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PROTECTIVE EFFECT OF GHRELIN ON TESTICULAR FUNCTIONS IN ADULT MALE DIABETIC ALBINO RATS

By

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ABSTRACT

Background: Oxidative stress is one of the important causes of type 1 diabetes which can induce changes in male reproductive system. Ghrelin is a peptide hormone that has been shown to have antioxidant properties. **Objective:** Evaluation of the ghrelin on testicular dysfunction of rat model of type1 diabetes mellitus induced by streptozotocin (STZ). **Material and Methods:** Forty adult male albino rats were divided into four equal groups: Group 1: served as normal control group received normal saline, group 2: Ghrelin –treated normal group, group 3: STZ-diabetic group, group 4: Ghrelin-treated diabetic group. At the end of experiment, rats were weighed and serum levels of blood glucose, insulin, luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone were determined. Also, epididymal sperm count and motility, testicular activity of superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (GPx), malondialdehyde (MDA), testicular weight and testicular histopathology were determined. **Results:** STZ-diabetic rats showed significant decrease in body weight, testicular weight, serum insulin, LH, FSH, testosterone, testicular activity of SOD, CAT, GPx, epididymal sperm count and motility, and significant increase in serum glucose level and testicular MDA level associated with impairment of testicular histoarchitecture as compared to control group. Ghrelin administration into diabetic rats resulted in significant increase in the body weight, testicular weight, serum insulin, LH, FSH, testosterone, testicular activity of SOD, CAT, GPx, epididymal sperm count and motility, and significant decrease in serum glucose, and testicular MDA levels associated with restoration of testicular histoarchitecture nearly to normal as compared to diabetic group. In the normal group, ghrelin resulted in significant increase in body weight, testicular weight, testicular activities of SOD, CAT, and GPx, sperm motility and significant decrease in testicular MDA, while it produced insignificant changes in the serum levels of glucose, insulin, FSH, LH, testosterone, and sperm count. **Conclusion**: Ghrelin has a protective effect against testicular dysfunction in STZ-induced diabetic rats which may be due to its antioxidant properties and improvement of insulin and glucose levels.

Keywords: STZ, Diabetes mellitus, Ghrelin, Oxidative stress, Testicular function.

INTRODUCTION

Diabetes is a common health problem that has been associated with decreased sexual function in both males and females such as penile erection loss, sexual disinclination, adverse effect on pregnancy outcomes, infertility, and decrease of clitoral sensitivity (Kim et al., 2006 and Amaral et al., 2009).

Oxidative stress (OS) increases in DM due to overproduction of reactive oxygen species (ROS) and decrease efficiency of anti-oxidant defenses (Amaral et al., 2008**)**. The generation of ROS is mainly due to glucose autoxidation, increased glycolysis, protein kinase C activation, activation of polyol pathway, and hexosamine pathway (Hakeem et al.,

2008). Increased ROS causes oxidation of proteins, lipids and damage of DNA in reproductive system of diabetic male (Suresh et al., 2013). Stability and capacity of the antioxidant defense against ROS during chronic diabetes plays an important role in the outcome of long term complications caused by ROS (Evans et al., 2002).

Ghrelin is the natural ligand of the growth hormone secretagogue receptor (GHS-R), and was first found to induce GH secretion in various species (Arvat et al., 2001). It also acts on the hypothalamus to increase food intake (Lim et al., 2013). Ghrelin has been primarily detected in the A-cells of stomach (S?nmez and Ozan, 2007). However, ghrelin secreting cells have also been identified in the pancreas (Arnes et al., 2012), hypothalamus (Cowley et al., 2003), pituitary gland (Korbonits et al., 2001), interstitial Leydig cells, and Sertoli cells (Tena-Sempere et al., 2002).

Ghrelin is involved in glucose metabolism, and its effect on the insulin is either inhibitory (Dezaki et al., 2004) or stimulatory (Date et al., 2002 and Granata et al., 2012). It has been reported that ghrelin has antioxidant and anti-inflammatory properties and functions as a free radical scavenger. The antioxidative effects of ghrelin via reduction in lipid peroxidation and increase of antioxidant enzyme activities have been reported in the liver (Alantary et al., 2014), rat ovary (Kheradmand et al., 2010), preadipocyte cell lines (Zwirska-Korczala et al., 2007), gastric injuries (Coskun et al., 2013), in primary cultured cardiomyocytes (Xu et al., 2008), and sensorimotor neuropathy (Tsuchimochi et al., 2013). Also, antioxidant properties of ghrelin in the testis were reported where ghrelin treatment protected testicular germ cells against damage induced by oxidative stress of heat exposure and ischemia reperfusion injury (Kheradmand et al., 2011 and Taati et al., 2012). The present study aimed to investigate the protective effects of ghrelin against testicular dysfunction in rat model of type 1 diabetes mellitus induced by streptozotocin.

MATERIAL AND METHODS

Animals and experimental design: Forty adult male albino rats of local strain weighing 130-150 g. Animals were kept in cages (20 x 30 x 50 cm –5 rats per cage) at room temperature, maintained on normal light/dark cycle, and fed on commercial rat pellets and water ad libitum. They were left for two weeks for acclimatization before experimental work.

The rats were randomly divided into four equal groups as follows: Group 1 **(**normal control group) received normal saline once every other day for 30 days by subcutaneous (S.C.) injection, group 2 (Ghrelin -treated normal group) where, each rat received single S.C. injection of ghrelin (Sigma Aldrich Co., USA) dissolved in saline at a dose of 2 nmol/ kg / 100 ?l saline every other day for 30 days (Kheradmand et al., 2011), group 3 **(**STZ-diabetic group), and group 4 **(**Ghrelin - treated diabetic group) where each rat was given the same dose of STZ, and ghrelin at a dose of 2 nmol /kg/100 ?l saline by S.C. injection every other day for 30 days (Kheradmand et al., 2011).

Induction of diabetes: Diabetes was induced by single intraperitoneal injection of freshly prepared STZ (Sigma Aldrich Co.,USA) at a dose of 60 mg/kg after fasting overnight (Shrilatha and Muralidhara, 2007). The STZ was dissolved in 0.1M citrate buffer (pH 4.5) immediately prior to injection. Three days later, a blood sample was collected from the tail vein, and fasting blood glucose was determined using a commercial glucometer (ACCU CHEK, Rhoche

Diagnostics, Germany). Rats with blood glucose level more than 300 mg/dl were included in this study. Blood glucose level was monitored regularly every week during the experiment.

Blood and tissue collection: Twenty four hours after the last dose of ghrelin, rats were weighed, blood was collected from retro-orbital plexus of veins and centrifuged at 3000 rpm for 20 minute to separate serum which was stored at - 20?C. It was used for measurement of the levels of serum glucose (Teitz, 1995), serum insulin (Temple et al., 1992), serum testosterone (Huang et al., 1995), serum FSH (Rebar et al., 1982), and serum LH (Teitz, 1995). Epididymes was used for measuring sperm count and sperm motility (WHO, 1992), while testes were weighed, and the right one was homogenized for measurement of testicular activities of MDA (Ohkawa et al., 1982), SOD (Flohe and Otting,1984), GPx ((Tappel, 1978), and catalase (Aebi, 1984), and the left one was used for histopathological examination.

Statistical analysis: Data were expressed as mean ± standard deviation (SD). Statistical analyses were carried out by using SPSS program (version 18 for windows) (SPSS Inc. Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to test for significance between the groups followed by Tukey's multiple comparison test. $P \leq 0.05$ was considered statistically significant.

RESULTS

The results of the present work showed that injection of STZ to rats in group 3 led to significant decrease in the body weight, testicular weight, serum insulin level, serum FSH, serum LH, serum testosterone, sperm count, sperm motility, testicular SOD activity, testicular GPx activity, and testicular CAT activity. Also, there were significant increase in the blood glucose level and testicular MDA activity as compared to control group (Tables 1 and 2).

Table (1): Effects of ghrelin on body weight, testicular weight, blood glucose level, insuli level, and serum levels of FSH, LH and testosterone in different studied groups (Mean ± SD).

Groups / Parameters	Control (group 1)	Ghrelin normal (group 2)	Diabetic (group 3)	Ghrelin-treated diabetic (group 4)
Body weight (g)	252. 8 ± 14.3	285. 6 ± 15.15*	157.32 ± 9.54*#	205.13 ± 11.14*+#
Testicular weight (g)	1.75 ± 0.4	2.3 ± 0. 48*	1.12 ± 0.12* #	1.56 ± 0.11* +#
Serum glucose (mg/dl)	103.13 ± 15.24	98.2 ± 14.23	389. 43 ± 18.21*#	184.21 ± 21.6*+#
Serum Insulin (?IU/mL)	24.7 ± 3.41	26.76 ± 4. 15	12.1 ± 2.54*#	19.7 ± 3.01*+#
Serum FSH (ng/mL)	5.7 ± 1.11	5.1 ± 0.96	2.48 ± 0.32*#	4.54 ± 0.54*+
Serum LH (ng /mL)	4.4 ± 0.5	4. 15 ± 0. 42	1.93 ± 0.23*#	3.64 ± 0.43*+#
Serum testosterone (ng/mL)	4.95 ± 0.50	4. 65 ± 0.75	1.52 ± 0.15*#	3.8 ± 0.32*+#

Number of rats in each group = 10.
* Significant as compared to control group.
+ Significant as compared to diabetic group.
# Significant as compared to ghrelin normal group.

The administration of ghrelin to diabetic rats led to significant increase in the body weight, testicular weight, serum insulin level, serum FSH, serum LH, sperm count, sperm motility, testicular SOD activity, testicular GPx activity, and testicular CAT activity and significant decrease in the blood glucose level, and testicular MAD activity as compared to diabetic group. Also, administration of ghrelin to normal group resulted in significant increase in body weight, testicular weight, testicular activities of SOD, CAT, and GPx, sperm motility and significant decrease in testicular MDA, while it produced insignificant changes in the serum levels of glucose, insulin, FSH, LH, testosterone and sperm count when compared to the control untreated group. Also, there were significant decrease in body weight, testicular weight, serum levels of insulin, FSH, LH, testosterone, testicular activities of SOD, CAT, and SOD, sperm count, sperm motility and significant increase in blood glucose level and testicular activity of MDA in STZ-diabetic and ghrelin–treated diabetic groups when compared with ghrelin normal group (Tables 1 and 2).

Table (2): Effects of ghrelin on testicular SOD, CAT, GP_X and MDA activity, epididymal sperm count and motility in different studied groups (Mean ± SD).

Groups / Parameters	Control (group 1)	Ghrelin normal (group 2)	Diabetic (group 3)	Ghrelin-treated diabetic (group 4)
Testicular SOD activity (U/mg protein)	125.9±13.8	158.34 ± 11.65*	52.4± 6.8* #	110.5± 10.6* + #
Testicular CAT activity (U/mg protein)	19.32 ± 2.13	27.26 ± 2.59*	10.46±1.89*#	17.87± 2.43+ #
Testicular GP_X activity (U/mg protein)	38.14 ± 3.11	49.21 ± 5.57*	17.51 ± 1.94*#	35.3 ± 2.69 * + #
Testicular MDA level (nmol/gm tissue)	118.21 ± 8.47	76.91 ± 9.12*	209.43 ± 16.32*#	140.12± 12.87*+#
Epididymal sperm count (million/ mL)	49.95 ± 5.78	48.67 ± 4.12	22.54 ± 3.01*#	40.45 ± 2.98* + #
Sperm motility (%)	62.21 ± 6.43	68.45 ± 6.11*	25.11 ± 4.32*#	51.87 ± 6.71*+ #

Number of rats in each group = 10
* Significant as compared to control group.
# Significant as compared to ghrelin normal group.
+ Significant as compared to diabetic group.

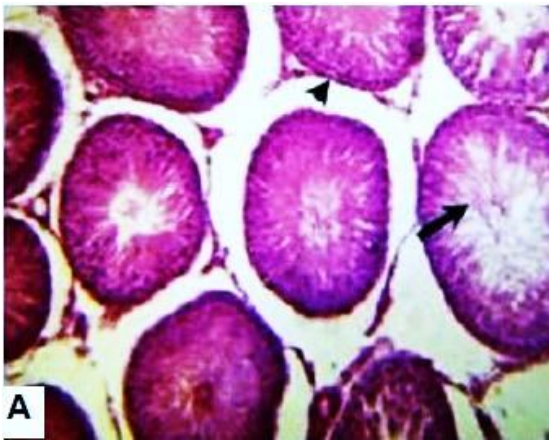

Figure (1): A photomicrograph of a section in rat testis (hematoxylin & eosin x 200) of untreated control group showing normal structure of testis with normal seminiferous tubule lined by normal spermatogenic cells, spermatids (arrow) and normal basement membrane (arrowhead).

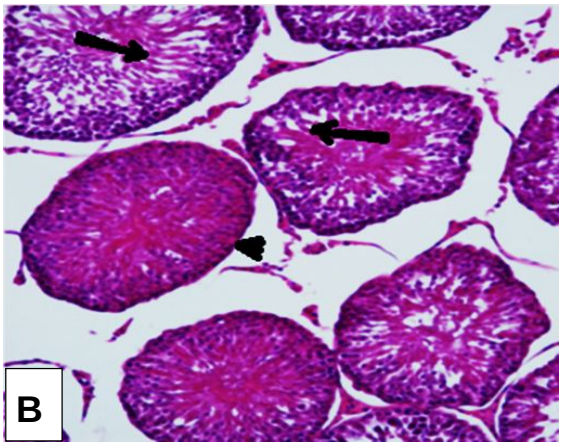

Figure (2): A photomicrograph of a section in rat testis (hematoxylin & eosin x 200) of ghrelin-treated normal group showing normal seminiferous tubules with normal spermatogenic cells, spermatids (arrow) and basement membrane (arrowhead).

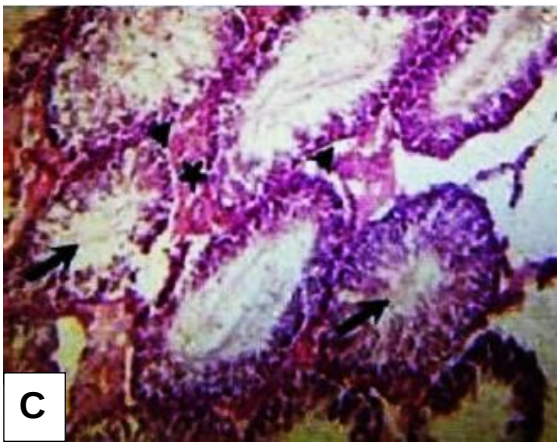

Figure (3): A photomicrograph of a section in rat testis (hematoxylin & eosin x 200) of STZ-diabetic group showing abnormal structure of seminiferous tubules with their lumina either empty or filled with necrotic shredded cells (arrows,), decrease of spermatogonia in the basal area (arrowheads) and excessive edema in interstitial compartment (star).

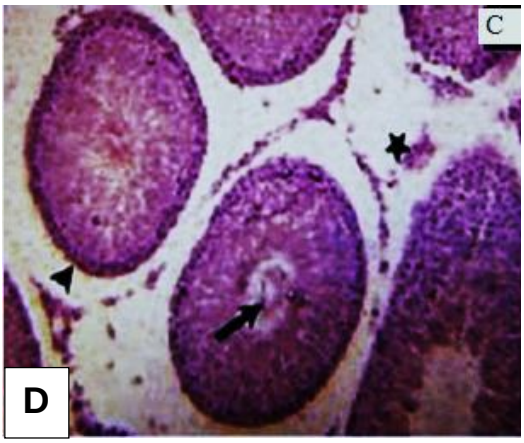

Figure (4): A photomicrograph of a section in rat testis (hematoxylin & eosin x 200) of group 3 (ghrelin treated diabetic group) showing normal appearance of seminiferous tubules with normal spermatids in the centre of the lumen(arrow), normal spermatogonia in the basal area (arrowhead) and normal extracellular matrix in the interstitial space (star).

DISCUSSION

Diabetes is a chronic metabolic disease leading to severe complications such as retinopathy, nephropathy, atherosclerosis and reproductive dysfunction (Ricci et al., 2009). DM interferes with male fertility on many levels. Endocrine control of spermatogenesis, penile erection, ejaculation distortion, sperm count and motility all are affected by diabetes (Rato et al., 2013). Various studies showed that experimentally induced diabetes with STZ had a destructive effect on testis tissue structure (Khaki et al., 2010). Previous

studies showed that testicular weight, sperm count, sperm motility and testosterone level are significantly reduced in diabetic subjects associated with vacuolization in spermatogonia and spermatocytes (Kanter et al., 2012).

Growing evidence indicates that oxidative stress increases in diabetes and plays a role in the development of its complications (Amaral et al., 2008). Excess amounts of ROS and free radicals have adverse effects on sperm motility and fertility due to damage to lipids and DNA of spermatozoa (Naziroglu, 2003). Ghrelin is a peptide hormone predominantly produced by the stomach, and it is the endogenous ligand for growth hormone secretagogue receptor**.** It was found that ghrelin has antioxidant properties (Kheradmand et al., 2009-a).

In the present study, body weights of diabetic rats significantly decreased when compared to control group, and ghrelin administration led to significant increase in the body weight in diabetic group and normal group. Reduction in body weight observed in diabetic rats might be the result of protein breakdown and wasting due to the unavailability of carbohydrate for utilization as an energy source (Andulla and Varadacharyulu, 2003). Antonyselvi et al. (2012) reported that body weight improved by the administration of ghrelin by stimulating food intake at central neuroendocrine level. It was reported that ghrelin induces this effect by acting on arcuate nucleus of the hypothalamus where it stimulates neurons containing the orexigenic factors neuropeptide Y and agouti related peptide, and inhibits neurons producing the anorexigenic peptides pro-opiomelanocortin, cocaine and amphetamine related transcript through binding to GHSR-1a (Chen et al., 2004).

Our study showed that administration of STZ to rats caused significant increase in the serum blood glucose and significant decrease in the serum insulin level as compared to the control group. STZ causes diabetes by rapid depletion of the beta cells mass probably through generation of ROS which leads to a reduction in insulin release and hyperglycemia (Gupta et al., 2004). Administration of ghrelin to STZ diabetic rats caused significant lower levels of the blood glucose and significant higher levels of the serum insulin, while its administration into normal rats produced insignificant changes in these parameters. These findings were in agreement with those observed by Irako et al. (2006) who stated that administration of ghrelin to STZ-treated newborn rats resulted in significant increases of insulin secretion and significant decrease of blood glucose level, and prevent development of diabetes at adult age by increasing pancreatic expression of insulin and Pdx1 mRNA together with increased replication of pancreatic beta cells. Increased insulin secretion by ghrelin may be partly attributed to increased Ca^{2+} level in beta cells via activation of phospholipase C (Date et al., 2002). Also, these results were in accordance with the results observed by Granata et al. (2010) who reported that treatment with either acylated ghrelin, unacylated ghrelin, or obestatin reduce blood glucose level, enhance insulin level and increase pancreatic islet area and number in STZ diabetic rats through upregulation of antiapoptic protein BCL2, and insulin &

Pdx1 mRNA expression which are the main regulators of β- cell function and survival suggesting that these peptides may either prevent β-cell death or stimulate β-cell regeneration.

The results of this study showed significant decrease in the serum levels of testosterone, FSH and LH in STZ diabetic group as compared to control group. These findings were parallel to that of the previous investigations (Baccetti et al., 2002, Olivares et al., 2009 and Khaki et al., 2014) which showed that these changes are due to dysfunction of hypothalamo-pituitary-testicular axis by direct effect of insulin deficiency associated with marked reduction of activities of Leydig cellular enzymes. Insulin plays a physiological role in the secretory activity of pituitary gland, and is needed for normal LH release and Leydig cell function (Perez-Diaz et al., 1982). Moreover, insulin receptors are expressed in the hypothalamic neurons expressing gonadotropin releasing hormone (GnRH), and insulin induces expression of GnRH suggesting that GnRH neurons are directly sensitive to insulin (Salvi et al., 2006). Thus, insulin deficiency in STZ-induced diabetes causes decrease in the pituitary gonadotropins secretion associated with decreased testicular steroidogenesis either due to decreased GnRH release or reduced responsiveness of pituitary to GnRH (Baccitti et al., 2002).

The present study showed that exogenous administration of ghrelin to STZ-induced diabetic group lead to significant increase in the plasma levels of FSH, LH and testosterone, while administration of ghrelin to normal group resulted in insignificant changes in the levels of these hormones as compared to control group. These results were in accordance with that reported by Maher et al. (2013) who stated that these effects of ghrelin in diabetic rats may be due to enhanced secretion of insulin and antioxidant effects of the ghrelin.

The results of the current study revealed significant decrease in the epididymal sperm motility and sperm count in STZ-diabetic rats as compared to control group. These results were in agreement with previous studies (Khaki et al., 2010, Akondi et al., 2011, Khaki et al., 2014 and Ghanbari et al., 2015) which showed that sperm count and sperm motility significantly decreased in rats treated with STZ. Spermatozoa are very susceptible to oxidative stress because its polyunsaturated fatty acids in plasma membrane, nuclear and mitochondrial DNA are susceptible to oxidation (Aitken and Koppers, 2011). Moreover, sperms are very poor in free radical scavengers (Bal et al., 2011). Amaral et al. (2006) reported that the cause of these changes is due to oxidative stress produced by hyperglycemia that causes changes in the sperm membrane specially lipid peroxidation, DNA damage in the sperm nucleus and errors in spermiogenesis affecting fertilizing potential. Also, damage to testicular mitochondria by oxidative stress decreases energy available for the sperms resulting in these changes (Palmeria et al., 2001).

Administration of exogenous ghrelin to diabetic rats resulted in significant increase in epididymal sperm count and sperm motility. These findings were supported by Kheradmand et al. (2009-b), Taati et al. (2012) and Maher et al. (2013)

who attributed these findings to the antioxidative effects of ghrelin on the plasma membrane of the sperms which protects it against oxidative damage. Administration of ghrelin to normal group resulted in insignificant changes in the sperm motility and significant increase in the sperm motility.

Increased oxidative stress is a commonly accepted contributor in the development and progression of DM and its complications (Ceriello, 2000). It was found that DM is accompanied by increased production of free radicals and impaired antioxidant defenses (Baynes and Thorpe, 1999). The findings of the present study proved that testicular activity of MDA significantly elevated and testicular activity of SOD, CAT, and GPx significantly decreased in the STZ-induced diabetic group as compared to control group. Increased oxidative stress causes damage of DNA in all tissues including retina, kidneys, myocardium, brain and testis (Amaral et al., 2006). Moreover, excess lipid peroxidation and reduced antioxidant enzymes concentration impair male fertility in type 1 DM (Aybek et al., 2008). The results of our study were consistent with other studies (Shrilatha & Muralidhara, 2007 and Khaki et al., 2014) which revealed that treatment of rats with STZ significantly increased testicular MDA and decreased testicular antioxidant enzymes. Administration of ghrelin into diabetic rats resulted in significant reduction in the activity of testicular MDA and significant increase in the activity of SOD, CAT and GPx. Also, administration of ghrelin into normal rats resulted in significant reduction in the activity of testicular MDA and significant increase in the activity of SOD, CAT and GPx as compared to normal control group. Kheradmand et al. (2015) reported that ghrelin enhances testicular antioxidant enzymes activities and reduces lipid peroxidation and consequently attenuates testicular injury in diabetic rats. Also, rat testicular damage following experimentally induced cryptorchidism and testicular ischemia/reperfusion was attenuated by ghrelin through its antioxidant effects (Kheradmand et al., 2014 and Taati et al., 2012). Also, it has been shown that ghrelin increases antioxidant defense system and reduces lipid peroxidation in different organs including ovary (Kheradmand et al., 2010), adipocyte tissue (Zwirska-Korczala et al., 2007), brain (Obay et al., 2008), stomach (Iseri et al., 2005), hypertensive rats (Kawczynska Drozdz et al., 2006), and liver (Alantary et al., 2014).

In this study, the histopathological sections showed abnormal structure of seminiferous tubules with necrotic shredded cells, decrease of spermatogonia and extensive edema in the interstitial tissue in STZ–diabetic group as compared to control group. These findings were in agreement with Shrilatha and Muralidhara (2007), and Khaki et al. (2010) who observed atrophy of seminiferous tubules with necrosis of spermatogenic cells and interstitial cells. These changes induced by diabetes are partially attributed to testicular oxidative stress, decreased testosterone level which is essential for growth of reproductive organs, and also decreased FSH level which is needed by Sertoli cells to secrete androgen binding protein which binds testosterone needed for differentiation of spermatids into mature sperms (Holdcraft and Braun, 2004). Administration of ghrelin into

diabetic rats resulted in marked recovery of testicular histoarchitecture of diabetic rats. These results were consistent with Kheradmand et al. (2014) who reported that ghrelin led to recovery of testicular histoarchitecture following experimentally-induced cryptorchidism. The enhancement of testicular histopathology may be due to restoration of the levels of FSH, testosterone, LH, insulin and glucose nearly to the normal values and antioxidant effect of ghrelin.

It has been shown that hyperglycemia, in addition to generation of ROS, causes overexpression of proinflammatory cytokines such as interleukin-1β, interleukin-6, and tumor necrosis factor α (TNFα) in rat testis that cause germ cell apoptosis and decrease of testosterone secretion from Leydig cells (Lysiak, 2004). Also, ghrelin receptors are expressed in lymphocytes, monocytes and macrophages (Dixit and Taub, 2005), and its binding to these receptors inhibits the production of TNFα IL1β and IL6 in testicular tissue (Taati et al., 2015). Therefore, the inhibitory effect of ghrelin on proinflammatory cytokines, besides its antioxidant properties, is another way to suppress the apoptosis of testicular cells.

In conclusion: Ghrelin exerted a potent protective effect against testicular dysfunction in STZ-induced diabetic rats by increasing levels of FSH, LH and testosterone, decreasing lipid peroxidation, increasing antioxidant enzymes SOD, GPx, CAT, enhancing carbohydrate metabolism as proved by significant increase in serum insulin level, and significant decrease of serum glucose level. The protective effect of ghrelin against pituitary-testicular dysfunction should be further investigated to assess its ability as new drugs in clinical medicine.

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REFERENCES

1. **Aebi H. (1984):** Catalase in vitro. Methods Enzymology,105:121-6.
2. **Aitken RJ, and Koppers AJ (2011):** Apoptosis and DNA damage in human spermatozoa. Asian Journal of Andrology, 13 (1): 36–42.
3. **Akondi R.B, 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. **Alantary A, Risk M and Soliman Kh (2014):** protective effect of ghrelin on paracetamol induced acute hepatotoxicity in rats. J. Physiol. Phathophysio., 5(2): 7-14.
5. **Amaral S, Moreno AJ, Santos MS, Seica R and Ramalho-Santos J. (2006):** Effects of hyperglycemia on sperm and testicular cells of Goto-Kakizaki and streptozotocin- treated rat models for diabetes. Theriogenology, 66(9):2056-67.
6. **Amaral S, Oliveira PJ and Ramalho-Santos J (2008):** Diabetes and the impairment of reproductive function: Possible role of mitochondria and reactive oxygen species. Curr Diabetes Rev., 4: 46–54.
7. **Amaral S, Mota PC, Lacerda B, Alves M, Pereira Mde L, Oliveira PJ and Ramalho-Santos. J. (2009):** Testicular mitochondrial alterations in untreated streptozotocin-induced diabetic rats. Mitochondrion, 9: 41–50.
8. **Andulla B and Varadacharyulu N.Ch (2003):** Antioxidant role of mulberry leaves in streptozotocin-diabetic rats. Clin. Chim. Acta., 338: 3-10.

9. Antonyselvi S, Kumar D, Shanthi A, and Anand P. (2012): Effect of intraperitoneal administration of Ghrelin hormone in testis of immature and mature male albino rats to study histoarchitecture. Int. J. Pharm. Biomed. Res.,3(2): 85-89

10. Arnes L, Hill J.T, Gross S, Magnuson M.A and Sussel L. (2012): Ghrelin Expression in the Mouse Pancreas Defines a Unique Multipotent Progenitor Population. PLoS ONE, 7: 520-26.

11. Arvat E, Maccario M, Di Vito L, Broglio F, Papotti M, Muccioli G and Dieguez C (2001): Endocrine activities of ghrelin, a natural growth hormone secretagogue (GHS), in humans: comparison and interactions with hexarelin, a nonnaturalpeptidyl GHS, and GH-releasing hormone. J Clin Endocrinol Metab., 86:1169–1174.

12. Aybek H, Aybek Z, Rota S, Sen N and Akbulut M. (2008): The effects of diabetes mellitus, age, and vitamin E on testicular oxidative stress. Fertil. Steril., 90:755–60.

13. Baccetti B, La Marca A, Piomboni P, Capitani S, Bruni E, Petraglia F and De Leo V. (2002): Insulin-dependent diabetes in men is associated with hypothalamo-pituitary derangement and with impairment in semen quality. Hum Reprod., 17(10):2673-2677.

14. Bal, G. Türk, M, Tuzcu M, Yilmaz O, Ozercan I and Kuloglu T. (2011): Protective effects of nanostructures of hydrated C_{60} fullerene on reproductive function in streptozotocin-diabetic male rats. Toxicology, 282 (3): 69–81.

15. Baynes J.W and Thorpe S.R. (1999): Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. Diabetes, 48:1–9.

16. Ceriello A. (2000): Oxidative stress and glycemic regulation. Metabolism, 49(2, Suppl 1):27–29.

17. Chen H.Y, Trumbauer M.E, Chen A.S, Weingarth D.T, Adams J.R, Frazier E.G,Shen Z, Marsh D.J, Feighner S.D, GuanX.M, Ye Z, Nargund R.P, Smith R.G, Van der PloegL.H, Howard A.D, MacNeil D.J and Qian S. (2004): Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. Endocrinol., 145: 2607–12.

18. Coskun Z.M, Sacanc O, Karatugd A, Turka N, RefiyeYanardagc R, Bolkentd S and Bolkenta S. (2013): Regulation of oxidative stress and somatostatin, cholecystokinin, apelin gene expressions by ghrelin in stomach of newborn diabetic rats. ACTHIS, 50697-50704.

19. Cowley M.A, Smith R.G, Diano S, Tschop M, Pronchuk N, Grove K.L Strasburger C.J Bidlingmaier M, Esterman M, Heiman M.L, Garcia-Segura L.M, Nillni E.A, Mendez P, Low M.J, Sotonyi P, Friedman J.M, Liu H, Pinto S, Colmers W.F, Cone R.D and Horvath T.L (2003): The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. Neuron, 37: 649–661.

20. Date Y, Nakazato M, Hashiguchi S, Dezaki K, Mondal MH, Hosoda H, Kojima M, Kangawa K, Arima T, Matsuo H, Yada T and Matsukura S. (2002): Ghrelin is present in pancreatic B-cells of humans and rats and stimulates insulin secretion. Diabetes, 51:124–129.

21. Dezaki K, Hosoda H, Kakei M, Hashiguchi S, Watanabe M, Kangawa K and Yada T. (2004): Endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca^{2+} signaling in beta-cells: implication in the glycemic control in rodents. Diabetes, 53: 3142–3151.

22. Dixit V.D and Taub D.D. (2005): Ghrelin and Immunity: A Young Player in an Old Field. Exp. Gerontol., 40:900–910.

23. Evans J.L, Goldfine I.D, Maddux B.A and Grodsky G.M. (2002): Oxidative stress and stress activated signaling pathways: A unifying hypothesis of type 2 diabetes. Endo. Rev., 23: 599-622.

24. Flohe L and Otting F.(1984): Superoxide dismutase assays. Methods Enzymol., 105:93–104.

25. Ghanbari E, Nejati V, Najafi,Gh, Khazaei M and Babaei M (2015): Study on the effect of royal jelly on reproductive parameters in

streptozotocin-induced diabetic rats. Int. J. Fertil. Steril., 9 (1): 113–120.

26. Granata R, Baragli A, Settanni F, Scarlatti F and Ghigo E. (2012): Unraveling the role of the ghrelin gene peptides in the endocrine pancreas. J. Mol. Endocrinol., 45: 107–118.

27. Granata R, Volante M, Settanni F, Gauna C, Ghe C, Annunziata M, Deidda1 B, Gesmundo I, Abribat T.L Muccioli G, Ghigo E and Papotti M. (2010): Unacylated ghrelin and obestatin increase islet cell mass and prevent diabetes in streptozotocin-treated newborn rats. Journal of Molecular Endocrinology, 45: 9–17

28. Gupta S, Kataria M, Gupta P.K, Murganandam R.C and Yashroy R.C. (2004): Protective role of extracts of neem seeds in diabetes caused by streptozotocin in rats. J. Ethnopharmacol., 90: 185–89.

29. Hakeem P, Sani H.A and Noor M.M. (2008): Effects of Gynuraprocumbens extract and glibenclamide on sperm quality and specific activity of testicular lactate dehydrogenase in streptozotocin-induced diabetic rats. Malaysian J. Biochemistry and Molecular Biology, 16:10–14.

30. Holdcraft R.W and Braun R.E. (2004): Androgen receptor function is required in Sertoli cells for the terminal differentiation of haploid spermatids. Development, 131(2):459-67.

31. Huang, H.F.S, Linsenmeyer T.A, Li M.T, Giglio W, Anesetti R, von Hagen J, Ottenweller, J.E and Pogach, L. (1995): Acute effects of spinal cord injury on the pituitary-testicular hormone axis and Sertoli cell functions: a time course study. J. Androl., 16: 148–157.

32. Irako T, Akamizu T, Hosoda H, Iwakura H, Ariyasu H, Tojo K, Tajima N and Kangawa K. (2006): Ghrelin prevents development of diabetes at adult age in streptozotocin-treated newborn rats. Diabetologia, 49: 1264–1273.

33. Iseri S, Sener G, Yuksel M, Contuk G, Cetinel S, Gedik N and Yegen B.C. (2005): Ghrelin against alendronate-induced gastric damage in rats. J Endocrinol., 187:399–406.

34. Kanter M, Aktas C and Erboga M. (2012): Curcumin attenuates testicular damage, apoptotic germ cell death, and oxidative stress in streptozotocin-induced diabetic rats. Mol. Nutr. Food Res., 56: 1–8.

35. Kawczynska-Drozdz A, Olszanecki R, Jawein J, Brzozowski T, Pawlik W.W, Korbut R and Guzik T.J. (2006): Ghrelin inhibits vascular superoxide production in spontaneously hypertensive rats. Am. J. Hypertens., 19:764–767.

36. Khaki, A., Fathiazad, F, Nouri, M., Khaki, A, Maleki, N.A., Khamnei, H.J, and Ahmadi P. (2010): Beneficial effects of quercetin on sperm parameters in streptozotocin-induced diabetic male rats. Phytother. Res., 24(9):1285-91.

37. Khaki A, Khaki A.A, Hajhosseini L, Golzar S and Ainehchi N. (2014): The anti-oxidative effects of ginger and cinnamon on spermatogenesis dysfunction of diabetic rats. Afr. J. Tradit. Complement Altern. Med., 11(4):1-8

38. Kheradmand A, Alirezaei M, Asadian P, RafieiAlavi E and Joorabi S. (2009-a): Antioxidant enzyme activity and MDA level in the rat testis following chronic administration of ghrelin. Andrologia, 41(6):335-340.

39. Kheradmand A, Alirezaei M and Birjandi M. (2010): Ghrelin promotes antioxidant enzyme activity and reduces lipid peroxidation in the rat ovary. Regulatory Peptides, 162: 84–89.

40. Kheradmand A, Alirezaei M and Dezfoulian O. (2015): Biochemical and histopathological evaluations of ghrelin effects following cadmium toxicity in the rat testis. Andrologia, 47(6): 634-43.

41. Kheradmand A, Dezfoulian O, Alirezaei M and Hadian B. (2014): Ghrelin is a suppressor of testicular damage following experimentally induced cryptorchidism in the rat. J Pediatr Surg., 49 (4):593-8.

42. Kheradmand A, Dezfoulian O and Tarrahi M.J. (2011): Ghrelin attenuates heat-induced degenerative effects in the rat testis. Regulatory Peptides, 167: 97–104.

43. Kheradmand, A, Taati, M and Babaei H. (2009-b): Ghrelin enhances viability of rat

spermatozoa during incubation at 37°C. Iranian Journal of Veterinary Research, 27: 103-9.

44. Kim N.N, Stankovic M, Cushman T.T, Goldstein I, Munarriz R, and Traish A.M. (2006): Streptozotocin-induced diabetes in the rat is associated with changes in vaginal hemodynamics, morphology and biochemical markers. BMC Physiol., 6: 1–9.

45. Korbonits M, Kojima M, Kangawa K, and Grossman A.B. (2001): Presence of ghrelin in normal and adenomatous human pituitary. Endocrine, 14: 101–104.

46. Lim C.T, Kola B, Feltrin D, Perez-Tilve D, Tsch?p M.H, Grossman A.B and Korbonits M. (2013): Ghrelin and cannabinoids require the ghrelin receptor to affect cellular energy metabolism. Molecular and Cellular Endocrinology, 365: 303–308.

47. Lysiak J.J. (2004): The role of tumor necrosis factor-alpha and interleukin-1 in the mammalian testis and their involvement in testicular torsion and autoimmune orchitis. Reprod. Biol. Endocrin., 2:1–10.

48. Maher N, Ali Kh, Dalia I and Suzan M.M. (2013): Effect of ghrelin on the testicular function in streptozotocin induced type 1 diabetic rats. International Journal of Diabetes Research, 2(6): 101-11

49. Naziroglu M. (2003): Enhanced testicular antioxidant capacity in streptozotocin-induced diabetic rats: protective role of vitamins C and E and selenium. Biol. Trace Elem. Res., 94: 61-72.

50. Obay B.D, Tasdemir E, Tumer C, Bilgin H.M and Atmaca M. (2008): Dose dependent effects of ghrelin on pentylenetetrazole-induced oxidative stress in a rat seizure model. Peptides, 29:448–455.

51. Ohkawa H, Ohishi N and Yagi K. (1982): Assay for lipid peroxides in animal tissues by thiobarbaturic acid reaction. Anal. Biochem., 95: 351-358.

52. Olivares A, Méndez J.P, C?rdenas M, Oviedo N, Palomino M.A, Santos I, Perera-Mar?n G, Gutiérrez-Sagal R and Ulloa-Aguirre A. (2009): Pituitary-testicular axis function, biological to immunological ratio and charge isoform distribution of pituitary LH in male rats with experimental diabetes. Gen. Comp. Endocrinol., 161(3):304-12.

53. Palmeira C.M, Santos D.L, Seiça R, Moreno A.J and Santos M.S.(2001): Enhanced mitochondrial testicular antioxidant capacity in Goto-Kakizaki (GK) diabetic rats: role of coenzyme Q. Am. J. Physiol., 28:C1023–C1028.

54. Pérez D?az J, Benitez A and Fern?ndez-Galaz C. (1982): Effect of streptozotocin diabetes on the pituitary-testicular axis in the rat. HormMetab Res., 14(9):479-82

55. Rato, L, Alves, M.G, Dias T.R, Lopes G, Cavaco J., Socorro, S and Oliveira P.F. (2013): High-energy diets may induce a pre-diabetic state altering testicular glycolytic metabolic profile and male reproductive parameters. Andrology,1(3):495-504.

56. Rebar R.W, Morandini I.C, Petze J.E and Erickson G.F. (1982): Hormonal basis of reproductive defects in athymic mice: reduced gonadotropins and testosterone in males. Biol. Repro., 5: 1267-76.

57. Ricci G, Catizone A, Esposito R, Pisanti F.A, Vietri M.T and Galdieri M. (2009): Diabetic rat testes: morphological and functional alterations. Andrologia, 41: 361-368.

58. Salvi R, Castillo E, Voirol M.J, Glauser M, Rey J.P, Gaillard R.C, Vollenweider P and Pralong F.P. (2006): Gonadotropin-releasing hormone-expressing neurons immortalized conditionally are activated by insulin: implication of the mitogen-activated protein kinase pathway**.** Endocrinology, 147(2):816-26.

59. Shrilatha B and Muralidhara E. (2007): Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: Its progression and genotoxic consequences. Reprod. Toxicol., 23: 578-587.

60. S?nmez M.F and Ozan E. (2007): Determination of ghrelin immunoreactivity in the rat stomach after fasting and refeeding. Acta Histochem., 109:193–199.

61. Suresh S, Prithiviraj E, Lakshmi NV, Ganesh, MK, Ganesh, L and Prakash S (2013): Effect of Mucunapruriens (Linn.) on

mitochondrial dysfunction and DNA damage in epididymal sperm of streptozotocin induced diabetic rat. J Ethnopharmacol., 9;145 (1):32-41.

61. Taati M, Moghadasi M, Dezfoulian O, Asadian P, Kheradmand A, Abbasi M and Zendehdel M. (2012): The effect of ghrelin pretreatment on epididymal sperm quality and tissue antioxidant enzyme activities after testicular ischemia/reperfusion in rats. Journal of Physiology and Biochemistry, 68: 91-97.

63. Taati M, Moghadasi M, Dezfoulian O, PaymanAsadian P and Zendehdel, M. (2015): Effects of Ghrelin on germ cell apoptosis and proinflammatory cytokines production in Ischemia-reperfusion of the rat testis.Iran J. Reprod. Med., 13(2): 85–92.

64. Tappel AL. (1978): Glutathione peroxidase and hydroperoxides. Methods Enzymol., 52:506–513.

65. Temple R.C, Clark P.M and Hales C.N. (1992): Measurement of insulin secretion in type 2 diabetes: problems and pitfalls. Diabetic Medicine, 9: 503-512.

66. Tena-Sempere M, Barreiro M.L, Gonzalez L.C, Gaytan F, Zhang F.P, Caminos J.E, Pinilla L, Casanueva F.F, Diéguez C and Aguilar E. (2002): Novel expression and functional role of ghrelin in rat testis. Endocrinology, 143: 717–725.

67. Tietz N.W. (1995): Clinical Guide to Laboratory Tests, 3rd Ed., Pbl, *W.B. Saunders Company*, Philadelphia, Pp:509–580.

68. Tsuchimochi W, Kyoraku I, Yamaguchi H, Toshinai K, Shiomi K, Kangawa K and Nakazato M. (2013): Ghrelin prevents the development of experimental diabetic neuropathy in rodents. European Journal of Pharmacology, 702 : 187–193.

69. WHO. (1992): Laboratory manual for the examination of human semen and sperm–cervical mucus interaction. (3rd ed.). Pbl; Cambridge University Press: Cambridge, United Kingdom, Pp:321

70. Xu Z, Lin S, Wu W, Tan H, Wang Z, Cheng C, Lu L and Zhang X. (2008): Ghrelin prevents doxorubicin induced cardiotoxicity TNF alpha/NF kB pathways and mitochondrial protective mechanisms. Toxicology, 147:133-8

71. Zwirska-Korczala K, Adamczyk-Sowa M, Sowa P, Pilc K, Suchanek R, Pierzchala K, Namyslowski G, Misiolek M, Sodowski K, Kato I, Kuwahara A and Zabielski R. (2007): Role of leptin, ghrelin, angiotensin Π and orexins in 3 T3 L1 preadipocyte cells proliferation and oxidative metabolism. J. Physiol. Pharmacol., 58: 53-64.

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خلفية البحث: يعتبر الإجهاد الناتج عن الأكسدة فى مرض السكرى من النوع الأول واحدا من أهم الأسباب التى تحدث تغيرات في الجهاز التناسلى الذكرى. والجريلين هرمون ببتيدى ثبت له خواصا مضادة للأكسدة.

الهدف من البحث: تقييم تأثير الجريلين على إضطرابات وظائف الخصية في النوع الأول من مرض السكرى المستحدث بواسطة ستربتوزوتوسين في ذكور الجرذان البيضاء.

مواد وطرق البحث: تم إجراء الدراسة على أربعين من ذكور الجرذان البيضاء البالغة، قسمت إلى أربع مجموعات متساوية: المجموعة الأولى: مجموعة ضابطة أعطيت محلولا ملحيا، المجموعة الثانية: مجموعة طبيعية عولجت بالجريلين، المجموعة الثالثة: مجموعة مصابة بالسكرى، المجموعة الرابعة: مجموعة مصابة بداء السكرى وعولجت بالجريلين. وبعد إنتهاء التجربة تم قياس وزن الجسم، ومستوى الجلوكوز، والإنسولين، و هرمون التستوستيرون، وإل إتش، وإف إس إتش فى الدم، وكذلك تم قياس حركة الحيوانات المنوية وعددها ونشاط إنزيمات كتالاز، سوبرأكسيد ديسميوتاز، وجلوتاثيون بيروكسيداز، ونشاط مالون داى ألداهيد فى الخصى، ووزن الخصية، وكذلك فحص مجهرى للخصي.

النتائج: اظهرت الجرذان المصابة بداء السكرى المستحدث بستربتوزوتوسين نقصا فى وزن الجسم ووزن الخصى ومستوى الإنسولين و إل إتش وإف إس إتش والتستوستيرون فى الدم، وكذلك نشاط كتالاز، سوبر أكسيد ديسميوتاز وجلوتاثيون بيروكسيداز فى الخصى وكذلك عدد وحركة الحيوانات المنوية كما أظهرت زيادة فى مستوى جلوكوز الدم ومستوى مالون داي ألداهيد فى الخصية بنسب دالة إحصائيا متبوعا بتدهور فى التركيب الهستولوجى للخصى بالمقارنة مع المجموعة الضابطة. عندما تم حقن الجرذان المصابة بداء السكرى بالجريلين أدى ذلك إلى زيادة وزن جسم وخصى الجرذان ومستوى الإنسولين وهرمونات التستوستيرون وإف إس إتش وإل إتش فى الدم وكذلك الإنزيمات المضادة للأكسدة فى الخصى وعدد وحركة الحيوانات المنوية، كما أدى إلى نقص مستوى الجلوكوز فى الدم ومستوى مالون داي ألداهيد فى الخصية بنسب دالة احصائيا، كما كان هناك إستعادة فى التركيب الخلوى للخصى تقريبا الى الحد الطبيعي بالمقارنة بالمجموعة المصابة بداء السكري ولم تتناول الجريلين. وقد أدى حقن الجريلين فى الجرذان الطبيعية إلى زيادة وزن جسم وخصى الجرذان ومستوى الإنزيمات المضادة للأكسدة فى الخصى وعدد وحركة الحيوانات المنوية، وكذلك نقص مالون داى ألداهيد بنسب دالة إحصائيا، بينما أحدث تغييرات فى مستوى جلوكوز الدم، والإنسولين، وإف إس إتش، وإل اتش، والتستوستيرون، وعدد الحيوانات المنوية بنسب غير دالة احصائيا.

الإستنتاج: الجريلين له تأثير وقائي على إضطرابات وظائف الخصية فى الجرذان المصابة بداء السكرى المستحدث وذلك ربما يكون بسبب تأثيره المضاد للأكسدة وتحسينه مستوى الإنسولين والجلوكوز فى الدم.