

PROTECTIVE EFFECTS OF POMEGRANATE (PUNICA GRANATUM) PEELS ON THE PITUITARY GONADAL HORMONAL AXIS OF STREPTOZOTOCIN-INDUCED DIABETES IN ADULT MALE ALBINO RATS

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ABSTRACT

Background: Type 1- diabetes mellitus more likely induces changes in the male reproductive system and alters the hormonal synthesis. Oxidative stress plays a crucial role in the pathophysiology of diabetic testicular dysfunction. Pomegranate (PG) is believed to have a variety of biologically active components with antioxidant properties.

Objectives: Evaluating the effects of PG peels on the hypogonadism and steroidogenesis in streptozotocin (STZ)-induced diabetes in adult male rats.

Materials and Methods: Forty adult male albino rats were divided into four equal experimental groups, i.e. control, non-diabetic-treated, diabetic and diabetic-treated. Diabetes was induced by a single dose of STZ injection. The treated rats received 100 mg/kg PG peels via oral gavages once daily for 10 weeks. At the end of the experimental period, blood samples were prepared for estimating fasting serum glucose (FSG), serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone (T) levels. Catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and steroidogenic enzymes (3β -HSD and 17β -HSD) activities were measured in the testicular homogenate. In addition, percentages of body weight (BW) gain and relative testicular weight were recorded.

Results: STZ- induced diabetic rats exhibited decreases in BW gain, testis weight, LH, FSH, T, CAT, SOD, GSH, 3β -HSD and 17β -HSD levels. However, PG peels administration improved the hyperglycemia, increased the LH, FSH and T levels. Also, the antioxidant enzyme activities and steroidogenic enzymes increased.

Conclusion: Diabetes had induced oxidative testicular dysfunction in adult rats and adversely affected the pituitary gonadal hormonal axis. Pomegranate peels has effectively reversed the diabetes- related testicular disturbances and improved gonadal steroidogenesis due to its antioxidant and antidiabetic effects.

Key words: Diabetes; Pomegranate; Antioxidants; Testes, Steroidogenesis; Pituitary gonadal axis.

INTRODUCTION

The reproductive system is one of the virulent systems that critically affected by insulin deficiency (Ghanbari *et al.*, 2016). The adverse effects of diabetes on testes

and sperms could be mediated due to its impact on the hypothalamic-pituitary-gonadal hormonal axis or the local effect on the testicular tissue (Nasrolahi *et al.*, 2013 and Ghanbari *et al.*, 2016).

Pomegranate (*Punica granatum* L) is a folk plant having medicinal properties and rich in phytochemical compounds (*Miguel et al., 2010*). Extracts of all parts of the fruit exhibit therapeutic properties (*Mosele et al., 2015*). However, most studies investigated the effects of pomegranates (PG) juice on various diseases (*Al-Olayan et al., 2014* and *Fernandes et al., 2015*). The inedible peels (PGP) represent almost 26-30% of the fruit. PGP have the highest antioxidant capacity i.e. 92% of the total antioxidant activity of the fruit (*Zahin et al., 2010*). This holds various types of ingredients including flavonoids, ellagitannins and proanthocyanidin compounds and minerals such as calcium, magnesium, phosphorus, potassium, sodium and vitamin C (*Elfalleh et al., 2012*).

The involvement of the oxidative stress and reactive oxygen species (ROS) production in the pathophysiology of various diabetic complications, makes focusing on natural antioxidants as a challenge to medicate such diseases and attenuate the side effects of traditional drugs.

So, the current work focused on exploring the antioxidant effects of PGP powder against hypogonadism in diabetic male rats and its role on the pituitary-testicular hormonal axis. Moreover, the effect of PGP on the steroidogenic process of testosterone synthesis was analyzed.

MATERIALS AND METHODS

Induction of diabetes:

Each rat was injected with a single intraperitoneal (IP) dose of a freshly prepared solution of streptozotocin (STZ) at a dose of 50 mg/kg BW in 5 mmol/L

citrate buffer (pH=4.5). After 72 hr of STZ injection, fasting blood glucose level was estimated using AccuChek glucometer (Roche, Germany). Rats with blood glucose levels more than 250 mg/dl were considered diabetic (*Yoruk et al., 2004*).

Preparation of pomegranate peel (PGP) powder:

PG fruits were collected from the local commercial market in Egypt (during October and November months). Fresh pomegranate fruits were cleaned, cut into pieces, and peeled manually. The collected peels were washed with excess water and then were sun-dried. Peels were powdered in a grinder to get 60-mesh size powder. Finally, powdered peels were blended for 2 min with distilled water to make required concentrated suspensions of 5mg/ml, 10mg/ml and 20mg/ml (*Endo et al., 2012*). PGP suspensions were used at the day of administration.

Experimental animals:

A total of forty adult male albino rats of Sprague-Dawley strain, weighing 170-200g were used for the present study. Rats were obtained from the Nile Center for Experimental Research in Mansoura city, Egypt. They were housed in stainless steel cages (37 x 26 x 18cm per 5 rats) and were kept under standard conditions for temperature (22-25°C) and relative humidity (55±5), with normal light/dark cycle. The animals were fed a commercial rat diet (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and tap water ad libitum. The animal care and procedure were in accordance to the ethical guidelines of the Nile Center for Experimental Research and approved by the international accreditation organization.

Experimental design:

After one week of acclimation, rats were randomly divided into the following groups:

- Group I (control): Consisted of 10 rats and were fed normal rat chow diet. Each rat received 1ml 0.9% saline by oral gavages for 10 weeks, in addition to a single IP injection of 1ml 0.9% saline.
- Group II (Non diabetic-treated): Consisted of 10 rats. Each rat received PGP powder at a dose of 100 mg/kg BW by oral gavages once a day for 10 weeks, in addition to a single IP injection of 1ml 0.9% saline.
- Group III: Rats received a single IP injection of STZ at a dose of 50 mg/kg BW. After 72 hr, diabetic rats were selected and divided into further two subgroups IIIA, IIIB.
 - Group IIIA (Diabetic): Consisted of 10 rats and served as a diabetic group and fed normal rat chow diet, in addition to 1ml 0.9% saline by oral gavages daily for 10 weeks.
 - Group IIIB (Diabetic-treated): Consisted of 10 rats and served as a diabetic group treated with PGP powder at a dose of 100 mg/kg BW by oral gavages daily for 10 weeks.

Body weight was recorded weekly, and the PGP dose was adjusted accordingly. At the end of the experiment, overnight fasted rats were weighed and then sacrificed under anesthesia. Blood samples were obtained by cardiac puncture, and serum was prepared by centrifugation at 3,000 rpm for 15 minutes at 25°C for biochemical analysis. The

testes were dissected out by laparotomy, weighed and stored at -70°C until be used for estimation of antioxidant and steroidogenic enzymes activities.

- Body weight (BW) gain (%): % of BW gain was calculated as a final BW-initial BW/ initial BW × 100.
- Testes index (%): Relative weight of testes was calculated as testes weight/Final body weight × 100.

Biochemical measurements:

Fasting serum glucose (FSG) was measured with a glucose GPO-POD enzymatic colorimetric assay kit (SPINREACT, S.A. Ctra, Santa Coloma, Spain) (*Kaplan, 1984*).

- Quantitative measurement of serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone (T) levels were carried by the enzyme linked immune fluorescent assay (ELIFA) technique using specific kits according to the protocol provided with each kit (*Butt, 1983, Wide, 1976 and Wheeler, 1995*) respectively. Kits are purchased from BioMerieux Egypt office (Masaken Al Mohandesin, Nasr City, Cairo Governorate). The company is in France (5 Rue des Aqueducs, 69290 Craponne, France).

Testes were homogenized according to (*Akondi et al., 2011*). They were rinsed in ice-cold PBS (0.01mol/L, pH 7.0-7.2), sliced into pieces then minced and homogenized in 5-10 ml cold buffer per gram tissue using a crusher (HeidolphSilentCrusher M type – Germany) to obtain 10% homogenate (w/v). The homogenates were centrifuged at 4000 rpm for 15 min. The supernatant was used for estimating the antioxidant

enzymatic activities. Catalase (CAT) was evaluated according to (Aebi, 1984), superoxide dismutase (SOD) according to (Beyer and Fridovich, 1987) and reduced glutathione (GSH) according to (Beutler, 1963). They were determined spectrophotometrically (nanophotometer p class implen GmbH, Munich, Germany). Assay kits were purchased from Biodiagnostic Company, Giza, Egypt.

The activities of steroidogenic enzymes i.e. 3β -hydroxysteroid dehydrogenase (3β -HSD) and 17β -hydroxysteroid dehydrogenase (17β -HSD) were measured spectrophotometrically

(nanophotometer p class implen GmbH, Munich, Germany) in the testes homogenate according to the method of (Bergmeyer et al., 1983).

Statistical analysis:

Data were presented in tables and figure as means \pm standard deviation (SD). They were determined by using SPSS software version 22. Differences among groups were analyzed by using one-way variance analysis (ANOVA), followed by Tukey's post hoc test analysis for multiple comparisons. Significance was statistically considered at $p \leq 0.05$.

RESULTS

• **Changes in percentages of body weight gain and testes index:** Both % of BW gain and % of testis weight showed significant decreases in diabetic rats (1.34 ± 2.15 & 1.71 ± 0.08) as compared to the control group (8.86 ± 4.00 & 1.87 ± 0.18) respectively. However, the non-diabetic group treated with PGP showed insignificant increases in BW gain % and testis weight % (10.06 ± 7.59 & 1.95 ± 0.14) as compared to changes in the control group (8.86 ± 4.00 & 1.87 ± 0.18) respectively. Diabetic rats treated with PGP powder demonstrated insignificant increases in both BW gain and testis weight % (5.88 ± 5.13 & 1.78 ± 0.16) when compared to the diabetic group (1.34 ± 2.15 & 1.71 ± 0.08) respectively (Table 1 and fig.1).

• **Changes in fasting serum glucose level (mg/dL):** FSG level significantly increased in diabetic group (331.25 ± 28.8) in comparison to the control group (111.88 ± 20.77). While in the non-diabetic group treated with PGP powder,

FSG was insignificantly changed from the control group (115 ± 17.88 & 111.88 ± 20.77 respectively). On the other hand, diabetic treated rats showed a significant decrease in FSG as compared to the diabetic rats (142.38 ± 18.63 & 331.25 ± 28.8 respectively-Table 1 and fig. 2).

• **Changes serum luteinizing hormone (mIU/ml), follicle stimulating hormone (mIU/ml) and serum testosterone (ng/ml) levels:** Diabetic rats exhibited a significant decrease in LH level (0.12 ± 0.044) and insignificant decrease in FSH level (0.08 ± 0.01) as compared to control rats (0.19 ± 0.03 , 0.12 ± 0.24 respectively). Also, serum T level significantly decreased in diabetic rats (0.38 ± 0.16) as compared to the control group (2.25 ± 0.94). While non-diabetic group treated with PGP powder showed insignificant decrease in both LH and FSH (0.18 ± 0.025 & 0.11 ± 0.029) respectively and insignificant increase in serum T (2.55 ± 1.05) as compared to the control

group (0.19 ± 0.03 , 0.12 ± 0.024 & 2.25 ± 0.94) respectively. On the other hand, diabetic rats treated with PGP powder demonstrated elevated levels of LH (0.17 ± 0.086) significantly, FSH (0.095 ± 0.013) insignificantly and testosterone (2.47 ± 0.95) significantly in comparison to the diabetic group (0.12 ± 0.044 , 0.08 ± 0.01 & 0.38 ± 0.16) respectively (Table 1 and fig. 3, 4).

● **Changes in the enzymatic activities of catalase (U/g tissue), superoxide dismutase (U/g tissue) and reduced glutathione (mg/g tissue):** Diabetic rats exhibited significant decreases in the activities of CAT (0.86 ± 0.51), SOD (2156.1 ± 379.85) and GSH (27.27 ± 2.79) as compared to those present in the control group (4.3 ± 0.84 , 3139.31 ± 175.72 & 46.12 ± 5.55) respectively. The non-diabetic treated group showed a significant increase in CAT (6.4 ± 1.09) and insignificant increases of SOD and GSH levels (3186.3 ± 371.32 & 53.37 ± 12.89) respectively in comparison to changed levels of the control group (4.3 ± 0.84 , 3139.31 ± 175.72 & 46.12 ± 5.55) respectively. On the other hand, diabetic treated rats with PGP powder exhibited

significant increases in the enzymatic activities of CAT (5.2 ± 1.16), SOD (3069.07 ± 206.15) and GSH (42.13 ± 4.7) as compared to diabetic rats (0.86 ± 0.51 , 2156.1 ± 379.85 & 27.27 ± 2.79) respectively (Table 1 and fig. 5,6,7).

● **Changes in 3β -hydroxysteroid dehydrogenase (nmol of NAD/min/mg tissue) and 17β -hydroxysteroid dehydrogenase (nmol of NADPH/min/mg tissue):** Both 3β -HSD and 17β -HSD showed significant decreases in diabetic rats (24.53 ± 4.02 & 34.17 ± 5.56) respectively in comparison to the control group (34.19 ± 4.97 & 45.59 ± 6.84) respectively. However, in the non-diabetic group treated with PGP powder, 3β -HSD increased significantly (42.62 ± 8.4) and 17β -HSD increased insignificantly (52.21 ± 5.78) in comparison to the control group (34.19 ± 4.97 & 45.59 ± 6.84) respectively. Also, diabetic rats treated with PGP powder demonstrated significant increases in enzymes levels (43.53 ± 7.4 & 52.1 ± 8.64) respectively as compared to the diabetic group (24.53 ± 4.02 & 34.17 ± 5.56) respectively (Table 1 and fig. 8).

Table (1): Mean changes in body weights, testis weight, serum glucose, luteinizing hormone, follicle stimulating hormone, testosterone, catalase, superoxide dismutase, glutathione, 3 β -hydroxysteroid dehydrogenase and 17 β -hydroxysteroid dehydrogenase levels in different rat groups

Groups	Group I (control)	Group II (Non- diabetic treated)	Group IIIA (diabetic)	Group IIIB (diabetic- treated)
Parameters				
Initial BW (g)	159.5 \pm 12.04	161.5 \pm 11.5	168.13 \pm 8.32	163.25 \pm 9.5
Final BW (g)	173.63 \pm 10.25	177.75 \pm 9.36	170.38 \pm 9.5	169.13 \pm 10.72
BW gain (%)	8.86 \pm 4.00	10.06 \pm 7.59	1.34 \pm 2.15 ^{*#}	5.88 \pm 5.13
Testes weight (g)	3.31 \pm 0.43	3.52 \pm 0.37	2.91 \pm 0.3	2.97 \pm 0.4
Testes index (%)	1.87 \pm 0.18	1.95 \pm 0.14	1.71 \pm 0.08 ^{*#}	1.78 \pm 0.16 [#]
FSG (mg/dL)	111.88 \pm 20.77	115 \pm 17.88	331.25 \pm 28.8 ^{*#}	142.38 \pm 18.63 ^{*#} ^{\$}
Serum LH (mIU/ml)	0.19 \pm 0.03	0.18 \pm 0.025	0.12 \pm 0.044 ^{*#}	0.17 \pm 0.086 ^{\$}
Serum FSH (mIU/ml)	0.12 \pm 0.024	0.11 \pm 0.029	0.08 \pm 0.01	0.095 \pm 0.013
Serum T (ng/ml)	2.25 \pm 0.94	2.55 \pm 1.05	0.38 \pm 0.16 ^{*#}	2.47 \pm 0.95 ^{\$}
CAT (U/g tissue)	4.3 \pm 0.84	6.4 \pm 1.09 [*]	0.86 \pm 0.51 ^{*#}	5.2 \pm 1.16 ^{\$}
SOD (U/g tissue)	3139.31 \pm 175.72	3186.3 \pm 371.32	2156.1 \pm 379.85 ^{*#}	3069.07 \pm 206.15 ^{\$}
GSH (mg/g tissue)	46.12 \pm 5.55	53.37 \pm 12.89	27.27 \pm 2.79 ^{*#}	42.13 \pm 4.7 [#] ^{\$}
3β-HSD (nmol of NAD/min/mg tissue)	34.19 \pm 4.97	42.62 \pm 8.4 [*]	24.53 \pm 4.02 ^{*#}	43.53 \pm 7.4 ^{*#} ^{\$}
17β-HSD (nmol of NADPH/min/mg tissue)	45.59 \pm 6.84	52.21 \pm 5.78	34.17 \pm 5.56 ^{*#}	52.1 \pm 8.64 ^{\$}

Data were represented as means \pm SD. Multiple comparisons were done according to Tukey's post hoc test analysis. FSG, fasting serum glucose; LH, luteinizing hormone; FSH, follicle stimulating hormone; T, testosterone; CAT, catalase; SOD, superoxide dismutase; GSH, reduced glutathione; 3 β -HSD, 3 β -hydroxysteroid dehydrogenase and 17 β -HSD 17 β -hydroxysteroid dehydrogenase.

*Significant values as compared with the control group.

#Significant values as compared with the non-diabetic-treated group.

\$ Significant values as compared with the diabetic group.

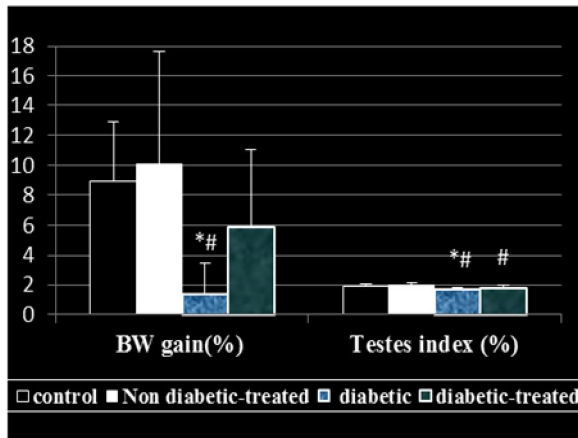


Figure (1): Mean changes (means ± SD) on body weight (%) and testes index.(%)
 *Significant values as compared with the control group.
 #Significant values as compared with the non-diabetic-treated group.

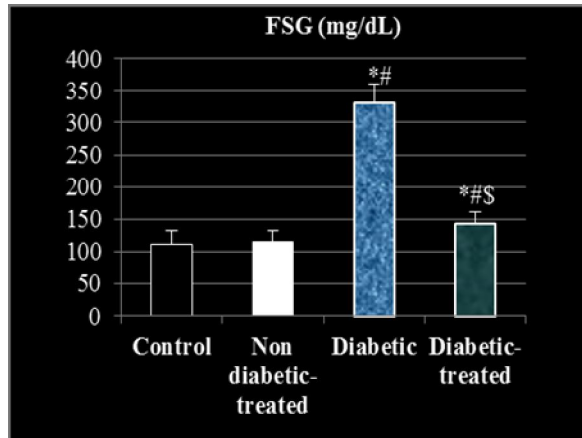


Figure (2): Mean changes (means ± SD) in fasting serum glucose levels (mg/dL).
 *Significant values as compared with the control group.
 #Significant values as compared with the non-diabetic-treated group.
 \$ Significant values as compared with the diabetic group.

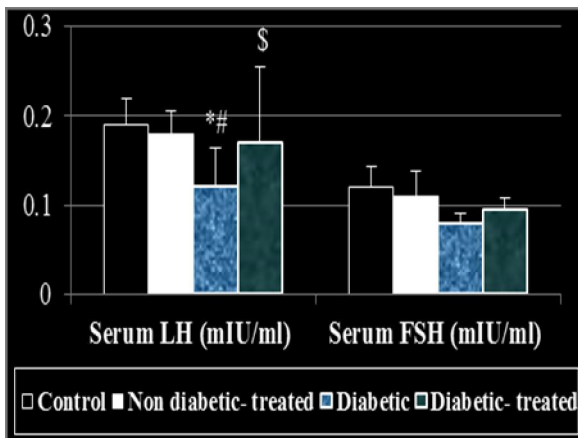


Figure (3): Mean changes (means ± SD) in serum luteinizing hormone and follicle stimulating hormone levels (mIU/ml).
 *Significant values as compared with the control group.
 #Significant values as compared with the non-diabetic-treated group.
 \$ Significant values as compared with the diabetic group.

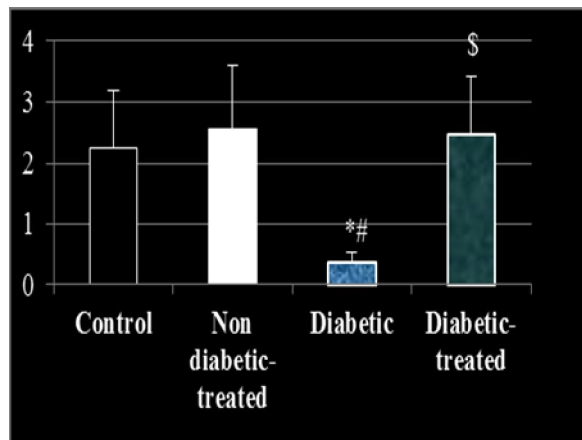


Figure (4): Mean changes (means ± SD) in serum testosterone levels (ng/ml)
 *Significant values as compared with the control group.
 #Significant values as compared with the non-diabetic-treated group.
 \$ Significant values as compared with the diabetic group.

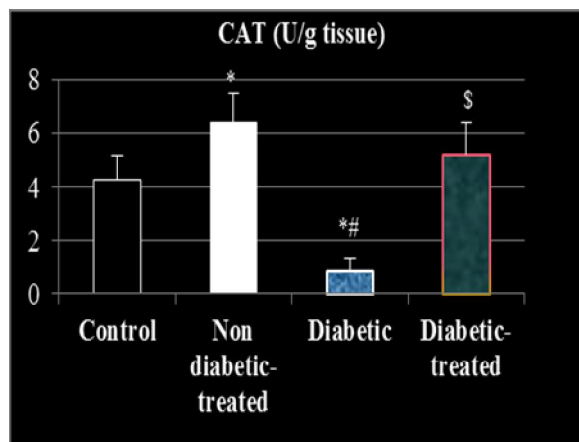


Figure (5): Mean changes (means ± SD) in the activity of tissue catalase (U/g tissue).

*Significant values as compared with the control group.
#Significant values as compared with the non-diabetic-treated group.
§ Significant values as compared with the diabetic group.

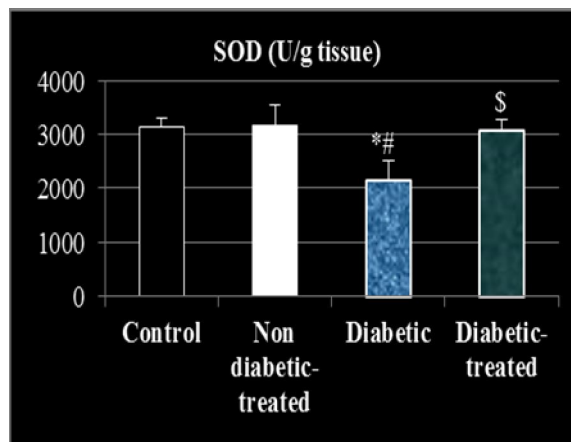


Figure (6): Mean changes (means ± SD) in the activity of tissue superoxide dismutase (U/g tissue).

*Significant values as compared with the control group.
#Significant values as compared with the non-diabetic-treated group.
§ Significant values as compared with the diabetic group.

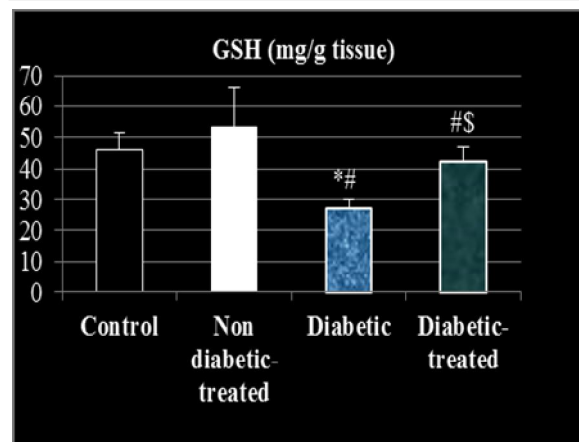


Figure (7): Mean changes (means ± SD) in the activity of testicular reduced glutathione (mg/g tissue).

*Significant values as compared with the control group.
#Significant values as compared with the non-diabetic-treated group.
§ Significant values as compared with the diabetic group.

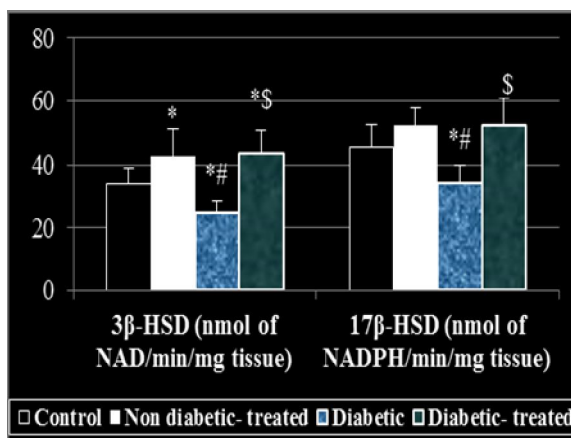


Figure (8): Mean changes (means ± SD) in levels of testicular 3β-HSD (nmol of NAD/min/mg/tissue) and 17β-HSD (nmol of NADPH/min/mg/tissue).

*Significant values as compared with the control group.
#Significant values as compared with the non-diabetic-treated group.
§ Significant values as compared with the diabetic group.

DISCUSSION

In the current study, we used STZ – induced diabetic rats as a model of type I diabetes. We found that body weights of diabetic rats decreased significantly throughout the experimental period. This is expected and could be related to the increased structural protein breakdown due to altered carbohydrate metabolism throughout the course of the disease (Salem *et al.*, 2017). Also, the relative testis weight (testes index) significantly decreased as it is correlated and depends on the body weight changes. Our findings were in agreement with previous studies (Ahmed *et al.*, 2013 and Salem *et al.*, 2017). On the other hand, we found improvements in BW gain and testis weight; although they insignificantly increased, upon administration of PGP powder in either non-diabetic or diabetic rats. Our finding is in consistent with (Ahmed *et al.*, 2013). Also, (Al-Olayan *et al.*, 2014) reported that pomegranate juice on its own did not change the testes weight and relative testes weight as compared with the control group.

The hyperglycemia induced by STZ continued throughout the experimental period. Non- diabetic and diabetic rats treated with PGP exhibited improvements of the FSG levels as compared to control and diabetic groups. The hypoglycemic effect of PG had been documented by (Jurenka, 2008).

Moreover, diabetic rats had demonstrated significant decreases in the levels of LH, FSH and T as compared to those in the control group. The decreased T level indicated that the number of Leydig cells was decreased (Al-Olayan *et al.*, 2014). The oxidative stress and ROS

production induced by diabetes could be implicated in testicular dysfunction (Nasrolahi *et al.*, 2013 and Ghanbari *et al.*, 2016). Recently, (Ding *et al.*, 2016) and (Zhao *et al.*, 2018) demonstrated that testicular apoptotic cell death in STZ-induced type 1 diabetes in rat or mouse model, occurs predominantly as a result of oxidative assault via activation of the mitochondrion-mediated cell death pathway. Despite of the significant decrease in serum T level, LH and FSH levels decreased which means that the negative feedback mechanism of the pituitary-testicular hormonal axis was interrupted. This finding put a light on the central effect of diabetes on the pituitary gland and other trophic hormones besides its oxidative effect on the testicular tissue. Such effects could be caused by insufficient insulin production and inadequate glucose utilization (Bashan *et al.*, 2009 and Wang *et al.*, 2012). Moreover, hyperglycemia promotes generation of non-enzymatic products between sugar and proteins, lipids and DNA, such products are advanced glycation end products (AGEs) (Singh *et al.*, 2014). Increased expression of AGEs and their receptors in the reproductive tract led to ROS production and tissue damage (Singh *et al.*, 2014 and Temidayo & du Plessis Stefan, 2018).

PGP administration to either non-diabetic or diabetic rats increased serum LH, FSH and T levels as compared to control or diabetic group. Effect of PGP might be through its lowering effect of FSG and AGEs levels and as antioxidant due to the presence of some biologically active components like tannins, phenolic acid and flavenoids (Souleman and Ibrahim, 2016). Our findings were in

consistent with (*Dkhil et al., 2013*) and (*Al-Olayan et al., 2014*) who found that PG juice improves serum LH, FSH, and T levels in rats treated with carbon tetrachloride.

Regarding the tissue antioxidant enzyme activities, CAT, SOD and GSH significantly decreased in testicular homogenate of diabetic group, indicating the occurrence of oxidative assault caused by glucose auto-oxidation with increased lipid peroxidation and ROS production as revealed by (*Gharagozloo & Aitken, 2011*) and (*Kangralkar et al., 2012*). On the other hand, PGP administration increased CAT, SOD and GSH levels. Our results agreed with other reports on PG juice against carbon tetrachloride (*Al-Olayan et al., 2014*) and PG juice and methanolic extraction on normal male testis (*Dkhil et al., 2013*). CAT and SOD are free radicals scavengers, which constitute a mutually defense system against oxidative assault (*Mohasseb et al., 2011*). The elevation of CAT, SOD and GSH upon PGP treatment could be due to the presence of flavonoids and tannins in the peels which by themselves having the ability to scavenge OH• and O₂•- radicals, this was in consistence with Turk et al. (2008). So, PGP might exert synergistic effects with CAT, SOD and other antioxidant metabolites to chelate metal ions, decrease lipid peroxidation, and maintain the oxidative balance.

The synthesis of testosterone in the mitochondria is under the influence of the steroidogenic enzymes including 3β-HSD and 17β-HSD (*Page, 2011*). In our study, both 3β-HSD and 17β-HSD significantly decreased in case of diabetes as an evidence of the involvement of the

testicular tissue in the diabetes induced oxidative damage besides the hyperglycemia and the decrease of glucose utilization. Similar results were obtained by (*Premalatha et al., 2013*). However, we found that rats treated with PGP showed increases in enzymes activities particularly in the diabetic treated group as compared to the diabetic group. The protective effect of PGP and its ability to attenuate the testicular damage and restores the steroidogenic pathway might be via means of the antioxidant and antihyperglycemic properties.

CONCLUSION

STZ-induced diabetes in rats led to an oxidative assault of the testes as evidenced by decreased activities of testicular antioxidant enzymes and steroidogenic enzymes. In addition, it adversely affected the pituitary gonadal hormonal axis. Also, pomegranate peels has ability to attenuate the diabetes induced testicular dysfunction. Besides, restoration of the pituitary gonadal hormonal axis was achieved due to antioxidant and antidiabetic properties of pomegranate peels.

REFERENCES

1. **Aebi H (1984):** Catalase in vitro. In *Methods in enzymology*, Packer L., ed., pbl. Academic Press, New York. pp. 121-126.
2. **Ahmed M, EL-Hakem A, Ewis S and Eid R (2013):** Effect of Punica granatum (pomegranate) juice on the ultrastructure of the testes in the streptozotocin induced diabetes in adult albino rat. *Int J Adv Res.*, 1(9): 831-851.
3. **Akondi R, Kumar P, Annapurna A and Pujari M (2011):** Protective effect of Rutin and Naringin on sperm quality in streptozotocin (STZ) induced type 1 diabetic rats. *Iranian*

- journal of pharmaceutical research, 10(3): 585-596.
4. **Al-Olayan E, El-Khadragy M, Metwally D and Moneim A (2014):** Protective effects of pomegranate (*Punica granatum*) juice on testes against carbon tetrachloride intoxication in rats. *BMC complementary and alternative medicine*, 14(1): 164-172.
 5. **Aybek H, Aybek Z, Rota S, Şen N and Akbulut M (2008):** The effects of diabetes mellitus, age, and vitamin E on testicular oxidative stress. *Fertility and sterility*, 90(3): 755-760.
 6. **Bashan N, Kovan J, Kachko I, Ovadia H and Rudich A (2009):** Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. *Physiological reviews*, 89(1): 27-71.
 7. **Bergmeyer HU, Grassl M and Walter HE (1983):** Enzymes. In: *Methods of Enzymatic Analysis*. Bergmeyer HU and Grassl, M. eds., pbl. Verlag Chemie, Weinheim, Germany. pp. 1007- 1009.
 8. **Beyer W and Fridovich I (1987):** Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Anal. Biochem.*, 161: 559-566.
 9. **Beutler E (1963):** Improved method for the determination of blood glutathione. *J. lab. clin. Med.*, 61, 882-888.
 10. **Butt WR (1983):** Gonadotrophins. *Hormones in Blood*, Gray CH, James WTH, eds., 3rd edition, pbl. Academic Press, London, 7, pp. 147-177.
 11. **Ding C, Wang Q, Hao Y, Ma X, Wu L, Li W, Wu Y, Guo F, Ma S, Huang F and Qin G (2016):** Vitamin D supplement improved testicular function in diabetic rats. *Biochemical and biophysical research communications*, 473(1):161-167.
 12. **Dkhil M, Al-Quraishy S and Moneim A (2013):** Effect of pomegranate (*Punica granatum L.*) juice and methanolic peel extract on testis of male rats. *Pakistan Journal of Zoology*, 45(5): 1343-1349.
 13. **Elfalleh W, Hannachi H, Tlili N, Yahia Y, Nasri N and Ferchichi A (2012):** Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower. *Journal of Medicinal Plants Research*, 6(32): 4724-4730.
 14. **Endo E, Ueda-Nakamura T and Nakamura C (2012):** Activity of spray-dried microparticles containing pomegranate peel extract against *Candida albicans*. *Molecules*, 17(9): 10094-10107.
 15. **Fernandes L, Pereira J, López-Cortés I, Salazar D and Ramalhosa E (2015):** Physicochemical changes and antioxidant activity of juice, skin, pellicle and seed of pomegranate (cv. Mollar de Elche) at different stages of ripening. *Food technology and biotechnology*, 53(4): 397-406.
 16. **Ghanbari E, Nejati V and Khazaei M (2016):** Antioxidant and protective effects of Royal jelly on histopathological changes in testis of diabetic rats. *International Journal of Reproductive Bio Medicine*, 14(8): 519-526.
 17. **Gharagozloo P and Aitken R (2011):** The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy. *Human reproduction*, 26(7): 1628-1640.
 18. **Jurenka J (2008):** Therapeutic applications of pomegranate (*Punica granatum L.*): a review. *Alternative medicine review*, 13(2): 128-144.
 19. **Kangralkar V, Patil S and Bandivadekar R (2012):** Oxidative stress and diabetes: a review. *Int J Pharm Appl.*, 1(1): 38-45.
 20. **Kaplan LA (1984):** Glucose. *Clinical chemistry*, pbl. The C.V. Mosby Co. St Louis, Toronto, Princeton, pp. 1032-1036.
 21. **Miguel M, Neves M and Antunes M (2010):** Pomegranate (*Punica granatum L.*): A medicinal plant with myriad biological properties-A short review. *Journal of Medicinal Plants Research*, 4(25): 2836-2847.
 22. **Mohasseb M, Ebied S, Yehia M and Hussein N (2011):** Testicular oxidative damage and role of combined antioxidant supplementation in experimental diabetic rats. *Journal of physiology and biochemistry*, 67(2):185-194.
 23. **Mosele J, Macià A, Romero M, Motilva M and Rubi? L (2015):** Application of in vitro gastrointestinal digestion and colonic

- fermentation models to pomegranate products (juice, pulp and peel extract) to study the stability and catabolism of phenolic compounds. *Journal of Functional Foods*, 14: 529-540.
- 24. Nasrolahi O, Khaneshi F, Rahmani F and Razi M (2013):** Honey and metformin ameliorated diabetes-induced damages in testes of rat; correlation with hormonal changes. *Iranian journal of reproductive medicine*, 11(12): 1013-1020.
- 25. Page S (2011):** Physiologic role and regulation of intratesticular sex steroids. *Current Opinion in Endocrinology, Diabetes and Obesity*, 18(3): 217-223.
- 26. Premalatha R, Jubendradass R, Rani S, Srikumar K and Mathur P (2013):** A phytoxyterol, 28-homobrassinolide modulates rat testicular steroidogenesis in normal and diabetic rats. *Reproductive Sciences*, 20(5): 589-596.
- 27. Salem M, Kolsi R, Dhouibi R, Ksouda K, Charfi S, Yaich M, Hammami S, Sahnoun Z, Zeghal K, Jamoussi K and Affes H (2017):** Protective effects of *Cynara scolymus* leaves extract on metabolic disorders and oxidative stress in alloxan-diabetic rats. *BMC complementary and alternative medicine*, 17(1): 328-346.
- 28. Singh V, Bali A, Singh N and Jaggi A (2014):** Advanced glycation end products and diabetic complications. *Korean J Physiol Pharmacol.*, 18(1): 1-4.
- 29. Souleman A and Ibrahim G (2016):** Evaluation of Egyptian pomegranate cultivars for antioxidant activity, phenolic and flavonoid contents. *Egyptian Pharmaceutical Journal*, 15(3): 143-149.
- 30. Temidayo S and du Plessis Stefan S (2018):** Diabetes mellitus and male infertility. *Asian Pacific Journal of Reproduction*, 7(1):6-14.
- 31. Turk G, Sonmez M, Aydin M, Yuce A, Gur S, Yuksel M and Aksoy H (2008):** Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. *Clinical nutrition*, 27(2): 289-296.
- 32. Wang X, Gu C, He W, Ye X, Chen H, Zhang X and Hai C (2012):** Glucose oxidase induces insulin resistance via influencing multiple targets in vitro and in vivo: the central role of oxidative stress. *Biochimie*, 94(8):1705-17.
- 33. Wheeler M (1995):** The determination of bio-available testosterone. *Ann Clin Biochem.*, 32, 345-357.
- 34. Wide L (1976):** Human pituitary gonadotrophins. *Hormone Assays and their Clinical Applications*, Loraine J A, Bell ET, eds., 4TH edition, pbl. Churchill Livingstone, Edinburgh, London and New York, pp. 87-140.
- 35. Yoruk M, Kanter M, Meral I and Agaoglu Z (2004):** Localization of glycogen in the placenta and fetal and maternal livers of cadmium-exposed diabetic pregnant rats. *Biological trace element research*, 96(1-3): 217-226.
- 36. Zahin M, Aqil F and Ahmad I (2010):** Broad spectrum antimutagenic activity of antioxidant active fraction of *Punica granatum L.* peel extracts. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 703(2): 99-107.
- 37. Zhao Y, Song W, Wang Z, Wang Z, Jin X, Xu J, Bai L, Li Y, Cui J and Cai L (2018):** Resveratrol attenuates testicular apoptosis in type 1 diabetic mice: Role of Akt-mediated Nrf2 activation and p62-dependent Keap1 degradation. *Redox biology*, 14, 609-617.

التأثيرات الوقائية لقشر الرمان على المحور الهرموني بين الغدة النخامية والغدد التناسلية في ذكور الجرذان البيضاء البالغة المصابة بداء السكري المستحث بالإستربتوزوتوسين

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خلفية البحث: يؤدي داء السكري من النوع الأول إلى تغييرات في الجهاز التناسلي الذكري، وإضطراب في تخليق الهرمونات. و يلعب دورا حاسما في الآلية الفسيولوجية المرضية لإختلال الخصية الوظيفي الناجم عن داء السكري. ويحتوي الرمان على مجموعة متنوعة من المكونات البيولوجية النشطة والتي لها خصائص مضادة للأكسدة.

الهدف من البحث: تقييم آثار قشر الرمان على قصور الغدد التناسلية وتكوّن الستيرويدات في ذكور الجرذان البالغة المصابة بداء السكري المستحث بالإستربتوزوتوسين.

مواد وطرق البحث: تم تقسيم أربعين من ذكور جرذان سبراغ-داولي البالغين إلى أربع مجموعات تجريبية. مجموعة ضابطة، مجموعة غير المصابين بمرض السكري الخاضعين للعلاج، مجموعة مرضى السكري و مجموعة مرضى السكري الخاضعين للعلاج. تم استخدام جرعة واحدة من حقن STZ لاحداث مرض السكري. تلقت الجرذان المعالجة 100 ميليجرام / كيلوغرام من قشور PG عن طريق الحقن بالفم مرة واحدة في اليوم لمدة 10 أسابيع. في نهاية التجربة، تم جمع عينات الدم لقياس مستوى الجلوكوز في الدم (FSG)، هرمون (LH)، الهرمون (FSH) ومستويات التستوستيرون (T). كما تم قياس مستوى نشاط (SOD، CAT) والجلوتاثيون (GSH)، والأنزيمات الستيرويدية المنشأ (β-HSD³ و β-HSD¹⁷) في نسيج الخصية. بالإضافة إلى ذلك، تم تسجيل النسب المئوية لوزن الجسم (BW) ووزن الخصية النسبي.

النتائج: أظهرت الجرذان المصابة بداء السكري نقصان في وزن الجسم ووزن الخصية ومستويات LH و FSH و T و CAT و SOD و GSH و β-HSD³ و β-HSD¹⁷، بينما اعطاء قشر الرمان قام بتحسين ارتفاع مستوى السكر في الدم، وأيضا زيادة مستويات LH، FSH، T، كما تم زيادة أنشطة الإنزيمات المضادة للأكسدة والإنزيمات الستيرويدية.

الاستنتاج: يحدث داء السكري خلا تأكسديا بخصية الجرذان البالغة يؤثر سلبا على المحور الهرموني بين الغدة النخامية والغدد التناسلية. وقد عكست قشور الرمان بفعالية الخلل الحادث بالخصية وحسنت إفراز الستيروئيدات التناسلية وذلك بسبب خواص الرمان كمضاد للأكسدة ومضاد لإرتفاع سكر الدم.