doi:10.4149/endo_2011_03_139

# Effect of ginseng extract supplementation on testicular functions in diabetic rats

[1]Sawiress FAR, [2]Ziada MS, [3] Bebawy WSF, [4]Amer, HA

*[1]Department of Physiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt; [2]Artificial Insemination and Embryo Transfer Department, Animal Reproduction Research Institute, Al Haram, Giza, Egypt; [3]General Organization of Veterinary Services, Giza, Egypt; [4]National Research Center, Theriogenology Department, Doki, Giza, Egypt*
*e-mail: francoisawiress@hotmail.com*

**Objective.** It was aimed to investigate the effect of standardized ginseng extract on fertility parameters in diabetic rats.

**Methods.** Thirty male rats were randomly allocated into three groups of 10 rats each: 1. controls, 2. diabetes (D) and 3. diabetes + ginseng (DG). The latter two groups were rendered diabetic by i.p. injection of streptozotocin (STZ; 50 mg/kg). Standardized ginseng extract (Dansk Droge A/S, Copenhagen, Denmark) was administered per os (100 mg/kg BW) by stomach tube daily for 90 days starting one week after STZ. Ninety days post STZ the rats were sacrificed, and testis, epididymis, prostate, and seminal vesicles were weighed and subjected to histological examination. In addition, spermiogram, testicular enzyme markers, intratesticular steroid hormonal profile and testicular antioxidant status were estimated.

**Results.** The administration of ginseng extract resulted in a significant improvement of fertility parameters and testicular antioxidants together with a decrease in malondialdehyde and testicular pathological signs including degenerative changes of the seminiferous tubules.

**Conclusion.** Ginseng extract may be a beneficial adjuvant therapy for diabetics suffering from infertility as a complication.

**Key words:** antioxidants, diabetes, ginseng extract, infertility, spermiogram, testicular enzyme markers.

Diabetes mellitus is prevalent globally and has been projected to become one of the world's main disablers and killers within next 25 years (Zimmet et al. 2001). Diabetes is a multifaceted multiorgan disorder declared as a disease of complications, where it affected testicular functions including steroidogenic and spermatogenic activities (Naziroglu 2003). These alterations in testicular function seemed to be multifactorial, where Leydig cells steroidogenic enzymes activities were lowered (Sudha et al. 2000). In addition, Leydig cells responsiveness and binding to LH were reduced (Tanaka et al. 2001). Moreover, progressive loss of viability in Sertoli cells was associated with hyperglycemia (Gondos et al. 1998). Also testis of diabetic rats showed increased apopotosis involving spermatogenic series (Koh 2007). This reproductive and sexual dysfunction, induced by diabetes, was associated with oxidative stress resulting in lipid and protein peroxidation which was evident two weeks after the onset of diabetes (Muralidhara 2007; Gumieniczek et al. 2008). Similarly, testicular antioxidant enzyme systems were altered (Gumieniczek et al. 2008). Thus, testicular weights, sperm counts and

**Corresponding author:** Francois Amin Riad Sawiress, Ph.D., [1]Department of Physiology, Faculty of Veterinary Medicine, Cairo University, Giza-12211, Egypt. Tel: 202-2391-5546 E-mail: francoisawiress@hotmail.com

motility were decreased in diabetic rats (Amaral et al. 2006; Koh 2007).

Panax ginseng is one of the most widely used herbs in oriental medicine (Kang et al. 2006). Moreover, it became increasingly popular in the western world due to its tonic effect and possible preventive and restorative properties (Kim et al. 2006). Researches had shown that ginseng administered to rats, resulted in significant increase in epididymal and testicular sperm counts (Park et al. 2006). Similarly, ginsenosides were shown to increase sperm motility in human subjects suffering from inferior motility (Chen et al. 2001).

Thus, the aim of the present study was to clarify the effect of standardized ginseng extract administration on fertility parameters in diabetic rats and to correlate this effect with testicular enzyme markers and antioxidant status.

## Materials and Methods

**Animals.** Thirty male Sprague-Dawley rats, weighing 150-200g were obtained from the National Research Center, Giza, Egypt, and housed in plastic cages with saw dust bedding, where food and water were provided ad-libitum. Rats were maintained on 12:12 hour light-dark cycle. After one week of adaptation, rats were randomly allocated into three equal groups of 10 rats each. First group served as normal control and was injected intraperitonealy with 0.2 ml of 0.05 M citrate buffer, pH4.5, while the second and third groups were rendered diabetic by intraperitoneal injection of 50 mg/kg streptozotocin (STZ) dissolved in 0.05 M citrate buffer, pH4.5 (Stephen-Morris et al. 1996). One week post STZ injection, blood samples were collected by orbital sinus technique for determination of blood glucose concentrations, thus confirming induction of diabetes. Third group is then supplemented with standardized Korean panax ginseng extract obtained from Dansk Droge A/S (Copenhagen, Denmark). Standardized ginseng extract was administered by a stomach tube at a rate of 100 mg/kg body weight and dissolved in 100 ml distilled water (Kang et al. 2006). Ginseng supplementation started one week post STZ injection and lasted for 90 days.

**Sampling.** Ninety days post STZ injection rats were anaesthetized by deep ether and, sacrificed by decapitation. Immediately, post decapitation, testis, epididymis, and accessory sexual glands were removed, washed with warm saline and their wet weight were obtained using analytical balance (Sartorius 1702) for determination of their relative and absolute weights. Epididymal sperm samples were taken from the *cauda epididymis* for determination of sperm counts (using Thoma ruling haemocytometer), percentage of live and abnormal spermatozoa in stained smears by eosin-nigrosine stain according to Blom (1983). One testicle was fixed in 10 % formol-saline for histopathological examination according to the method of George (1981), while the other testicle was kept in liquid nitrogen (-196 °C) for the determination of testicular enzyme markers activity and antioxidant status.

**Testicular tissue tests.** Testicular tissue protein concentration was determined according to the procedure adopted by Lowry et al. (1951).

**Testicular enzyme markers.** Activities of acid phosphatases (ACP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and gamma-glutamyl transferase (γGT) were determined in testicular homogenate (Hodgen and Sherins 1973) according to the method of Babson and Read (1959), Teitz (1970), Allain (1973), and Szasz (1969) respectively.

**Testicular hormonal profile.** Testicular testosterone and estradiol concentrations were determined by radioimmunoassay according to the method of Jaffe and Behrman (1974) and Xing et al (1983) using commercial testosterone and estradiol kits purchased from Diagnostic Products Corporation (DPC, Los Angeles, USA) and Diagnostic System Laboratories (DSL, Webster, TX, USA) respectively. Count/minute was measured by Gamma Tec. II (Model 600B, The Nucleus, INC., Oak Ridge, TN, USA).

**Testicular antioxidant status.** This was accomplished by measuring malondialdehyde, as one of the main end products of lipid peroxidation (Yoshioka et al. 1979), superoxide dismutase (Jewett and Rocklin 1993) and glutathione S-transferase (Habig et al. 1974) activities.

**Statistical evaluation.** Data are presented as means ±S.E. and analyzed by one way ANOVA using Costate computer program version 3.03 (copyright 1986 Cottort software) according to the method of Snedecor and Cochran (1980). Groups were compared by the least significant difference test (LSD) at the 5 % level of probability.

## Results

Table 1 shows that paired epididimotesticular, prostatic and seminal vesicle absolute weights were significantly lower in the diabetic group when compared either to controls or to ginseng treated group. Moreover, Table 1 also shows that the absolute weights of the above men-

**Table 1**

**Effect of ginseng extract administration on reproductive organs weight of diabetic rats.**

| Parameter | Paired epididymotesticular weight | | Prostate weight | | Seminal vesical weight | |
|---|---|---|---|---|---|---|
| | Absolute (g) | Relative (%) | Absolute (g) | Relative (%) | Absolute (g) | Relative (%) |
| Control | 5.16±0.09[a] | 1.50±0.01[a] | 0.50±0.03[a] | 0.15±0.01[a] | 0.94±0.02[a] | 0.27±0.00[a] |
| Diabetes | 3.49±0.15[b] | 1.39±0.01[b] | 0.21±0.03[b] | 0.01±0.01[b] | 0.69±0.03[b] | 0.28±0.01[a] |
| Diabetes + GE (100mg) | 6.16±0.09[c] | 1.76±0.03[c] | 0.62±0.04[c] | 0.18±0.01[c] | 1.15±0.09[c] | 0.32±0.02[b] |
| L.S.D. | 0.330 | 0.050 | 0.088 | 0.019 | 0.162 | 0.032 |

Data represent mean ± SE, n=10, $p<0.05$.
Means with different superscripts are significantly different.

tioned ginseng group organs were significantly higher than those of controls. Similarly, apart from the seminal vesicles, the relative weights of above mentioned control group organs were significantly higher than those of the diabetic group, and significantly lower than those of the ginseng group. Relative weight of seminal vesicles of the diabetic group did not differ significantly from that of the control; however it was significantly lower than that of the ginseng group.

Table 2 shows that diabetes resulted in a significant decrease in sperm counts, and percentage of live sperms in eosin-nigrosine stained smears and also in an increase of abnormalities percentage compared to the controls and ginseng group. Similarly the percentage of live sperms was significantly lower, while the abnormalities were significantly higher in the ginseng group compared to controls. In addition, the highest significant sperm count was recorded for the ginseng group.

Table 3 shows that the activities of testicular enzyme markers were reduced in the diabetic group as compared to controls. Ginseng group showed significantly higher ACP, ALP and γGT activities when compared either to controls or the diabetic groups; but the lactate dehydrogenase activities did not differ significantly from those of the control group.

Table 4 shows that the lowest intratesticular testosterone and estradiol concentrations are in the diabetic group compared to the other experimental groups. In contrast, however, the ginseng group showed the highest intratesticular estradiol compared to the other two groups. Additionally, there was no significant difference in intratesticular testosterone concentrations between the control and ginseng groups.

Table 5 shows that the lowest intratesticular malondialdehyde (MDA) concentration was found in the control group, while that in the diabetic group was the highest. Inversely, the highest activity of superoxide dismutase

**Table 2**

**Effect of ginseng extract administration on spermiogram of diabetic rats.**

| Parameter | Count (X $10^5$) | Live (%) | Abnormalities (%) |
|---|---|---|---|
| Control | 5.05± 0.09[a] | 78.00±2.26[a] | 12.70±0.65[a] |
| Diabetes | 1.95±0.27[b] | 55.20±1.45[b] | 23.00±1.59[b] |
| Diabetes+ GE(100mg) | 5.78±0.18[c] | 65.00±1.67[c] | 16.50±0.69[c] |
| L.S.D. | 0.559 | 5.297 | 3.102 |

Data represent mean ± SE, n=10, $p<0.05$.
Means with different superscripts are significantly different.

(SOD) and glutathione S-transferase (GST) was found in the control group, while the lowest activities were recorded in the diabetic group.

Concerning the histopathological picture, diabetes resulted in degenerative changes of germinal epithelium which ranged from the absence of most of germinal epithelium from the wall of some seminiferous tubules (Fig. 1) or desquamated in the lumen of the seminiferous tubules (Fig. 2). Some seminiferous tubules of the diabetic group showed spermatid giant cells in the lumen (Fig. 3).The testes of the ginseng group showed normal histological picture in which the layers of germinal epithelium were intact with considerable numbers of sperms in the lumen of seminiferous tubules (Fig. 4).

## Discussion

Reproductive dysfunction is a well-recognized consequence of diabetes mellitus (Muralidhara 2007). In attempts to reveal this dysfunction, an evaluation of epididymal sperm samples was done in the experimental groups to detect counts, live % and abnormalities.

**Table 3**

**Effect of ginseng extract administration on testicular enzyme markers of diabetic rats.**

| Parameter | Acid phosphatase (U/mg protein) | Alkaline phosphatase (U/mg protein) | Lactate dehydrogenase (U/mg protein) | γ Glytamyl transferase (U/mg protein) |
|---|---|---|---|---|
| Control | 24.51±0.88[a] | 907.58±14.30[a] | 996.64±17.01[a] | 3.37±0.28[a] |
| Diabetes | 6.61±0.71[b] | 597.20±18.78[b] | 697.99±19.96[b] | 0.65±0.09[b] |
| Diabetes+ GE(100mg) | 28.99±0.61[c] | 976.21±6.19[c] | 1010.54±21.48[a] | 4.37±0.26[c] |
| L.S.D. | 2.153 | 40.890 | 56.787 | 0.650 |

Data represent mean ± SE, n=10, p<0.05.
Means with different superscripts are significantly different.

**Table 4**

**Effect of ginseng extract administration on testicular hormonal profile of diabetic rats.**

| Parameter | Intratesticular testosterone (ng/g testicular tissue) | Intratesticular estradiol (pg/g testicular tissue) |
|---|---|---|
| Control | 8.13±0.28[a] | 16.80±0.55[a] |
| Diabetes | 1.10±0.23[b] | 9.80±0.53[b] |
| Diabetes+ GE(100mg) | 8.48±0.25[a] | 20.90±0.69[c] |
| L.S.D. | 0.741 | 1.730 |

Data represent mean ± SE, n=10, p<0.05.
Means with different superscripts are significantly different.

**Table 5**

**Effect of ginseng extract administration on testicular antioxidant status of diabetic rats.**

| Parameter | Malondialdehyde (μmol/mg tissue) | Superoxide dismutase (EU/mg protein) | Glutathione S-transferase (EU/mg protein) |
|---|---|---|---|
| Control | 1.85±0.12[a] | 89.19±4.24[a] | 0.92±0.03[a] |
| Diabetes | 8.49±0.24[b] | 47.34±2.58[b] | 0.25±0.02[b] |
| Diabetes+ GE(100mg) | 4.11±0.32[c] | 67.49±1.65[c] | 0.56±0.03[c] |
| L.S.D. | 0.696 | 8.760 | 0.085 |

Data represent mean ± SE, n=10, p<0.05.
Means with different superscripts are significantly different.

Regarding the sperm cell concentration which is considered the most sensitive tests for spermatogenesis and it is highly correlated with fertility (El-Kashoury 2009) diabetes was found to induce a decrease in sperm conc. and live % and to increase abnormalities. The reported results coincided with that of Scarano et al. (2006) who showed that diabetes resulted in lower sperm quantity and quality. These alterations, which correlated with the above mentioned decrease in testicular enzymes markers and were further documented by histopathological findings, seemed to result from several factors including lower gonadotrophins, intratesticular testosterone and increased oxidative stress.

Since a long time it is known that testicular function is primarily controlled by the follicle-stimulating hormone (FSH) which regulates spermatogenesis, and the luteinizing hormone (LH) which controls Leydig cell function (Ward et al. 1991). It was reported that diabetic rats showed decrease in levels of serum insulin, testosterone, FSH, and LH when compared with healthy controls (Ballester et al. 2004). Insulin seems to be important as a direct effect of insulin and/ or glucose on the pituitary biosynthesis and/or secretion of FSH is evidenced, moreover, transgenic mice that lack brain insulin receptors showed a strong impairment of spermatogenesis (Sudha et al. 1999; Brüning et al. 2000). Moreover, decreased levels of gonadotrophins must have affected spermatogenesis resulting in low sperm counts as sperm production is an FSH-regulated process that requires normal Sertoli cell function (Ward et al. 1991). This result appeared to be true since γ GT (Sertoli cell enzyme marker) was altered in the present investigation. Gamma-glutamyl transferase (GGT) measurements have been proposed as a useful diagnostic test in a recognition of Sertoli cell dysfunction (Sherins and Hodgen 1976). Since, progressive loss of viability in Sertoli cells was associated with hyperglycemia (Gondos et al. 1998), moreover diabetes was reported to be associated with increase in intratesticular glucose (Mallick et al. 2007), one would speculate a decrease in activities of Sertoli cells which was evidenced in the present study by the decrease in GGT activities. As, Sertoli cells play

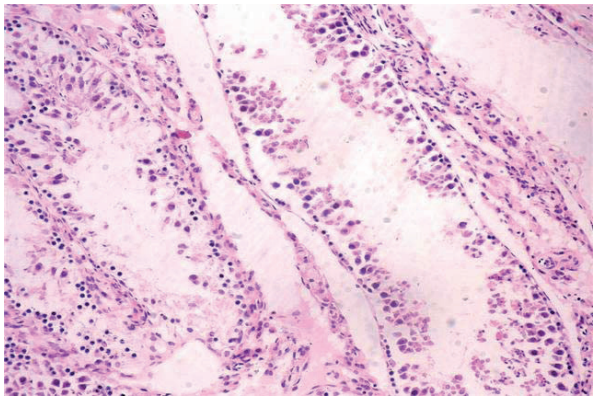

**Fig 1 Testes of diabetic rat showing absence of most of germinal epithelium lining seminiferous tubules and sperms in the lumen. H&E X 100.**

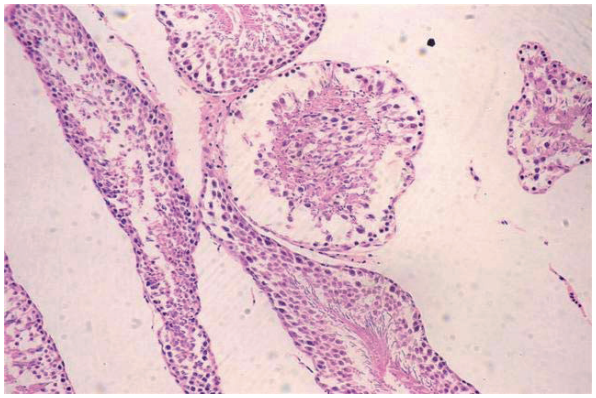

**Fig 2 Testes of diabetic rat showing desquamation of germinal epithelium in the lumen of some seminiferous tubules. H&EX100.**

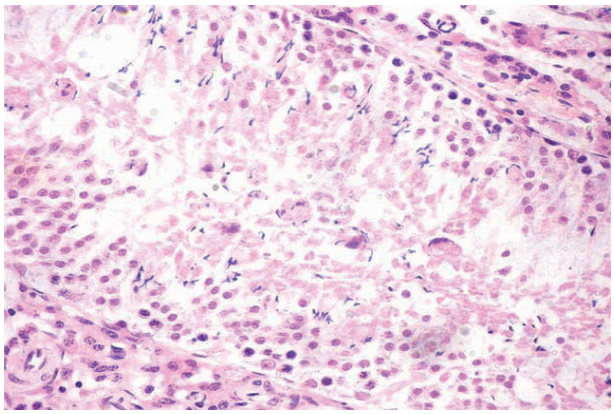

**Fig 3 Testes of diabetic rat showing spermatid giant cells in the lumen of seminiferous tubules. H&EX400.**

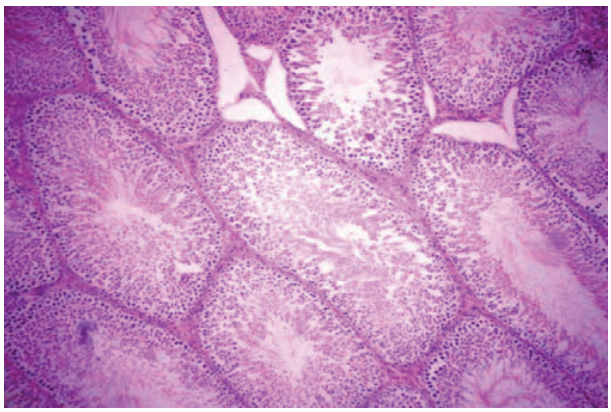

**Fig 4 Testis of the diabetic rats receiving ginseng extracts showing normal histological picture. H&EX100.**

a crucial role in maintaining high intratubular levels of testosterone (through androgen binding protein) and estrogen production (through aromatase activity), the present study investigated the intratesticular levels of both testosterone and estradiol. Intratesticular estradiol levels were decreased in the diabetic group, and this coincided with a decrease in GGT activities. Concerning intratesticular testosterone, previous studies demonstrated that diabetes resulted in a decrease in serum testosterone levels (Aybek et al. 2008; Farrell et al. 2008), additionally, the present investigation demonstrated a decrease in intratesticular testosterone in the diabetic group. This decrease may be the result of both a decrease in the total number of Leydig cells and in the rhythm of androgen biosynthesis by the remaining functional cells (Hurtado de Catalfo et al. 1998; Ballester et al. 2004). This decrease was further documented by the fall in activities of ACP enzymes in the diabetic group.

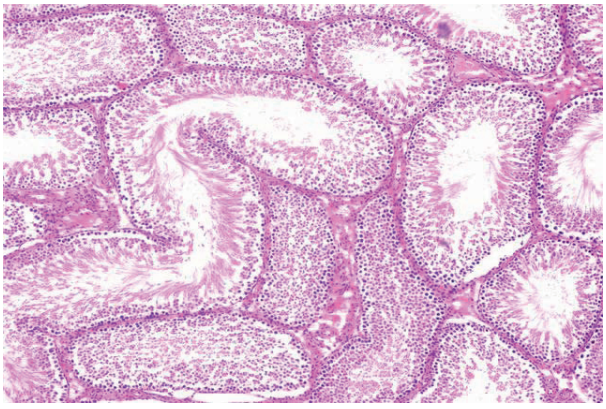

**Fig 5 Testis of the controls showing normal histological picture. H&EX100.**

These enzymes marker, which is regulated by levels of gonadotrophins, correlated not only with post meiotic

development of spermatids, but also with testicular steroidogenesis (Latchoumycandane et al. 1997; Yousef et al. 2001). The above mentioned results showed that diabetes mellitus alters the activity of ACP, which correlated with the reported decrease in intratesticular steroidogenic activity and lower sperm counts and viability. The mentioned results coincided with those of Bener et al. (2009) and Wojtowicz et al. (2009), who showed an alteration in ACP activity in diabetic rabbits which correlated lower sperm quality and fertilizing capacity. The current decrease in intratesticular testosterone goes hand by hand with that of Sanguinetti et al. (1995) who reported that administration of streptozotocin to male rats induces a decrease in testicular testosterone production. In this concern, intratesticular testosterone is crucial for the process of spermatogenesis, since high level of testosterone in testis is essential for the maintenance of structural morphology and normal physiology of seminiferous tubule (Sharpe et al. 1992). Additionally, in experimental circumstances in which the intratesticular testosterone concentration is reduced a significant germ cell detachment and apoptosis is seen (Blanco-Rodriguez and Martinez-Garcia 1998; Kim et al. 2001). However, the same result contradicts with that of Mansour et al. (2009) who failed to report a similar decrease in testicular testosterone in diabetic rats despite a recorded decrease in the serum levels of the same hormone. Alkaline phosphatase and LDH, another two testicular enzyme markers (El-Kashoury 2009), behaved similarly, in the present investigation, to ACP in the diabetic group. Within the testis the ALP activity was of significant importance for the process of spermatogenesis, moreover, the decrease in its activity was correlated with the state of germ cell loss (Kumar et al. 2003). The present investigation showed that diabetes resulted in decrease in ALP activities which correlated with reduction in testicular weights, and sperm counts together with the pathological findings. In rats, testicular LDH is localized in mitochondria of primary spermatocytes and subsequent stages, where it is associated with the maturation of germinal epithelial layer of seminiferous tubules (Sinha et al. 1997). Also LDH was correlated with motility and living sperm where it ensures metabolism of spermatozoa (Pesch et al. 2006). Thus lower activity of LDH pointed to a lower sperm quality together with alteration in spermatogenesis documented by histopathology. Lower counts and live % may not be only attributed to alteration in hormonal profile but also to the injury induced by oxidative stress (Gumieniczek et al. 2008). In attempts to reveal the testicular oxidative stress- antioxidant status in diabetic rats we measured malondialdehyde, as lipid peroxidation product, and the antioxidant enzymes SOD and GST in the testis (Bauche et al. 1994). Diabetes induced a state of oxidative stress which was evidenced by an increase in levels of malondialdehyde and a decrease in SOD and GST activities in testicular tissues. This state of oxidative stress was reflected not only by lower sperm counts but also by a decrease in the viability of sperms (Ghosh et al. 2002). The present findings concerning the oxidative status agreed with those of Zhao et al. (2005), Muralidhara (2007), and Aybek et al. (2008) who reported similar results concerning testicular SOD and malondialdehyde, but contradicted that of Muralidhara (2007) who showed an increase in GST enzyme activities in diabetic rats.

The present investigation pointed to a reduction in prostatic and seminal vesicles weights. The current results were in agreement with those of Barkeley and Goldmass (1977) and Singh et al. (2005) who showed that Streptozotocin induced diabetes in male rats resulted atrophy of sex organ, changes in histoarchitecture of ventral prostate. This decrease in absolute and relative weights of these organs may be due to low serum level of testosterone in diabetic rats, as testosterone is the prime regulator of normal growth of these organs (Sanguinetti et al. 1995; Mansour et al. 2009). Similarly, testicular weights of the diabetic group were reduced an effect which was further documented by the testicular histopathological findings of germinal epithelial degeneration that varied from desquamation to absence of most of germinal epithelium. The present findings go hand by hand with that of Sujatha et al. (2001) and Mansour et al. (2009) who reported that the decrease in testicular weight in diabetic rats may be due to reduced tubule size, spermatogenic arrest and inhibition of steroid biosynthesis of Leydig cells, together with consistent structural disorganization of germinal epithelium evident by abnormal accumulation of germ cells in the lumen of seminiferous tubules in diabetic rats. This testicular pathology may have been resulted from a decrease in levels of gonadotrophins in diabetic rats and/or diabetes-induced oxidative stress previously recognized in diabetic rat testis (Ballester et al. 2004; Muralidhara 2007). Reactive oxygen species was reported to cause damage to DNA, impairment of the protein function and peroxidation of lipids which results in rupture of the membranes and cause structural and functional alterations in the testis (Wei et al. 2007; Kim et al. 2010).

The recorded ameliorative effect of ginseng extract on the above mentioned spermiogram, testicular enzyme markers, intratesticular testosterone, estradiol and organ weights, in the present investigation, may be attributed to its stimulatory effect on hypothalamopituitary gonadal axis as shown by Salvati et al. (1996) who reported an increase in sperm counts and motility, serum FSH, LH and testosterone after ginseng administration in oligoastenospermic individuals. The reported results agreed with those of Chen et al (2001) who reported that ginsenoside enhanced sperm motility in humans with inferior sperm motility, Hwang et al. (2004) and Park et al. (2006) who showed that ginseng increased sperm numbers, fertility, and decreased spermatogenic disorders and testicular pathology in guinea pigs and rats exposed to dioxin. Additionally, an antioxidant property of ginseng cannot be ignored. Ginseng and its extract has been shown to exhibit a variety of antioxidant activity in both lipid and aqueous media via the chelation of metal ions and scavenging of hydroxyl and superoxide free radicals (Kitts et al. 2000; Kim et al. 2002b). Moreover a direct effect of ginseng and its extract through stimulating gene expression and protein synthesis of antioxidant enzyme SOD was reported previously (Yamamoto et al. 1977; Chang et al. 1999). The present findings of the ameliorative effect of ginseng extract on oxidative stress induced injury is in agreement with those of Siddique et al. (2000) and Kim et al. (2002a) who showed that ginseng extracts are known to scavenge hydroxyl and superoxide radicals and to have a remarkable capacity to protect brain tissue proteins from oxidative damage in vitro, furthermore, the crude saponin fraction of Korean red ginseng was found to abrogate the generation of NADPH-driven superoxide in rats brain and Kim et al. (2010) who showed that ginseng is a potent antioxidant agent that protects against oxidative damage to the testis tissue via decreased production of NADPH oxidase and superoxide.

In conclusion, ginseng extract proved to be of benefit for diabetic individuals suffering from diabetes induced infertility.

## Acknowledgements

The authors are grateful to Dr. Eman Bakr, professor of pathology, for the interpretation of histopathology.

## References

Allain CC: Quantitative determination of lactate dehydrogenase in serum. Clinical Chemistry 19, 223-228, 1973.

Aybek H, Aybek Z, Rota S, Sen N, Akbulut M: The effect of diabetes mellitus, age and vitamin on testicular oxidative stress. Fertil Steril 90, 755-760, 2008. doi:10.1016/j.fertnstert.2007.01.101

Babson AL, Read AP: Colorimetric determination of acid phosphatase. Am J Clin Pathol 32, 89-91, 1959.

Ballester J, Munoz MC, Dominguez J, Rigau T, Guinovart JJ, Rodriguez-Gil JE: Insulin-dependent diabetes affects testicular function by FSH- and LH-linked mechanisms. J Andrology 25, 706-719, 2004.

Barkeley MS, Goldmass BD: A quantitative study of serum testosterone, sex accessory organ growth, and the development of intermale aggression in the mouse. Horm Behav 8, 208-218, 1977. doi:10.1016/0018-506X(77)90038-1

Bauché F, Fouchard M, Jégou B: Antioxidant system in rat testicular cells Fed Europ Biochem Soc Lett 349, 392-396, 1994.

Bener A, Al-Ansari AA, Zirie M, AL Hamaq AO: Is male fertility associated with type 2 diabetes mellitus? Internat Urol Nephrol 41, 777-784, 2009. doi:10.1007/s11255-009-9565-6

Blanco-Rodriguez J, Martinez-Garcia C: Apoptosis precedes detachment of germ cells from the seminiferous epithelium after hormonal suppression by short-term oestradiol treatment of rats. International J Andrology 21,109-115, 1998. doi:10.1046/j.1365-2605.1998.00109.x

Blom E: The spermiogram of the bull. Vordisk Veterinaer, 35, 105, 1983.

Brüning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Müller-Wieland D, Kahn CR: Role of brain insulin receptor in control of body weight and reproduction. Science 289, 2122-2125, 2000. doi:10.1126/science.289.5487.2122

Chang MS, Lee SG, Rho HM: Transcriptional activation of Cu/Zn superoxide dismutase and catalase genes by panaxadiol ginsenosides extracted from Panax ginseng. Phytother Res 13, 641-644, 1999. doi:10.1002/(SICI)1099-1573(199912)13:8<641::AID-PTR527>3.0.CO;2-Z

Chen J, Chen L, Tsauer W, Tsai C, Chen B, Chen Y: Effects of ginsenoside Rb2 and Rc on inferior human sperm motility in vitro. Am J Chin Med 29, 155-160, 2001. doi:10.1142/S0192415X01000174

El-Kashoury AA: Influence of subchronic exposure of profenofos on biochemical markers and microelements in testicular tissue of rats. Nature & Science 7, 16-29, 2009.

Farrell JB, Deshmukh A, Baghaie AA: Low testosterone and the association with type 2 diabetes. The Diabetes Educator 34, 799-806, 2008. doi:10.1177/0145721708323100

George C: Staining procedure 4th ed. Williams and Wilkins, Baltimore, 1981.

Ghosh D, Das UB, Mallick M, Debnath J: Testicular gametogenic and steroidogenic activities in cyclophosphamide treated rat: a correlative study with testicular oxidative stress. Drug and Chem Toxicol 25(3), 281-292, 2002. doi:10.1081/DCT-120005891

Gondos B, Rivkind Y, Jovanovic L: Effect of increasing glucose concentrations on Sertoli cell viability in the non-obese diabetic mouse. Ann Clin Lab Science 24, 236-241, 1998.

Gumieniczek A, Hopkala H, Zabek A: Protective effects of a PPARγ agonist pioglitazone on antioxidative systems in testis of diabetic rabbits. Pharmazie 63, 377-378, 2008.

Habig WH, Pabst MJ, Jacoby WB: Glutathione s-transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem 249, 7130-7139, 1974.

Hodgen DG, Sherins JR: Enzymes as markers of testicular growth and development in the rat. Endocrinology 93, 985-989, 1973. doi:10.1210/endo-93-4-985

Hurtado de Catalfo G, Nelva I, De Gomez Dumm T: Lipid dismetabolism in Leydig and Sertoli cells isolated from streptozotocin-diabetic rats. Internati J Biochem Cell Biol 30, 1001-1010, 1998. doi:10.1016/S1357-2725(98)00055-7

Hwang S, Kim W, Wee J, Choi J, Kim S: Panax ginseng improves survival and sperm quality in guinea pigs exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Brit J Urol International, 94, 663-668, 2004.

Jaffe BM, Behrman NR: Methods of hormone radioimmunoassay, Academic press, 1974.

Jewett SL, Rocklin AM: Variation of one unit activity with oxidation rate of organic substrate in indirect superoxide dismutase assays. Analyt Biochem 212, 555-559, 1993. doi:10.1006/abio.1993.1368

Kang KS, Kim HY, Yamabe N, Nagai R, Yokozawa T: Protective effect of sun Ginseng against diabetic renal damage. Biological & Pharmaceutical Bulletin 29, 1678-1684, 2006. doi:10.1248/bpb.29.1678

Kim CS, Park JB, Kim KJ, Chang SJ, Ryoo SW, Jeon BH: Effect of Korean red ginseng on cerebral blood flow and superoxide production. Acta Pharmacologica Sinica 23, 1152-1156, 2002a.

Kim JH, Cho SY, Kang CW, Yoon IS, Lee JH, Jeong SM, Lee BH, Lee JH, Pyo MK, Choi SH, Quan SF, Lee JH, Choi CB, Rhim H, Nah SY: Ginseng saponins diminish adverse vascular effects associated with chronic methionine-induced hyperhomocysteinemia. Biol Pharmac Bull 29, 2425-2431, 2006. doi:10.1248/bpb.29.2425

Kim JM, Ghosh SR, Weil ACP, Zirkin BR: Caspase- 3 and caspase activated dioxyribonuclease are associated with testicular germ cell apoptosis resulting from reduced intratesticular testosterone. Endocrinology 142, 3809-3816, 2001. doi:10.1210/en.142.9.3809

Kim Y, Kim G, Shin J, Kang-Sup Kim K, Lim J: Effect of Korean Red Ginseng on Testicular Tissue Injury after torsion and detorsion. Korean J Urol 51, 794-799, 2010. doi:10.4111/kju.2010.51.11.794

Kim YK, Guo Q, Packer L: Free radical scavenging activity of red ginseng aqueous extracts. Toxicology 172,149-56, 2002b. doi:10.1016/S0300-483X(01)00585-6

Kitts DD, Wijewickreme AN, Hu C: Antioxidant properties of a North American ginseng extract. Mol Cell Biochem 203, 1-10, 2000. doi:10.1023/A:1007078414639

Koh PO: Streptozotocin induced diabetes increases apoptosis through JNK phosphorylation and Bax activation in rat testes. J Vet Med Science 69, 969-971, 2007. doi:10.1292/jvms.69.969

Kumar M, Sharma MK, Saxena PS, Kumar A: Radioprotective effect of Panax ginseng on the phosphatases and lipid peroxidation level in testis of Swiss Albino mice. Biol Pharmaceut Bull, 26, 308-312, 2003.
doi:10.1248/bpb.26.308

Latchoumycandane C, Gupta SK, Mathur PP: Inhibitory effects of hypothyrodism on the testicular functions of postnatal rats. Biomed Lett 56, 171-177, 1997.

Lowry OH, Rosebnough NJ, Farr AL, Randall RJ: Protein measurement with the folin phenol reagent. J Biol Chem 193, 265-275, 1951.

Mallick C, Mandal S, Barik B, Bhattacharya A, Ghosh D: Protection of testicular dysfunctions by MTEC, a formulated herbal drug, in streptozotocin induced diabetic rat. Biol Pharmaceut Bull 30(1), 84-90, 2007. doi:10.1248/bpb.30.84

Mansour M, Coleman E, Dennis J, Akingbemi B, Schwartz D, Robert Judd T, Plaisance E, Stewart LK, Morrison E: Activation of PPARγ by Rosiglitazone does not negatively impact male sex steroid hormones in diabetic rats. Hindawi Publishing Corporation Article ID 101857, 8 pages doi:10.1155/2009/101857, 2009.

Muralidhara SB: Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: its progression and genotoxic consequences. Reprod Toxicol 23, 578- 587, 2007. doi:10.1016/j.reprotox.2007.02.001

Naziroglu M: Enhanced testicular antioxidant capacity in Streptozotocin induced diabetic rat: Protective role of vitamins C, E and selenium. Biological Trace Element Research 94, 61-72, 2003. doi:10.1385/BTER:94:1:61

Park J, Hwang S, Lee W, Yu K, Paek K, Hwang B, Han K: The therapeutic effect of tissue cultured root of wild panax ginseng C.A. Mayer on spermatogenic disorder. Arch Pharm Res, 29,800-807, 2006. doi:10.1007/BF02974082

Pesch S, Bergmann M, Bostedt H: Determination of some enzymes and macro-and microelements in stallion seminal plasma and their correlations to semen quality. Theriogenology 66, 307-313, 2006. doi:10.1016/j.theriogenology.2005.11.015

Sanguinetti RE, Ogawa K, Kurohmaru M, Hayashi I: Ultrastructural changes in mouse Leydig cells after streptozotocin administration. Experimental Animals 44, 71-73, 1995. doi:10.1538/expanim.44.71

Savati G, Genovesi G, Marcellini L, Paolini P, De Nuccio I, Pepe M, Re M: Effects of Panax Ginseng C.A. Meyer saponins on male fertility. Panminerva Medica 38, 249-254, 1996.

Scarano WR, Messias AG, Oliva SU, Klinefelter GR, Kempinas WG: Sexual behavior, sperm quantity and quality after short-term streptozocin-induced hyperglycaemia in rats. Int J Andrology 29, 482-488, 2006. doi:10.1111/j.1365-2605.2006.00682.x

Sharpe RM, Maddocks S, Millar M, Saunders PTK, Kerr JB, Mckinnell C: Testosterone and spermatogenesis: identification of stage dependent, androgen-regulated proteins secreted by adult rat seminiferous tubules. J Andrology 13,172-184, 1992.

Sherins RJ, Hodgen GD: Testicular gamma glutamyl-transpeptidase: an index of Sertoli cell function in man. J Reprod Fert 48,191-193, 1976. doi:10.1530/jrf.0.0480191

Shrilatha B, Muralidhara: Occurrence of oxidative impairments, response of antioxidant defenses and associated biochemical perturbation in male reproductive milieu in the Streptozotocin-diabetic rat. Int J Andrology 30, 508-518, 2007. doi:10.1111/j.1365-2605.2007.00748.x

Siddique MS, Eddeb F, Mantle D, Mendelow AD: Extracts of Ginkgo biloba and Panax ginseng protect brain proteins from free radical induced oxidative damage in vitro. Acta Neurochir, Suppl 76, 87-90, 2000.

Singh S, Yuvaraj S, Malini T, Balasubramanian K: Experimental diabetes has adverse effects on the differentiation of ventral prostate during sexual maturation of rats. The anatomical record. Part A, Discoveries in molecular, cellular, and evolutionary biology 287(2), 1281-1289, 2005.

Sinha N, Narayan R, Saxena DK: Effect of endosulfan on the testes of growing rats. Bull Environ Contamin Toxicol 58, 79-86, 1997. doi:10.1007/s001289900303

Snedecor GM, Cochran WG: Statistical analysis 7th ed, pp.215-237, 1980.

Stephen-Morris G, Hasten L, Hegsted M, Guidry K: Chromium picolinate supplementation improves cardiac metabolism, but not myosin isoenzyme distribution in the diabetic heart. The J Nutrit Biochem 7, 617-622, 1996. doi:10.1016/S0955-2863(96)00121-0

Sudha S, Valli G, Julie MP, Arunakaran J, Govindarajulu P, Balasubramanian K: Influence of streptozotocin-induced diabetes and insulin treatment on the pituitary-testicular axis during sexual maturation in rats. Endocrinol Diabetes 107, 14-20, 1999.

Sudha S, Valli G, Julie P, Balasubramanian K: Influence of Streptozotocin induced diabetes and insulin treatment on the pituitary testicular axis during sexual maturation in rats. Exp Clin Endocrinol Diab 108, 14-20, 2000.

Sujatha R, Chitra KC, Latchoumycandane C, Mathur PP: Effect of lindane on testicular antioxidant system and steroidogenic enzymes in adult rats. Asian J Andrology 3, 135-138, 2001.

Szasz G: Kinetic determination of serum γ glutamyl transferase. Clin Chem 15, 124-136, 1969.

Tanaka M, Nakaya S, Kumai T, Kobayashi S: Impaired testicular function in rats with diet induced hypercholesterolemia and/or Streptozotocin induced diabetes mellitus. Endocrine Res 27, 109-117, 2001. doi:10.1081/ERC-100107174

Tietz NW: Fundamentals of clinical chemistry. W.B. Saunders, Philadelphia, 1970.

Ward DN, Bousfield GR, Moore KH. Gonadotropins. In: Reproduction in Domestic Animals (Ed. PT Cupps) pp. 25-67, Academic Press, San Diego, Calif 1991.

Wei SM, Yan ZZ, Zhou J: Beneficial effect of taurine on testicular ischemia-reperfusion injury in rats. Urology 70, 1237-1242, 2007. doi:10.1016/j.urology.2007.09.030

Wojtowicz Z, Dworzanski W, Kędzierawska-Kurylcio A, Dworzanski J, Kis JG, Hermanowicz-Dryka T, Kis JZ, Lisiecka J, Jedrzejewska EA, Burdan F: Activity of testicular lysosomal enzymes in the course of experimental diabetes in rabbits. Annales Universitatis Mariae Curie-Skłodowska Lublin, Polonia, 22, 135-140, 2009.

Xing S, Cekan SZ, Diczfalusy EA: Validation of radioimmunoassay for estradiol 17β by isotope dilution-mass spectrometry and by a test of radiochemical purity. Clin Chim Acta, 135, 189-201, 1983. doi:10.1016/0009-8981(83)90135-3

Yamamoto M, Kumagai A, Yamamura Y: Stimulatory effect of Panax ginseng principles on DNA and protein synthesis in rat testes. Arzneimittelforschung 27(7):1404-5, 1977.

Yoshioka T, Kawada K, Shimada T, Mori M: Lipid peroxidation in maternal and cord blood and protective mechanisms against activated oxygen toxicity in blood. Am J Obstet Gynecol 135, 372-376, 1979.

Yousef GM, Diamandis M, Jung K, Eleftherios P: Molecular cloning of a novel human acid phoshatase gene that is highly expressed in the testes. Genomics 74(3), 385-395, 2001. doi:10.1006/geno.2001.6556

Zhao HG, Liu GW, Liu SC, Wang ZC, Liu Y, Wang ZQ, Li C, Cai L, Gong SL: Changes of cycle and apoptosis of spermatogenic cells and antioxidant capacity in male rats with diabetes mellitus. Zhonghu Nan Ke Xue, 11: 735-739, 2005.

Zimmet P, Albert K, Shaw J: Global and societal implications of the diabetes epidemic. Nature (London) 414, 782-787, 2001. doi:10.1038/414782a