The Impact of Alcohol on Sperm Parameters in diabetic mice

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Abstract: Diabetes mellitus (DM) is demonstrated one of the important stress to modern universal health. Reproductive disorder in diabetic males is fixed. Found that insulin-dependent diabetes is connected to reduce ejaculated semen and decreased vitality and motility of the spermatozoa. Alcohol consumption can have an adverse effect on sperm production. Alcohol abuse is considered as one of the problems associated with poor semen production and sperm quality. **Objective:** Given the importance of sperm in reproductive and Generation health other hand, diabetes and alcohol interaction aim of this study is survey the impact of alcohol on sperm parameters in mice with diabetes. **Material and methods:** 32 Adult male mice (10 weeks old, 35g) that they divided to 4 groups, mice of group 1 served as control fed on basal diet, group 2 received streptozotocin (STZ) (200 mg/kg, single dose, intraperitoneal) and basal diet, group 3 received alcohol (10 mg/kg, Water-soluble) and basal die and group 4 received streptozotocin and alcohol for 35 days. Finally, right tail of epididymis was cut in Ham's F10. Released sperm were used to analyse number, motility, morphology (Pap-staining) and viability (eosin-Y staining) of the sperm. **Result:** In diabetes + alcohol mice, a significant decrease was found in sperm viability and sperm morphology compared to control, diabetes and alcohol mice, the morphology of sperm diabetes +alcohol more than other groups were beheaded. In diabetic and alcohol mice, a significant decrease was found in sperm motility and sperm count compared to control mice. **Conclusion:** Alcohol consumption in men with diabetes exacerbate damage to sperm, particular makes to increase the sperm beheaded

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Key words: Mice, Sperm parameters, Diabetes, Alcohol.

Introduction:

It is demonstrated that Diabetes mellitus (DM) is one of the important stress to modern universal health. Its rate is going up quickly and according to the World Health Organization (WHO) report, 177 million people were exaggerated by diabetes worldwide in 2000. But by 2025; this figure will be raised to over 300 million (Petroianu A 2009). The large number (90%) of patients with type-1 diabetes is diagnosed before the age of 30. This type of diabetes is growing by 3% every year in European children, with a growing number being diagnosed in early childhood (WHO 2007). As a consequence diabetes will involve additional men prior to and during their reproductive years (Agbaje I 2007, shrilatha 2007). The occurrence of reproductive disorder in diabetic males is approved. Several experimental works have demonstrated different kinds of male reproductive dysfunctions both structurally and physiologically (Neill J.o 2009, Ricc.G 2009). The DM may affect male reproductive function at multiple levels as a result of its detrimental effects on endocrine control of spermatogenesis or by impairing erection and ejaculation (Petroianu A 2009). Ricc et al. found that insulin-dependent diabetes is connected to reduce ejaculated semen and decreased vitality and motility of the spermatozoa with no change in seminal viscosity (Ricc.G 2009). Another work from Queen's University has revealed that excess sugar in the blood may affect sperm quality and therefore male fertility potential (Agbaje I 2007). There are some confirmations indicating higher rates of infertility in diabetic men (Joao Ramalho 2009).

Recently, the substances in the environment that can disturb male fertility have been increased. Ethanol is among the most widely abused drug, which can suppress reproductive function and sexual behaviour in laboratory animals and humans (Fadem 1993). Alcohol is toxic for testes and causes fertility disturbances through low sperm count and motility in men (Maneesh 2006). Irregular diameter of the seminiferous tubules and high amount of death cells in the lumen of alcoholic men were also noticed by Martinez et al. (2009). Studies have shown that chronic ingestion of ethanol results in a variety of pathological changes in male reproduction in humans and laboratory animals (Srikanth V 1999). These changes include altered spermatogenesis and sperm motility, altered testicular and accessory gland morphology, reduction of reproductive organ weight, reduced cauda epididymal sperm content and impaired epididymal sperm maturation. Furthermore, alcohol abuse by males has been correlated with hypogonadism, feminization, and alterations in

reproductive hormonal homeostasis, reduced libido, ejaculation problems and impotence (Ren J-C 2005).

Materials and methods:

Animals:

Totally 32 Adult male mice (10 weeks old, 35g) that they divided to 4 group, mice of group 1 served as control fed on basal diet, group 2 received streptozotocin (STZ) (200 mg/kg, single dose, intra peritoneal) and basal diet, group 3 received alcohol (10 mg/kg, Water-soluble) and basal die and group 4 received streptozotocin and alcohol for 35 days. Groups were housed in a controlled environment with a temperature range of 25±3 ℃ and mean relative humidity of 50±5%.

Epididymal sperm preparation:

After 35 days (one duration of spermatogenesis in mice is about 32 days), a small part of the cauda epididymal of each mouse was dissected and located in 1 mL of pre-warmed Hams F10 medium (37℃, 5% CO2). Gentle tearing of the tissue was done to make spermatozoa swim out into the culture medium. The dishes were placed in the incubator for 15 min.

Induction of diabetes:

In experimental group, the diabetes was induced via a single intraperitoneal (i.p) injection of buffered solution (0.1 mol/l of citrate, pH 4.5) of STZ at a dosage of 200 mg/kg body weight. At this dose, STZ induces considerable hyperglycemia (blood glucose > 250 mg/dl) in mice that was measured at 72 hours post-injection (shrilatha 2007).

Sperm analysis:

Sperm count:

The dissected epididymis of each animal was transferred into 10 ml Ham's F10 medium and cut to small slices, in order to swim out the sperm into the medium. After 10 min of diffusion, 1 ml of the solution was diluted with 9 ml formaldehyde fixative. The diluted solution was transferred into each chamber of Neubauer hemocytometer and sperm heads was manually counted under a microscope. Sperm count was performed according to WHO guidelines (WHO 1999) and data were expressed as the number of sperm per ml (Hamid Reza Momeni 2012).

Sperm motility:

Assessment of sperm motility was done according to WHO protocol (WHO 1999). In brief, 10 µl of the sperm suspension was placed on a microscopic slide and cover slipped. A minimum of five microscopic fields were assessed to evaluate sperm motility on at least 200 sperm for each animal. The percentage of sperm motility was analysed for following motion parameters: Motility was expressed as percentage of progressive (fast and slow) and non-progressive spermatozoa (Hamid Reza Momeni 2012).

Sperm viability:

Eosin-nigrosin staining was used to asses' sperm viability according to WHO protocol (WHO 1999). Briefly, eosin (1%, Merck, Germany) and nigrosin (10%, Merck, Germany) was prepared in distilled water. One volume of sperm suspension was mixed with two volume of 1% eosin. After 30 second, an equal volume of nigrosin was added to this mixture. Thin smears were then prepared and observed under a light microscope at ×100 magnification. Viable sperm remained colorless while nonviable sperm stained red (Hamid Reza Momeni 2012).

Sperm morphology:

For studying the sperm morphology, a drop of sperm suspension was smeared onto a clean glass slide. The smear was then air dried and fixed in a mixture of equal parts ethanol and ether. The slides were then stained with Papanicolaou stain. Dried stained slides were scanned under oil immersion (100 objectives) for morphological abnormalities. A total of 100 sperms per sample were classified according to their morphology; such as normal, coiled mid piece, hair pin (a kink at the annulus, usually 180°), bent tail (a kink at the annulus, usually 90°), coiled tail, double head, amorphous head, triangular head, pin head and cytoplasmic droplet. Sperm abnormality was expressed as percent (Zohre Zare 2010).

Statistical analysis:

Results are expressed as mean±SD for 8 animals per group. One-way analysis of variance (ANOVA) was used to assess the statistical significance of the data. $p<0.05$ was considered significant.

Results and Discussion:

Table 1 shows the means and statistical analysis of the various sperm parameters Between the 4 groups.

Diabetes is the most common endocrine disease that leads to metabolic abnormalities involving regulation of carbohydrate metabolism Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular diseases (Khaki A 2009). Since streptozotocin causes testicular dysfunction and degeneration under situations of experimentally

induced diabetes in animal models (Shrilatha B 2007). Diabetic rats showed a reduction in seminiferous tubule diameter, increased thickening of the basement membrane in seminiferous tubules and degenerated germ cells. TUNEL-positive cells were significantly more numerous in diabetic rats than in control rats. Melatonin significantly attenuated the diabetes-induced morphological changes and germ cell apoptosis in the diabetic rat testis (Ganeli E 2008). It is indicated that the level of oxidative stress is high in hyperglycemia state (Steger RW 1997), due to excess production of reactive oxygen species (ROS) and decreased efficiency of anti-oxidant enzyme defences (Steger RW 1997). Oxidative stress is harmful to sperm function and a significant factor in the etiology of male infertility (Kartikeya Makker 2009). In addition, oxidative stress impairs male fertility by changing the cell function like sperm motility (Kartikeya Makker 2009); it may consequently lead to the destruction of sperm mitochondria, resulting in sperm ATP depletion (Das J 2009) and reduced sperm motility and viability. It is therefore likely to assume that reduced sperm motility and viability induced by diabetes has been due to the ability of this toxicant in the induction of oxidative stress. It is noteworthy that diabetes-related alterations in Leydig cells are also related to changes in the pituitary-testicular axis (Steger RW 1997). Thus, this disease induces a decrease in the serum levels of luteinizing hormone (LH), which is responsible for normal Leydig cell function (Steger RW 1997). Diabetic testicular dysfunction might be transient or permanent depending on the degree and duration of the disease. Erectile dysfunction (ED) is a well-recognized complication of diabetes mellitus (DM). The low incidence of diabetes in infertile patients might be the reason for the limited amount of current research (Altay B 2003). However, an altered testicular axis was noted in experimental studies Seethalakshmi et al. (Seethalakshmi L 1987) found that testicular weight, sperm count and motility significantly decreased in diabetic rats, our results a significant decrease was found in sperm count and motility compared to control. Moreover, Cameron et al. (Cameron DF 1985) defined increasing tubule wall thickness, germ cell depletion and Sertoli cell vacuolization in diabetic human testicular biopsies and in diabetic rats.in diabetic men the concentration, motility, vitality and the proportion of normal shape spermatozoa is also lower (Garcia-Diez LC 1991).

Table1: Statistical analysis of various sperm parameters in the groups studied

Variables	Control group	Diabetes group	Alcohol group	Diabetes + Alcohol group	p-value
Rapid motility(%)(Grade a)	19.5±3.505	4.125±1.959[a]	6.125±3.907[a]	6.75±2.549[a]	0
Slow motility(%)(Grade b)	23.25±4.862	16.625±6.345	10.875±4.086[a]	16.25±3.882[a]	0
Non progressive (%) (Grade c)	32±4.375	23.625±3.814	33.375±8.534	29.75±7.285	0.023
Immotile sperm(%)(Grade d)	25.875±4.389	55.625±4.983[ab]	52.125±5.667[a]	47.375±7.443[a]	0
Total motility(%)(Grade a,b,c)	76±4.276	44.375±4.983[ab]	47.875±5.667[a]	52.625±7.443[a]	0
Count($\times 10^6$)	110±17.492	23.375±8.105[ab]	15.25±8.497[a]	8.25±1.035[a]	0
Viability (%)	78.125±5.083	47.75±6.627[ab]	38.125±6.22[ab]	27.375±10.702[a]	0
Normal morphology	75.875±6.728	60.75±4.267[ab]	51±2.777[ab]	40.625±4.955[a]	0

a: Significant compared to control group in the same row. b: Significant compared to diabetes+alcohol group in the same row. ($P \leq 0.05$).

Ethanol consumption produces a significant decrease in the percentage of motility, nuclear maturity and DNA integrity of spermatozoa in rat (Ali Reza Talebi 2011), and normal morphology in human and animal spermatozoa (Nagy 1986). Martinez et al. (2009) reported histological abnormalities in testicular tissue of alcoholic animals. These included intense intercellular spaces, irregular diameter of the seminiferous tubules, and high amount of necrotic cells in the lumen compared with controls. In addition, they showed that the epididymal sperm motility is decreased in ethanol treated rats. In another study, Srikanth et al. (1999) demonstrated that the rate of spermatozoa with forward motility that were extracted from cauda epididymitis, significantly decreased in ethanol-consuming rats. They also showed that ethanol consumption in male rats may decrease pregnancy rate (to 60%) in their cohabitated female rats and number of pups delivered (to 50%). Brzek (1987) also reported that a reduction of semen volume, density, and motility were caused by alcohol consumption. In addition, it was reported that daily alcohol consumption decreases normal sperm morphology (Goverde 1995). Carlsen et al. (1992) found that ethanol users showed a significant decrease in mean sperm count, ranging from 113 to 66 million/mL. Our findings were also in agreement with the aforementioned investigations that have shown that epididymal sperm motility was significantly decreased in rats following ethanol consumption. However. J. Villalta et al. observed that alcohol could affect the normal development of sperm, especially

decreased sperm vitality, increased the rates of abnormal morphology and decreased count in sperm, our results also showed a significant decrease in the total sperm number in rats treated with alcohol. Our results did show in diabetes + alcohol mice, a significant decrease in sperm morphology and viability compared to control, diabetes and alcohol mice.

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