovarian stromal cell and oocyte showed brown cytoplasmic staining and were marked as positive for NF-kB. At a magnification of 100, the number of NF-kB positive cells has been estimated by scoring at least 100 ovarian stromal cells per field in 10 fields of tissue sections systematically (Wang et al.2017).

5.2. B cell lymphoma (Bcl-2), and vascular endothelial growth factor (VEGF) in the uterus

For an immuno-histochemical demonstration of Bcl-2&VEGF. To reduce non-specific antibody binding, the uterine sections have been blocked by goat serum for 20 minutes at 37 °C. After that, the sections were incubated separately with primary antibodies (mouse-anti-human BCL-2) at 4 °C overnight. Then washed three times in PBS and incubated with the biotin-labeled goat anti-mouse IgG at 37 °C for 30 minutes. Repeat washing in PBS and incubated with streptavidin peroxidase complex for 30 minutes at 37 °C. Staining with 3, 3'-diaminobenzidine (DAB) for 10 minutes at room temperature was noticed. Finally, staining with hematoxylin solution took place.

For VEGF, 0.1M PBS at pH 7.4 for 30 minutes, the uterine sections were incubated with 1% BSA and with primary polyclonal goat anti-mice VEGF antibody (diluted 1:30, Sc-1836, Santa Cruz Biotechnology) for 12 hours at 4°C. Moreover, washing the sections in PBS then incubated for 1 hour at room temperature with secondary biotinylated donkey anti-goat IgG (diluted 1:300, Sc-2042, Santa Cruz Biotechnology). Sub sequentially, the material was washed in Tris-HCl buffer (TBS) and treated with peroxidase streptavidin (diluted 1:300, Vector SA-5004). The peroxidase was exposed with 0.5mg diaminobenzidine tetrahydrochloride (DAB; Sigma) in 0.3% H₂O₂ in TBS. Carrazi's Hematoxylin was used as counterstained. Then the slides were dehydrated and mounted with Entellan (Merck, Darmstadt). Negative controls were performed by the exclusion of the primary antibody incubation step (Shalash et al.2020).

5.4. Computer-Assisted digital image analysis

Slides of immunohistochemical stain were photographed by aiding of Olympus® digital camera installed on Olympus® microscope with 1/2 X photo adaptor, utilizing objective lens 40 X. For stain quantification and area measurement, Intel® Core I3® based computer using Video-Test Morphology® software (Russia) was used for

analyzing the resulting images. From each case, 5 slides were prepared and from each slide, 5 random fields were analyzed (Bokhary et al. 2021).

5.5. Statistical analysis

All values have been expressed as mean \pm S.E. Analysis of data was carried out using SPSS (version 18) by one-way ANOVA to test the significant difference between groups. Significance is declared by probability (p) levels of ≤ 0.05 was considered statistically significant (Kangralkar, 2012).

RESULTS

The following data shows changes in different parameters measured as follow:

Bodyweight:

In the diabetic group, the mean body weight was significantly ($P < 0.001$) decreased if compared with control, while in diabetic supplemented with DPP group the body weight was significantly ($P < 0.001$) increased when compared to the diabetic group as shown in (Fig.1).

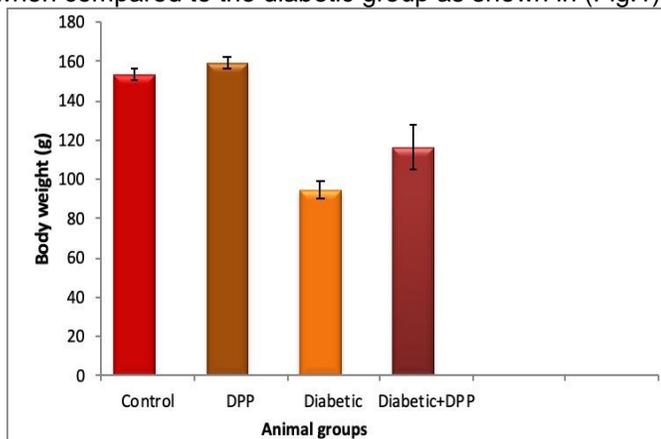


Figure 1: Histogram illustrates the mean body weight (g) changes of control and other experimental groups.

2. Changes in serum biochemical parameters

2.1. Blood glucose level

The diabetic group showed the highest serum glucose level in comparison with the control and DPP group. Otherwise, the serum glucose level of the diabetic group that supplemented with date palm pollen was indicated significantly lower than the diabetic group (Fig.2).

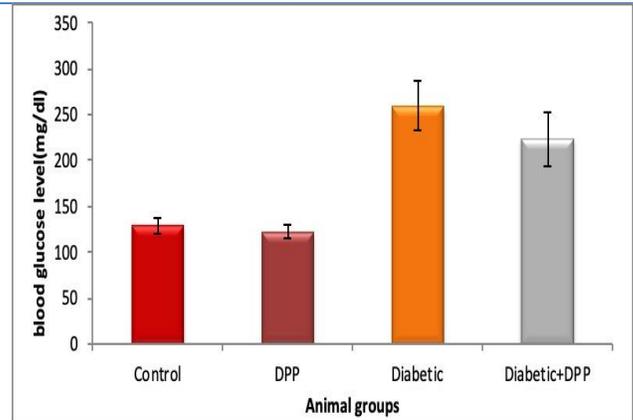


Figure 2: Histogram showing the blood glucose level (mg/dL) of control and other experimental groups.

2.2. Insulin hormone

The results of serum insulin level showed that the diabetic group significantly ($P < 0.001$) decreased compared with the control group. In contrast, the serum insulin level in the ameliorated group with palm pollen was highly significant ($P < 0.001$) in comparison with the diabetic group (Fig.3).

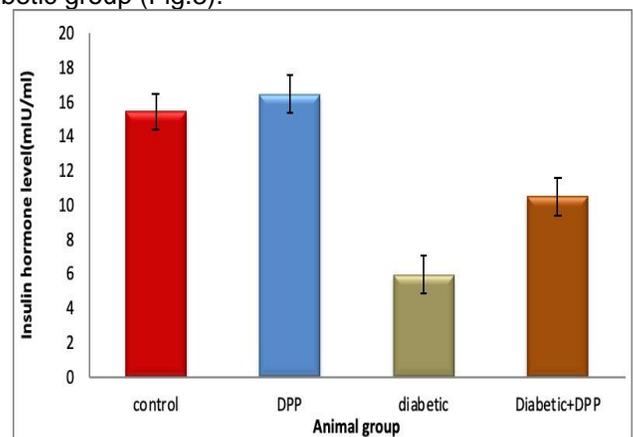


Figure 3: Histogram showing serum insulin hormone level (mIU/ml) of control and other experimental groups.

2.3. The changes in lipid profile

The following data illustrated that the diabetic group's HDL level has a significant ($P < 0.001$) decrease compared with the control. While the level of LDL was significantly ($P < 0.001$) higher than that of the control group. On the other hand, the ameliorated group with date palm pollen showed a normal range as the control in HDL level while the serum LDL level showed a significant ($P > 0.001$) decrease compared to the diabetic group (Fig.4).

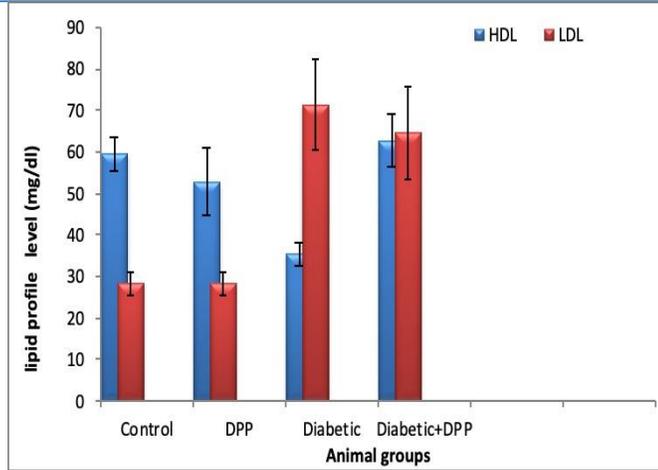


Figure 4: Histogram showing serum lipid profile HDL-C (mg/dl) and LDL –C (mg/dL) in serum of control and other experimental groups.

In addition, the serum cholesterol and triglycerides levels of the date palm pollen supplemented group showed non-significant changes with the control. In contrast, the serum cholesterol and triglycerides levels in the diabetic group indicated highly significant ($P < 0.001$) in comparison to the control group. Further results showed a significant decrease ($P < 0.001$) in ameliorated date palm pollen group compared with the diabetic group (Fig.5).

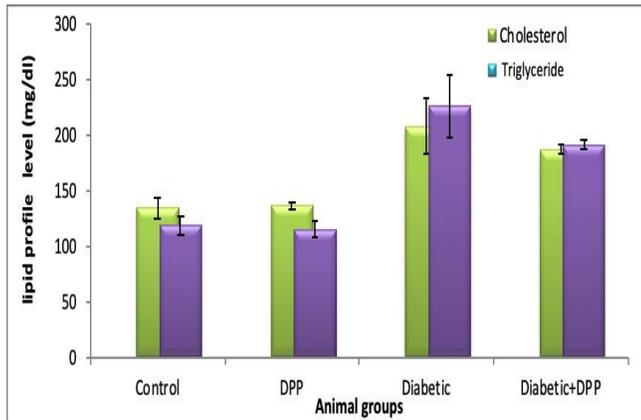


Figure 5: Histogram showing serum lipid profile cholesterol (mg/dL) and Triglyceride (TG) (mg/dL) in serum of control and other experimental groups.

2.4. The changes in female hormones

a- Serum follicle-stimulating hormone (mlu/ml) luteinizing hormone (mlu/ml) levels

The data represented in Figure 6 showed that the induction of diabetes by STZ in female rats exhibit a significant decrease ($P < 0.001$) in LH & FSH serum levels as compared to the control group. While the diabetic rats that supplemented with date palm pollen revealed a non-significant change compared with the diabetic group.

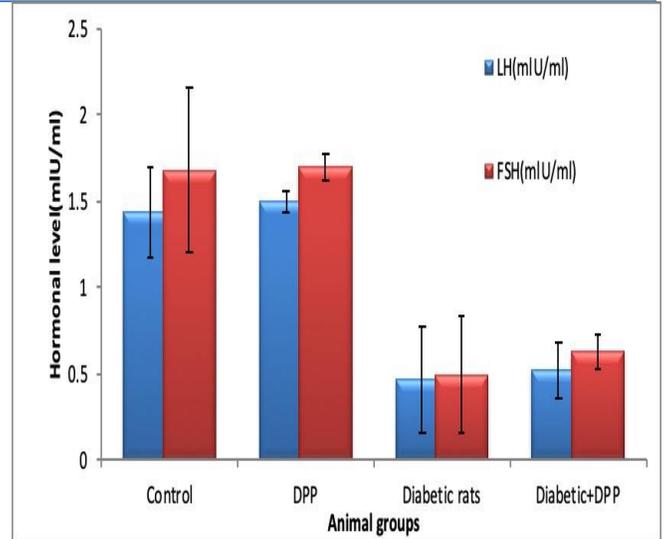


Figure 6: Histogram showing serum FSH (mlu/ml) and LH (mlu/ml) concentrations in control and other experimental groups.

b- Serum estrogen (pg/ml) and progesterone (ng/ml) levels:

Both the serum estrogen and progesterone levels of the STZ induced diabetic group showed a significant depletion as compared to the control group. The serum estrogen level of the date palm pollen supplemented group was significantly higher than the diabetic group, (Fig.7). Similarly, the serum progesterone level in the ameliorative group; diabetic rats that supplemented with date palm pollen significantly increase in comparing with the diabetic group (Fig.8).

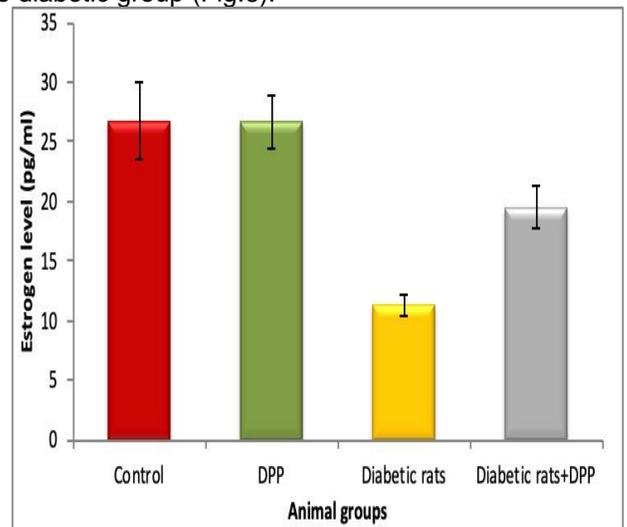


Figure 7: Histogram showing serum estrogen (pg/ml) concentrations in control and other experimental groups.

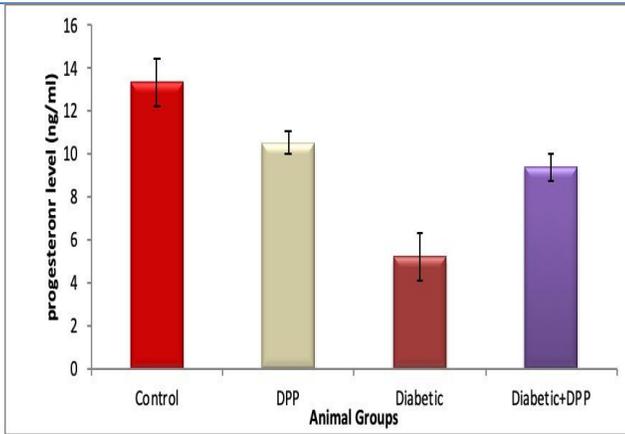


Figure 8: Histogram showing serum progesterone (ng/ml) concentrations in control and other experimental groups.

2.5. Changes in MDA and Anti-oxidants

As shown in Figs. (9,10,11) the serum levels of CAT and SOD in the diabetic group displayed a significant ($P<0.001$) decrease, while the level of MDA was significantly higher than the control. In contrast, the two diabetic groups that supplemented with date palm showed significant ($P<0.001$) elevation in both CAT and SOD and a significant ($P<0.001$) depletion in MDA level if compared with the diabetic group.

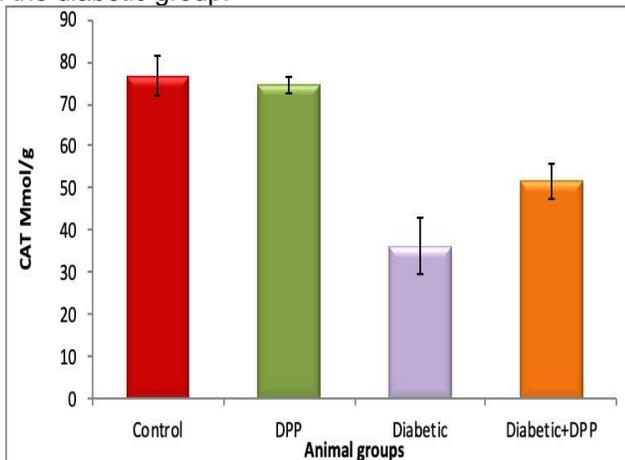


Figure 9: Serum catalase (CAT) activity in different studied groups.

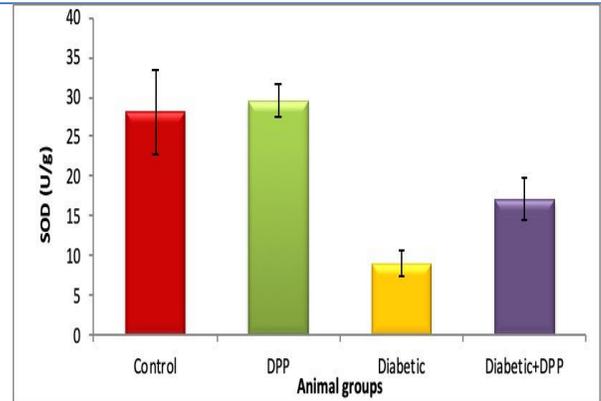


Figure 10: Serum superoxide dismutase activity (SOD) in different studied groups.

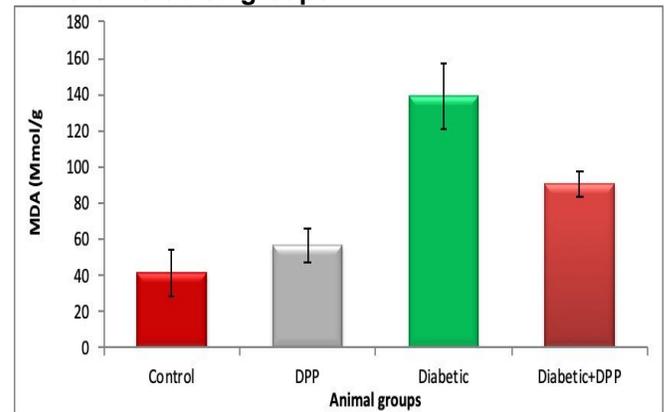


Figure 11: Serum lipid peroxidation product (MDA) activity in different studied groups.

3. The changes in flow cytometric analysis for caspase-8, Pi and TGF- β

3.1. Caspase-8

The obtained flow cytometric data was revealed that the mean percentage value of the ovarian tissue caspase-8 activity was the highest ($P<0.001$) in the absolute diabetic group. While in the diabetic groups that supplemented with DPP, the mean percentage values of caspase-8 activity were significantly ($P<0.001$) lower than that of the absolute diabetic group (Fig .12).

3.2. Propidium iodide (Pi)

In the absolute diabetic group, the mean percentage values of Pi in ovarian tissue were significantly ($P<0.001$) higher than that of the control group. On the other hand, the mean percentage values of Pi in the ameliorated group with DPP were significantly lower than that of the absolute diabetic and control group (Fig.12)

3.3. Transform growth factor (TGF)

The present results revealed that the mean percentage value of TGF- β in the uterine tissue of the

diabetic group was significantly ($P < 0.001$) higher than that of the control and the supplemented groups. In the diabetic groups that supplemented with DPP, the mean percentage values of TGF- β were significantly ($P < 0.001$) lower than that of the diabetic group but showed non-significant changes with the control group (Fig.13).

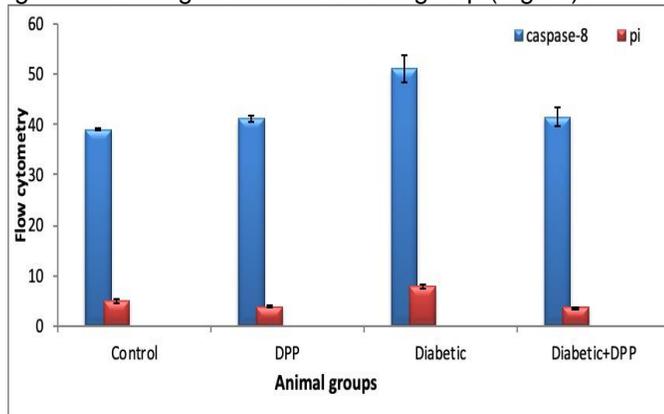


Figure 12: Histogram showing flow cytometric analysis for caspase-8 and Pi in the ovary.

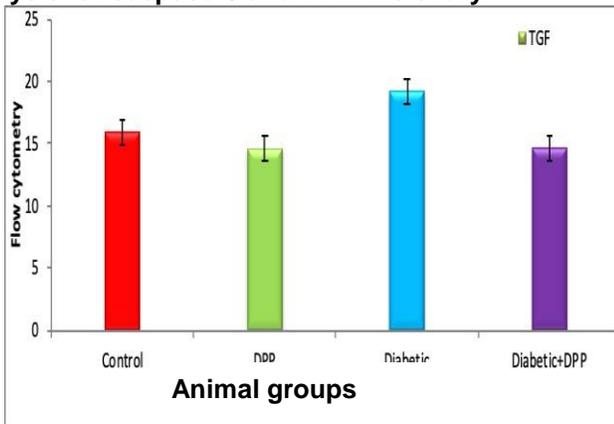


Figure 13: Histogram showing flow cytometric analysis for TGF in the uterus.

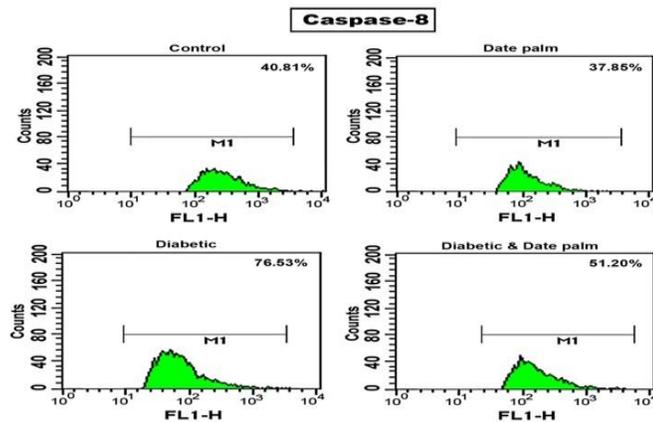


Figure 14: Flow cytometric chart showing the mean percentage values of caspase-8 activity in the ovary of different studied groups.

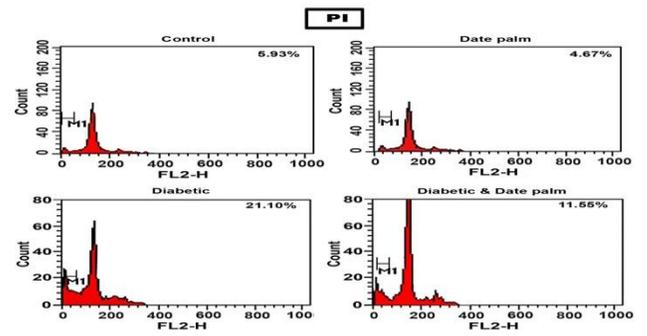


Figure 15: A flow cytometric chart showing the mean percentage values of Pi activity in the ovary of different studied groups.

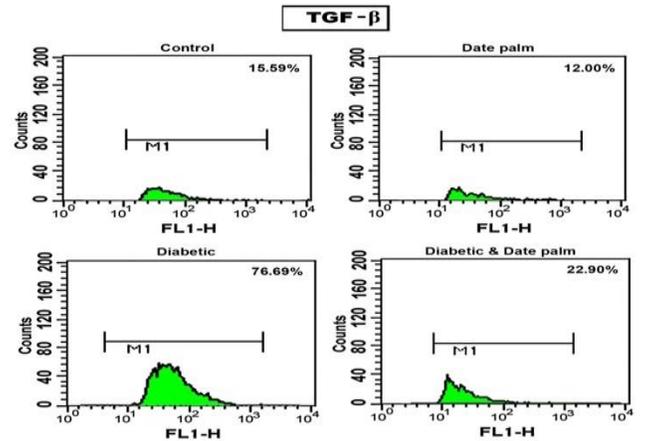


Figure 16: A flow cytometric chart showing the mean percentage values of TGF activity in the uterus of different studied groups.

4. Histological Observations

4.1. The ovary:

Plate1: (A - E): The ovary of the control and the supplemented group with date palm pollen in different developmental stages exhibited normal follicles comprising secondary follicles, Graafian follicles, and fresh corpora lutea. Medullary areas are rich in blood and lymphatic vessels and also loose connective tissue (A&B). The ovary of the diabetic group, showing cysts formation and a high degree of degeneration of follicular atresia. The follicular cysts with virtually no granulosa cell layer of huge cystic follicles with scant granulosa cells. Also, vacuoles in stromal cells and damage in most Graafian follicles were observed (C&D). In the diabetic rats that supplemented with date palm pollen, the ovarian tissue exhibited marked amelioration of the histological architecture but still not closely similar to the control group, some amelioration in its architecture exhibited a decrease in atretic cysts when compared to the diabetic group. The ovary displayed an obvious follicular exhalation with healthy developed oocytes as well as intact structure of granulosa and thecal layer.

6.2. The uterus

Plate2: (A - E): A histological examination of the uterus from control and the supplemented group with date palm pollen, exhibited normal histological structure of rodent uterus. Endometrium consisted of pseudostratified columnar cells and underlying highly cellular connective stroma filled with blood vessels and endometrial glands lined by simple columnar epithelial cells.

A microscopic examination of the uterus in the diabetic group showed histopathological lesions in the uterus include necrosis in stromal mesenchymal cells, few numbers of glands, damaged and hyperplasia of luminal epithelial cells (C&D). The observations on experimental groups revealed that administration of date palm to the diabetic rats resulted in improvement of the uterine tissue exhibited normal stroma, glands, and luminal epithelial cells.

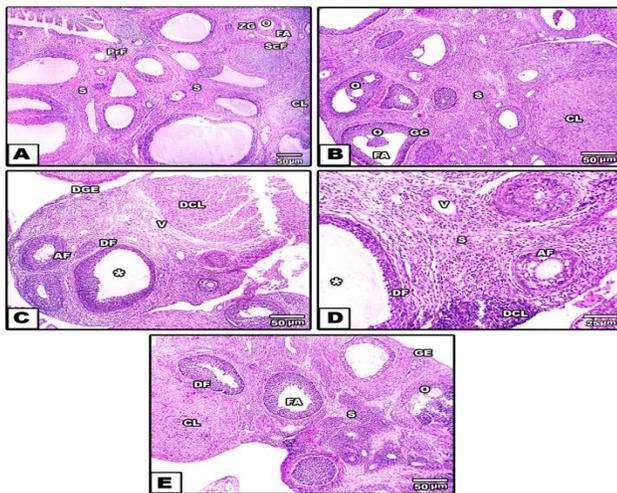


Plate 1: Photomicrograph of histological sections in the ovary of control (A), DPP (B), diabetic (C & D), diabetic & DPP (E). Note the normal histological structure in the ovary Figs. (A, B). In Figs. (C & D)

the ovarian section shows deleterious histological alterations; atretic follicles, hemorrhage, fragmented germinal epithelium, and cellular hypertrophy. In Fig. (E) the ovarian sections showed remarkable amelioration in their histological organization. (Abbreviations: Atretic follicles (AF), Granulosa cells (GC), Follicular Antrum (FA), Zona Pellucida (ZP), Zona Granulosa (ZG), Stroma (S), Blood Vessel (BV), Primordial Follicle (PF), Primary Follicle (Prf), Corpus Luteum (CL), Oocyte (O), Germinal Epithelium (GE), Degenerated Germinal Epithelium (DGE), Degenerated Corpus Luteum(DCL), Degenerated Follicles (DF), Vacuolated Stroma (V), Secondary Follicles (SCF), Asterisk (Cystic Follicles). (H&E staining, scale bar=50µm, Fig.D: bar=25µm).

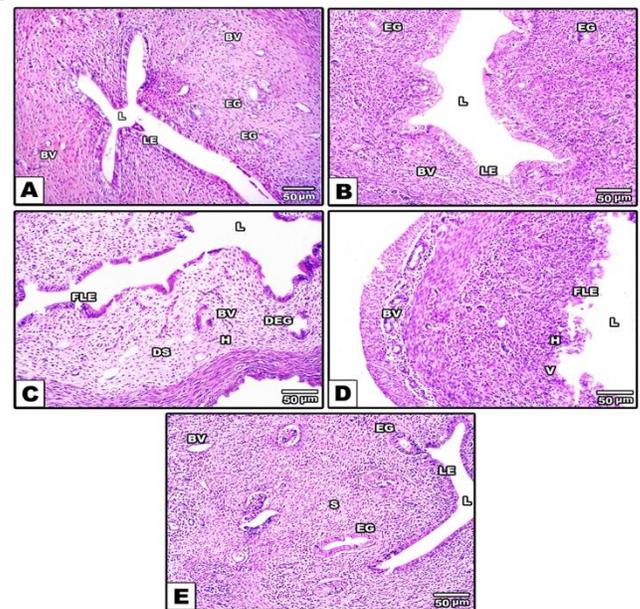


Plate 2: Photomicrograph of histological sections in the uterus of control (A), DPP (B), diabetic (C & D), and diabetic & DPP (E).

Note the normal histological structure of the uterus in Figs. (A & B). In Figs. (C & D) the uterine section shows deleterious histological alterations including fragmented uterine epithelium, hemorrhage, damaged glands, stromal necrosis, and hypertrophy. In Figs. (E) the uterine sections showed remarkable amelioration in their histological organization. (Abbreviations: Endometrial glands (EG), Blood vessels (BV), Hyperplasia (H), Damage stroma (DS), Luminal epithelium (LE) and Fragmented luminal Epithelium (FLE), Lumen(L), Stroma (S), Damage Endometrial Glands (DEG). (H&E staining, scale bar = 50µm).

5. Immunohistochemical Observations

5.1. NF-KAPPA in the ovary

Plate 3: (A-D): As shown in the control and the supplemented group with DPP (A, B) the ovarian sections were exhibited a weak to moderate immune expression for the antibody of NF-KAPPA. In contrast, the ovarian section of the diabetic group (C) was indicated a strong immune-histochemical reaction for NF-KAPPA antibody. Such immune expressions for NF-KAPPA antibodies were more confined to the Graafian follicles and ovarian stroma. Otherwise, the diabetic group that supplemented with date palm pollen (D) ovarian sections were displayed a moderate immune-histochemical reaction for NF-KAPPA stain especially in the Graafian follicles, and weak immune expression in the ovarian stroma.

5.2. VEGF in the uterus

Plate 4: (A-D): In the control and dietary

supplemented group (A, B) the uterine sections displayed weak to moderate VEGF immune positive reaction in the endothelial capillaries of the uterine endometrium. In the diabetic group (C), an intense immune positive expression for VEGF was recorded in the endometrial capillaries. Moreover, in the uterine sections of the diabetic group that supplemented with date palm pollen (D) the VEGF immune-reactivity was weak to moderate.

5.3. BCL-2 in the uterus

Plate 5:(A-D): In the current work, the uterine sections of the control and supplemented group (A&B), the immune-histochemical expression for the antibody of BCL-2 indicated a moderate to a strong positive reaction. Such reaction was more localized in the endometrial stroma. The uterine sections of diabetic (C) showed a very weak BCL-2 immune expression. While the uterine sections of ameliorated group (D) were revealed a weak to moderate immune-reactivity for the antibody of BCL-2.

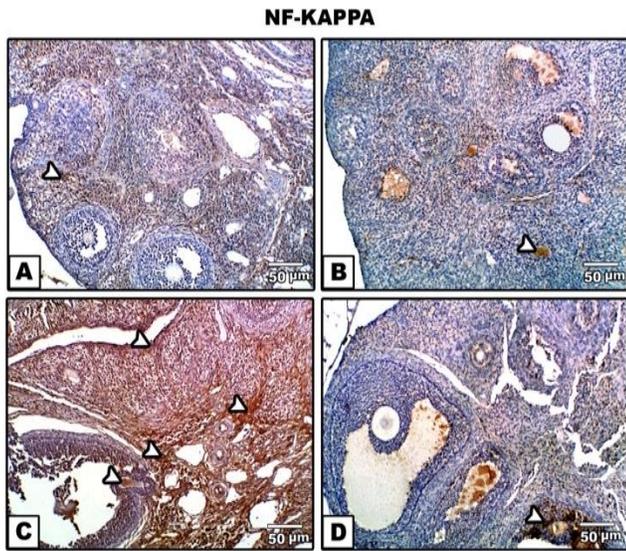


Plate 3: Photomicrograph of histological paraffin sections in the ovary of control (A), DPP (B), diabetic (C), diabetic & DPP (D) stained with KAPPA antibody. Note A, B the ovarian sections showing a weak to moderate immune reaction for KAPPA antibody. In C, the ovarian sections show strong immune expression for KAPPA. In D the ovarian sections show a moderate reaction in the ovarian stroma. The arrowhead refers to the degree of reaction (NF-kAPPA immunostaining, scale bar=50μm).

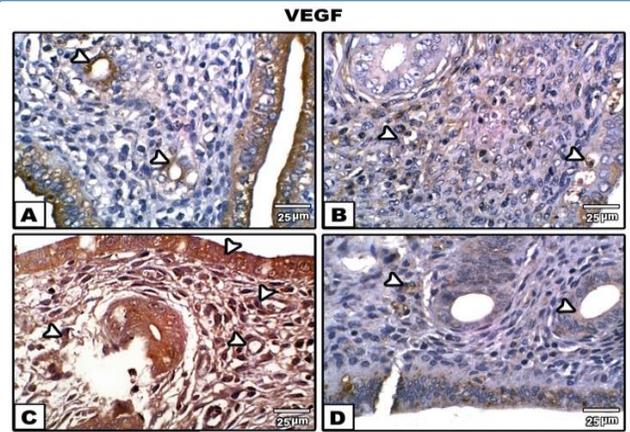


Plate 4: Photomicrograph of histological paraffin sections in the uterus of control (A), DPP(B), diabetic (C), and diabetic &DPP (D) stained with VEGF antibody. Note weak to moderate immune reaction for VEGF antibody in Figs. A& B. In (C) the uterine sections showed strong positive immune expression for BCL-2 (D) the uterine sections showed a moderate to a strong reaction. The arrowhead refers to the degree of reaction (VEGF immunostaining, scale bar=25μm).

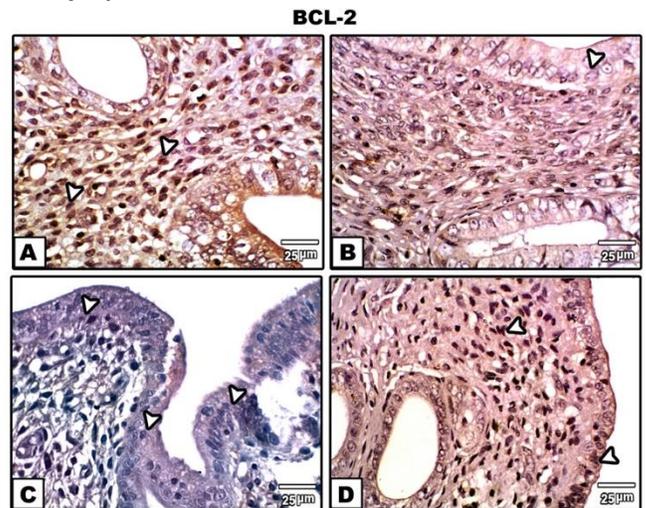


Plate 5: Photomicrograph of histological paraffin sections in the uterus of control (A), DPP (B), diabetic (C), and diabetic &DPP(D) stained with BCL-2 antibody.

Note in A&B the uterine sections a moderate to strong immune reaction for BCL-2 antibody. In (C) the uterine sections show weak immune expression for BCL-2. In (D) the uterine sections showed a moderate to a strong reaction in the uterine endometrium.

The arrowhead refers to the degree of reaction (BCL-2 immunostaining, scale bar=25μm).

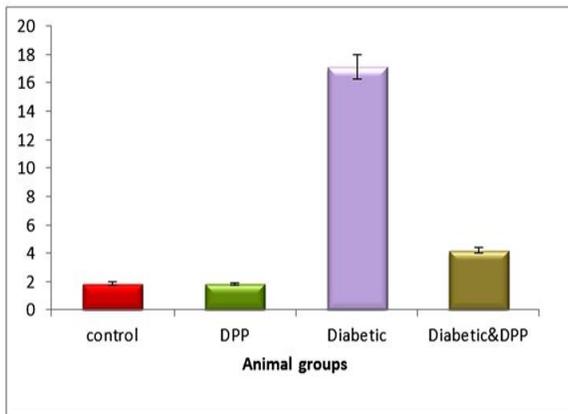


Figure 22: Histogram showing image analysis of immune histochemical staining of NF-KAPPA in the ovary in the control & other experimental groups.

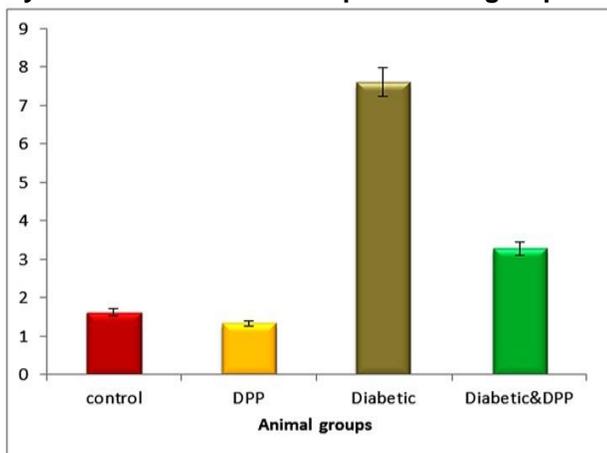


Figure 23: Histogram showing image analysis of immune histochemical staining of VEGF in the uterus in the control & other experimental groups.

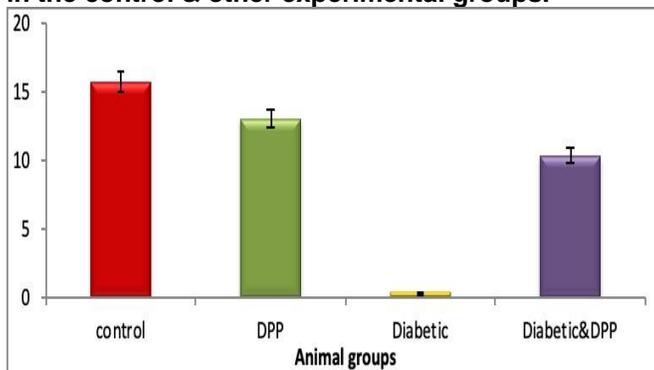


Figure 24: Histogram showing image analysis of immune histochemical staining of BCI-2 in the uterus in the control & other experimental groups.

DISCUSSION

Chronic hyperglycemia is followed by functions and

structural deterioration of different body organs, particularly the sexual organs in addition to kidneys, nerves, and cardiovascular system. Recently, several researchers assured that the products of certain natural plants and fruits like date palm pollen known as medicinal plants could ameliorate or treat some chronic diseases (Yildiz and Sandikci, 2016).

Our current study showed that the bodyweight of the diabetic group was significantly lower than that of the other studied groups. Previous studies found that STZ decreases the bodyweight through induction of hyperglycemia and decreased glucose uptake by the cells and stimulation of lipolysis (Shima, et al. 2011). Another study explained that the bodyweight depletion in the individuals suffering from diabetes may be mainly attributed to the breaking down of tissues proteins (Abbas and Ateya, 2011; Tariq et al. 2016). Moreover, the diabetic group that supplemented with date palm pollen showed markedly recovered in their body weights. Such observation agreed with the results of other studies (Peng et al.2015). The role of date palm pollen in maintaining body weight may be attributed to its high contents of fructose sugar and also the moderate value of protein that maintains bodybuilding (Eleazu et al.2013; Wu et al. 2017).

In addition, the blood glucose level in STZ – the induced diabetic group was significantly increased in comparison with the other studied groups. Several studies had been assured that streptozotocin leads to inhibition of the secretory power of β -cells in the pancreas via the negative feedback mechanism and also decreases glucose uptake by the cells (Babes et al. 2018). Different findings discussed that STZ destroys pancreatic β -cells leading to insulin production deficiency and thus, blood glucose level has been elevated (Frangogiannis, 2011). In contrast, the diabetic group that supplemented with date palm pollen showed a remarkable amelioration in the blood glucose levels to have appeared near to the normal range as the control. Previous researches confirmed that flavonoids and polyphenols in some medicinal plants could activate glucose metabolism through activation of β -cells and increase glucose uptake by the cells (Peng et al.2015).

Furthermore, A depletion in serum insulin levels occurred in the diabetic group when compared with control and DPP supplemented groups. The present findings are approved with the work of other researchers (Frangogiannis, 2011; Peng et al.2015; Yildiz and Sandikci, 2016). Moreover, a remarkable amelioration in serum insulin level was recorded in the diabetic group that supplements with date palm pollen due to the polyphenols and flavonoids compounds that could stimulate the secretory power of the pancreas (Yildiz and Sandikci, 2016)

In STZ induced diabetic group, the data concerning lipid profiles showed a significant increase in LDL, cholesterol, and triglycerides. Several studies were

confirmed that lipolysis is a major complication in diabetic individuals and consequently is accompanied by elevated lipid profiles (Moein, 2015). In the diabetic group that was supplemented with date palm pollen, the lipid profiles were still significantly higher than the control. Such findings may explain that the nutritional compounds of date palm could not have a remarkable inhibitory effect on the lipolysis process (Erbaş et al. 2014).

Herein, the present work was indicated that serum FSH, LH, estrogen, and progesterone levels of diabetic neonatal rats were significantly lower than the control group. The present findings are following other previous researches (Nardi et al. 2020). As assured by Farias SP, et al. (2014) that the diabetic rats had low serum levels of FSH and LH and this is due to that the STZ causing inhibition of the anterior pituitary secretory ability. As a general aspect, the diminished levels of serum estrogen and progesterone levels are a normal result for the consequential decreased level of secreted FSH and LH (Desouky et al. 2015). El-Beltagy and El-Ghaweet (2016) reported that, estrogen and progesterone levels are associated with diabetes mellitus and altered glucose tolerance.

In the present investigations, the estimated antioxidant capacities were revealed that in the diabetic group the MDA level was significantly higher than that of the control while the level of catalase CAT and superoxide dismutase SOD were significantly lower than the normal group. Ishurd and Kennedy (2005) found that diabetic hyperglycemia is a major factor for the development of oxidative stress which is followed by elevated malondialdehyde (MDA; is a lipid peroxidation end product). Another study confirmed that diabetic individuals had marked depletion in superoxide dismutase SOD and catalase CAT levels (Marth and Jose, 2010). Previously several types of research had confirmed that the antioxidant powerful capacity of polyphenols and flavonoids is achieved by inhibition of free radical's liberation so thus, oxidative stress suppression (Trinder, 1969).

Currently, a significant elevation in the two antioxidants; CAT and SOD but significant depletion in the MDA has been recorded in the ameliorated group with date palm pollen if compared with the absolute diabetic group. Several lifestyle disorders like metabolic syndrome, type II diabetes mellitus, obesity, and coronary artery disease are accompanied by oxidative stress.

In the current work, several histopathological observations were noticed in the ovarian sections of the diabetic group in form of degenerated germinal epithelium atretic follicles and decreased the number of oocytes. Also, the ovarian stroma appeared hemolytic with hemorrhage area and scattered vacuoles. The obtained conclusions agreed with the results of previous work (Flier et al. 1976). Also, other studies confirmed that deficiency in insulin secretion or its action had a direct deleterious effect on the ovarian structure through inhibition of FSH

and LH secretion (Meiattini, 1978).

Furthermore, in STZ induced diabetic group, the uterine sections showed severe damage especially in the endometrial layer that represented by degenerated endothelium with numerous hypertrophied and vacuolated cells. As mentioned above, the decreased secretion of estrogen may be considered as the main factor for the destruction of the uterine endometrium (Van and Zilverstmit, 1957). Supplementation of date palm pollen to the diabetic groups was showed ameliorative signs for the uterine reconstructions. The ameliorative capacity of such supplements may be related to their enhancing role in the secretion of FSH and estrogen (Young et al. 1994).

Flow cytometry can be applied to evaluate chromatin condensation, cytoplasmic dehydration, and shrinkage during apoptosis. The obtained results of flow cytometric analysis revealed that the mean percentage values of caspase-8 & propidium iodide in ovarian tissues and TGF in the uterine tissues were significantly higher in the diabetic group if compared with other groups. Caspases are programmed cell death sensors and apoptotic markers. Hyperglycemia has been shown to activate caspase 8 that implicated in P53-mediated apoptosis (Ahmed et al. 2008). According to Satheesh and Pari (2008) reported that diabetes leads to abnormal ovarian function causing granulosa cells apoptosis and inhibition of angiogenesis of the ovaries. Another hypothesis stated that STZ stimulates cellular apoptosis by Bcl-2 down-regulation as it inhibits apoptosis by sequestering preforms of death-driving cysteine proteases or caspases (Cohen et al.1970).

The flow cytometric assay for propidium iodide has been vastly used to evaluate apoptosis in various experimental models. In the recent work, the mean percentage values of PI analysis by flow cytometry were highly significant in the ovarian tissues of STZ induced diabetic rats. The obtained results go parallel with the previous study which confirmed that the highly significant flow cytometric analysis of PI is a good sign for DNA fragmentation and consequently loss of nuclear DNA content that is confirmed apoptosis (Sun et al.1988). It had been concluded that the diabetic rats had a high rate of apoptosis which plays a crucial role in the pathophysiology of female gonads dysfunction (Eleazu et al. 2013).

Further study by flow cytometry revealed that the mean percentage value for TGF- β was significantly higher in the uterine tissues of the diabetic group. A similar observation was recorded in the uterine tissues of the dehydroepiandrosterone-induced uterus (Eleazu et al. 2013). Moreover, it had been confirmed that overexpression of TGF- β protein in various tissues is considered a good key marker for fibrosis (Ohkawa et al. 1982).

The diabetic group that supplemented with date palm pollen revealed that the level of apoptotic markers, caspase-8 & PI as well as pro-fibrotic protein TGF- β significantly reduced although such markers did not reach

statistical significance. Several studies indicated that the flavonoids and polyphenol compounds in medicinal plants and fruits play a significant role in the inhibition of apoptosis and tissues fibrosis by induction of cellular division (Juan et al. 2012).

Nuclear factor-KB protein is correlating with the extrinsic cell death pathway, which may be stimulated by pro-apoptotic signal, including death receptors activation. The immunohistochemical study of the present work revealed that nuclear factor-KB was highly expressed in the ovarian follicles and stroma of diabetic rats. Such results have been agreed with Bancroft Gamble (2008) who found that NF-kB immune-expression is highly expressed in the ovarian sections of diabetic rats. The over-expression of NF-kB is an indicator marker for follicular inflammatory pathways induced by oxidative stress in diabetic individuals.

The uterine endometrium is the functional layer of the uterus that is rich with glands and blood capillaries. The vascular endothelial growth factor (VEGF) is one of the protein markers that are more localized in the capillary's endothelium and responsible for the formation of new capillaries in case of oxidative stress and inflammatory response and it is well documented that diabetes mellitus causes an increased expression of VEGF in numerous tissues as a response to both hyperglycemia and tissues ischemia (Wu et al.2008). The immunohistochemical study of the present work showed an intense immune positive expression for VEGF in the endometrial capillaries of the STZ induced diabetic group (Prentice et al. 1992).

Bcl-2 is an oncoprotein that inhibits the rate of apoptosis (Nour et al. 2017). The present results indicated that the immune expression for the Bcl-2 antibody was very weak in the uterine endometrial cells of the diabetic group. Such results indicate that cellular apoptosis is enhanced in cases of diabetes leading to weight loss (Hayes, 1994). The immune-reactivity for VEGF of the diabetic group supplemented with date palm pollen was moderate if compared with the diabetic group however the immune-reactivity for BCL-2 was moderately expressed. This result may be attributed to the anti-inflammatory and antioxidant capacities induced by the nutritional ingredients of the supplemented food to maintain apoptosis.

CONCLUSION

Diabetes mellitus (DM) is a metabolic disorder described by a high level of blood glucose that affects carbohydrates, proteins, and lipids metabolism with a high risk of vascular diseases complications. Blood glucose level is tightly controlled by insulin that is secreted from β -cells of the pancreas. Beta-cell dysfunction is the main factor across the spectrum of pre-diabetes to diabetes. Some medicinal plants like date palm pollen can control blood sugar and lifespan than neither by insulin nor hypoglycemic drugs and thus, DPP was qualified as a very strong antioxidant and antidiabetic.

CONFLICT OF INTEREST

The authors declare that they have no competing of interests.

ACKNOWLEDGEMENT

Authors acknowledge Zoology Department, Faculty of Science, Mansoura University.

AUTHOR CONTRIBUTIONS

The contribution of work in this study is equal between authors. The final manuscript was written, revised, and approved by all the authors.

Copyrights: © 2022@ author (s).

This is an open access article distributed under the terms of the [Creative Commons Attribution License \(CC BY 4.0\)](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

REFERENCES

- Abbas FA, Ateya AM. (2011). Estradiol, Estriol and Estrone and Novel flavonoids from Date Palm Pollen. *Aust J Basic and App. Sci.*; 5(8): 606-614.
- Abdel-Shaheed, Abdalla ES, Khalil AF, El-Hadidy EM. (2021). Effect of Egyptian Date Palm Pollen (*Phoenix dactylifera* L.) and Its Hydroethanolic Extracts on Serum Glucose and Lipid Profiles in Induced Diabetic Rats. *Food Nutr Sci.*; 12; 147-161.
- Ahmed B, Khan S, Masood MH, Siddique AH. (2008). Anti-hepatotoxic activity of cichotyboside, a sesquiterpene glycoside from the seeds of *Cichorium intybus*. *J. Asian. Nat. Prod. Res.*; 10 (3-4):223-31.
- Andulla B, Varadacharyulu NCH. (2003). Antioxidant role of mulberry leaves in streptozotocin-diabetic rats. *Clin Chim Acta.*; 338: 3-10.
- Babes RM, Sandu, RG, Baran, OE, Cosoreanu, V, Ilie, MT, Duta AI, Ceausescu MC, Ciucur PM, Costache S, Ganea C, Baran I (2018) Simple discrimination of sub-cycling cells by propidium iodide flow cytometric assay in Jurkat cell samples with extensive DNA fragmentation. *Cell cycle.*; 17(6):766-779.
- Bancroft JD, Gamble M. (2008). *Theory and practice of histological techniques*. Elsevier Health Sciences.
- Bayoumy S, Habib M, Abdelmageed R. (2020). Impact of maternal diabetes and obesity on fetal cardiac functions. *Egypt Heart J.*; 72 (1), 1-7.
- Beylot M. (2005). Effects of inulin-type fructans on lipid metabolism in animal and human beings. *Br J Nutr.*; 93: 163-S168.
- Bhaskarachary K, Joshi AKR. (2018). *Natural Bioactive Molecules with Antidiabetic Attributes: Insights into*

- Structure-Activity Relationships. Studies in natural product chemistry.; Chapter 11, 57: 353-388.
- Bokhary K, Aljaser F, Abudawood M, Tabassum H, Bakhsh A, Alhammad S, Aleyadhi R, Almajed F, Alsubki R. (2021). Role of Oxidative Stress and Severity of Diabetic Retinopathy in Type 1 and Type 2 Diabetes. *Ophthalmic Res.*; 64(4):613–621.
- Cheng Y, Sibusiso L, Hou L, Jiang H, Chen P, Zhang X, Wu M, Tong H. (2019). Sargassum fusiform fucoidan modifies the gut microbiota during alleviation of streptozotocin-induced hyperglycemia in mice. *Int J Macromol.*; 12(8): 3572-3585.
- Cohen G, Dembiec D, Marcus J. (1970). Measurement of catalase activity in tissue extracts. *Anal Biochem.*; 34: 30–38.
- Desouky KM, Anwar IR, Algaidi AS. (2015). Immunohistochemical Expression of Bcl-2 and Microvessel Density in Uterine Fibroids in Saudi Patients. *WIMJ Open.*; 2(3): 121-126.
- El-Beltagy BMA, El-Ghaweet AH. (2016). Adverse effects of monosodium glutamate on the reproductive organs of adult Female albino rats and the possible ameliorated role of carob (*Ceratonia Siliqua*). *J of Biosc and Applied. Res.*; 2(3): 2356-9182.
- Eleazu CO, Eleazu KC, Chukwuma S, Essien UN. (2013). Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use, and potential risk to humans. *J Diabetes Metab Disord.*; 12 (1):60.
- Erbas O, Pala GH, Pala EE, Oltulu F, Aktug H, Yavasoglu A, Taskiran D. (2014). Ovarian failure in diabetic rat model: Nuclear factor-kappaB, oxidative stress, and pentraxin-3. *Taiw J of Obs and Gyne.*; 53: 498:503.
- Farias SP, Souza SK, Marçal CA, Fioretto TE Aires, BM (2014). Altered placental morphology and VEGF expression in diabetic rats. *Microscopy. Advan in Scie Res and Edu.*; 285-290.
- Flier JS, Kahn CR, Jarrett DB, Roth J. (1976). Characterization of antibodies to the insulin receptor: A cause of insulin-resistant diabetes in man. *J Cdin Invest.*; 58:1442.
- Frangogiannis NG. (2020). Transforming growth factor-β in tissue fibrosis. *J Exp Med.*; 217(3): 4-16.
- Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. (2011). Polycystic ovary syndrome: etiology, pathogenesis, and diagnosis. *Nat Rev Endocrinol.*; 7 (4): 219-231.
- Hayes W. (1994). Principles and methods of Toxicology .3rd edition. Raven Press. New York.; 521-550.
- Ishurd O, Kennedy JF. (2005). The Anti-Cancer Activity of Polysaccharide Prepared from Libyan Dates (*Phoenix dactylifera L.*). *Carbohy Polym.*; 59: 531-535.
- Juan WS, Lin HW, Chen YH, Chen HY, Hung YC, Tai SH, Huang SY, Chen TY, Lee EJ. (2012). Optimal Percoll concentration facilitates flow cytometric analysis for annexinV/propidiumiodine-stained ischemic brain tissues. *Cytometry Part A.*; 81: 400–408.
- Kangralkar VA, Patil SD, Bandivadekar RM. (2012). Oxidative Stress and Diabetes: A Review. *Int J Pharma Appl.*; 1: 38–45.
- Marsh TG, Straub RK, Villalobos F, Hong MY. (2011). Soy protein supports cardiovascular health by downregulating hydroxymethylglutaryl–coenzymeA reductase and sterol regulatory element-binding protein–2 and increasing antioxidant enzyme activity in rats with dextran sodium sulfate-induced mild systemic inflammation. *Nutr Res.* 2011; 31(12): 922-928.
- Marth RP, Jose MMA. (2010). Attenuation of hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats by chloroform extract of fruits of *Ferocactus latispinus* and *Ferocactus histrix*. *Boletín Latinoamericano y del Caribe de Plantas Medi y Aromáticas.*; 9 (6): 475 – 484.
- Meiattini F. (1978). The 4-hydroxybenzoate 14-aminophenazone chromatographic system. *Clin Chemi.*; 24 (12): 2161-2165.
- Mellembakken JR. (2011). Sustained fertility from 22 to 41 years of age in women with the polycystic ovarian syndrome. *Hum Reprod.*; 26 (9): 2499-2504.
- Mia MA, Mosaib MG, Khalil MI, Islam MA, Gan SH. (2020). Potentials and Safety of Date Palm Fruit against Diabetes: A Critical Review. *Foods.*; 9(11): 1557.
- Michael HN, Salib JY, Eskander EF. (2016). Bioactivity of diosmetin glycosides isolated from the epicarp of date fruits, *Phoenix dactylifera*, on the biochemical profile of alloxan diabetic male rats. *Phytother Res.*; 27(5):699-704.
- Moein, S. (2015). Polyphenols, and cancer. *Biochemistry Department, Molecular Medicine Research Center.*; 1(1): 6-12.
- Mohamed NA, Ahmed OM, Hozayen WG, Ahmed MA. (2018). Ameliorative effects of bee pollen and date palm pollen on the glycemic state and male sexual dysfunctions in streptozotocin-Induced diabetic Wistar rats. *Biomed. Pharmacother.*; 97:9-18.
- Nagarchi K, Ahmed S, Sabus A, Saheb HS. (2015). Effect of Streptozotocin on Glucose levels in Albino Wister Rats. *J of pharm Sci & Res.*; 7(2):67-69.
- Nardi GM, Ferrara E, Converti I, Cesarano F, Scacco S, Grassi R, Gnoni A, Felice Roberto Grassi FR, Rapone B. (2020). Does Diabetes Induce the Vascular Endothelial Growth Factor (VEGF) Expression in Periodontal Tissues? A Systematic Review. *Int J Environ Res Public Health.*; 17(8): 2765.
- NIDDK. (2008). National Institute of Health. Publication No., (9):5135.
- Nour MS, Sarhan NR, Mazroa SA, Gawish SA. (2017). Histological and immunohistochemical study of cardiac telocytes in a rat model of isoproterenol –

- induced myocardial infarction with a reference to the effect of grape seed extract. *Acta histochemical*.; 119(7):747-758.
- Ohkawa H, Waka T, Kaneada C. (1982). Assay for lipid peroxides in animal tissue by the thiobarbituric acid reaction. *Analy Biochemistry*.; 95 (2): 351-358.
- Peng J, Li X., Zhang D, Chen J Su Y, Smith SB, Dong Z. (2015). Hyperglycemia, p53, and mitochondrial pathway of apoptosis are involved in the susceptibility of diabetic models to ischemic acute kidney injury. *Kidney Int.*; 87(1): 137-150.
- Petersen MC, Shulman GI. (2018). Mechanisms of Insulin Action and Insulin Resistance. *Physiol Rev.*; 98(4): 2133–2223.
- Prentice A, Randall BJ, Weddell A, McGill A, Henry L, Home CHW, Thomas EJ. (1992). Ovarian steroid receptor expression in endometriosis and two potential parent epithelia: endometrium and peritoneal mesothelium. *Hum. Reprod.*; 7: 1318-1325.
- Pushparaj P, Tan CH, Tan BK. (2000). Effects of Averrhoa bilimbi leaf extract on blood glucose and lipids in Streptozotocin diabetic rats. *J Ethnopharmacol.*; 72(1-2):69-76.
- Satheesh AM, and Pari L. (2008). Effect of pterostilbene on lipids and lipid profiles in Streptozotocin – Nicotinamide induced type 2 diabetes mellitus. *J App Biomed.*; 6(1): 31-37.
- Shalash M, Badra M, Imbaby S, EIBanna E. (2020). Malondialdehyde in type-2 diabetics and association with cardiovascular risk factors. *J M R I.*; 41 (2): 21-30.
- Shehzad M, Rasheed H, Naqvi SA, Al-Khayri JM, Lorenzo, JM, Alaghbari MA, Manzoor MF, Aadil RM. (2021). Therapeutic potential of date palm against infertility: A REVIEW. *Metabolites.*; 11(6): 408.
- Shi LE, Zheng W, Aleid SM, Tang ZX. (2013). Date Pits: Chemical Composition, Nutritional and Medicinal values, Utilization. *Crop Sci.*; 54 (4): 1322-30.
- Shima H, Naeem E, Hassan M, Hossein N. (2011). Aloe vera gel protects ovarian structure in diabetic rat. *Am Euros J Toxicol Sci.*; 3 (3): 197–203.
- Stefanska A, Cembrowska P, Kubacka J, Prusinska MK, Sypniewska G. (2019). Gonadotropins and Their Association with the Risk of Prediabetes and Type 2 Diabetes in Middle-Aged Postmenopausal Women. *Disease Markers.*; doi.org/10.1155/2019/2384069.
- Sun Y, Oberley LW, Li YA. (1988). A simple method for clinical assay of superoxide dismutase. *Clin Chem.*; 34: 497 - 500.
- Tariq S, Nurulain MS, Rashed H, Lotfy M, Emerald BS, Koturan S, Adeghate EK. (2016). Diabetes-induced changes in the morphology and nociceptinergic innervation of the rat uterus. *J of Molec Histo.*; 47 (1): 21 - 33.
- Trinder P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem.*; 6: 24-25.
- Uniuofin JO, Lebelo SL. (2020). Mechanisms of medicinal plants and their bioactive compounds for the prevention and treatment of type 2 diabetes: An updated over Review. *Oxid Med Cell Longev.*; 36pages.
- Van HE, Zilversmit DB. (1957). Micro method for the direct determination of serum triglycerides. *J Lab Clin Med.*; 50(1):152–157.
- Wang X, Xian T, Jia X, Zhang L, Liu L, Man F, Zhang X, Zhang J, Pan Q, Guo L. (2017). A cross-sectional study on the associations of insulin resistance with sex hormone, abnormal lipid metabolism in T2DM and IGT patients. *Medicine Balt.*; 96(26): e7378.
- Wu C, Wilson S, Walker B, Dawid I, Paisley T, Zimarino V, Ueda H. (1987). Purification and Properties of Drosophila Heat Shock Activator Protein. *Sci.*; 238: 1247-1253.
- Wu Y, Li Y, Liao X, Wang Z, Li R, Zou S, Jiang T, Zheng B, Duan P, Xiao J. (2017). Diabetes induces abnormal ovarian function via triggering apoptosis of granulosa cells and suppressing ovarian angiogenesis. *Int J Biol Sci.*; 13 (10): 1297-1308.
- Yildiz M, Sandikci M. (2016). Changes in rat ovary with experimentally induced diabetes and the effects of lycopene on those changes. *Rom J Morphol Embryol.*; 57: 703-713.
- Young SG, Bihain B, Flynn LM, Sanan DA, Ayrault-Jarrier M, Jacotot B. (1994). Asymptomatic homozygous hypobetalipoproteinemia associated with apolipoprotein B45.2. *Hum Mol Genet.*; 3:741-744.
- Zatalia SR, Sanusi H. (2013). The role of antioxidants in the pathophysiology, complications, and management of diabetes mellitus. *Acta Med Indones-Indones J Intern Med.*; 45(2): 141–147.
- Zhang CR, Aldosari SA, Vidyasagar PS, Nair KM, Nair, MG. (2013) Antioxidant and anti-inflammatory assays confirm bioactive compounds in Ajwa Date fruit. *J Agric Food Chem.*; 61: 5834 - 5840.