The Effect of Water Avoidance Stress (WAS) on Histomorphometric Changes of Testicular Tissue in Rat

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INTRODUCTION

Testis is the reproducing gland in male animals and it has both exocrine and endocrine components. The exocrine part contains seminiferous tubules. These tubules are lined with layer of germ cells that from puberty to the old ages. The main function of these tubules is produce and free spermatozoids, and its endocrine part contains lydic and sertoli cells that produce and excretion of hormones like testosterone, estrogen and inhibin [1, 2].

Several factors may have harmful effects on sperm normal production. Antibiotic drugs [3], anti-inflammatory drugs [4, 5], anti-cancer drugs [6], pesticides [7], irradiation [8], toxins [9], air pollution [10] and vitamins deficiency [11]. Moreover, other factors such as heavy metals [12] and alcohol consumption [13] have important role in dysfunction of spermatogenesis.

The body’s reaction to external or internal signals is called Stress. The effects of acute and chronic stress on physiologic function, development, and perpetuation of many human diseases have been become reported in several papers [14-16]. Stress can be either psychological or physical. Different forms of the stress by effect on activity of hypothalamic-pituitary-adrenal (HPA) axis [17] can affect male fertility and reproduction system. So it can lead to disruption of germinal epithelium, degeneration of the seminiferous epithelium, leydig cell hypertrophy, local dilations of the intercellular spaces between sertoli cells junctions and modifications in the interstitial tissue [16, 18]. It is indicated that semen quality and specific aspects of psychological stress are related together inversely [19]. Khandve and colleagues showed that immobilisation stress for 60 days period (4hr/day) can disturb spermatogenesis in Swiss albino mice [20]. In another study, following the stress exposure in rats, there was progressive disruption in structure and functions of the testis [21].

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Evaluating effects of different forms of stress on animal models can lead to identification and recognition of harmful effects of them on human’s body. WAS (psychological stress) can be count a model of life stress [22, 23]. Various symptoms such as rearing and grooming, occasionally urination serving and fecal output are occurred by emotional reactivity due to psychological stress [24, 25]. In a study, it is reported that chronic social stress can disorganize grooming behavior in mice [26]. In another study, Smith and et al. indicated that during 1 month WAS in rats, urination frequency and fecal pellet excretion and anxiety-like behavior increased significantly compared to sham and baseline [27].

The purpose of this study was to determine the effect of Water avoidance stress (WAS) -represent potent psychological stressor- on Histomorphometric Changes of Testicular Tissue, Corticosterone levels and Fecal pellet output in rat.

**MATERIALS AND METHODS**

**Animals:**
Adult male Wistar rats (200–250 g.) were used for all experiments. The animals were housed under standardized conditions of temperature (20 ± 1 C), and lighting (12-h day/12-h night) and fed a commercial rat pellet. All of the experimental procedures were done between 7 am to 9 am to avoid the effect of diurnal variations.

**Experimental groups:**
Three experimental groups were designed as acute (n=12), chronic (n=12) and normal (n=6) groups. Thereafter, acute and chronic groups divided into the control (n=6) and WAS (n=6) groups. In acute WAS group (T1), rats were exposed to WAS for 2 hr in 1 day and in chronic WAS group (T2) they were exposed to WAS for 2 hr daily for six days. In the control groups, rats were placed on the same platform above a waterless container for 2 hr in one day (acute C1) or 6 days (chronic C2). In the normal group, rats were handled daily but remained in their home cages.

**Water Avoidance Stress:**
Rats were handled 2 weeks before the study [28]. The procedure involved placing the rat on a glass platform (8 × 6 cm) in the middle of a plastic container 90cm in diameter and 50cm high, filled with warm water (25°C) to 1 cm below the height of the platform. Rats were placed on the platform for avoided access to stimulus (water). At the end of the experiments, rats were euthanized by decapitation immediately after the final imposed stress.

**Corticosterone analysis:**
The corticosterone levels from normal, control and WAS-treated groups were measured by Corticosterone rat/mouse ELISA Kit (IB79175).

**Fecal pellet output:**
A number of fecal pellets expellants were counted in the tank at the end of the last day in all groups.

**Histopathology and Light microscopy:**
The middle portions of testicular parenchyma were placed in Formaldehyde and Bouin's solution for at least 24 h. After that they were dehydrated in an alcohol series, followed by xylenes infiltrations. Then the tissue samples were embedded in paraffin. A microtome (Leica RM2145, Berlin, Germany) was used to sections the Paraffin blocks in to 5 μ thick and stained with hematoxylin and eosin. The samples were analyzed under a light microscope (Nikone) and measurements were performed on the basis of digitalized microphotographs obtained with a microscope-coupled digital camera and using the software “Image Tool 3.0”. The morphometrical measurements of 200 cross-sectioned seminiferous tubules were taken. For each individual, the diameter of the seminiferous tubule was measured across the minor and major axes, and the mean diameter obtained. Also the thickness of interstitial tissue and seminiferous duct epithelium were determined.

**Statistical analysis:**
Comparison of the mean of corticosterone levels, number of the fecal pallets and morphometrical measurements of the testis tissues between groups was performed using mann-whitney U. The results were expressed as mean ± S.E.M (standard error of means). P-value less than 0.05 were considered as significant.
Results:

Corticosterone:

Corticosterone level in the normal rats was 10.13±0.24 (ng/ml) which was significantly lower than the controls and WAS-treated animals (P<0.05). The mean ±S.E.M of corticosteron in the WAS-treated animals was significantly lower than the control groups (P<0.05). In the WAS-treated groups, the corticosteron level in T1 (13.92±0.22 ng/ml) is significantly lower T2 (15.26±0.5 ng/ml) (P<0.05), also in the control groups, the corticosteron level in C1 (16.32±0.27 ng/ml) is lower than C2 (18.3±0.1 ng/ml) (P<0.05) (Fig1).

Fecal pellet output:

The number of fecal pellets, quantified at the end of the first day (in acute group), and sixth day (in chronic group). It was significantly higher in chronic groups compared with the acute groups (T1 (2.12 ± 0.24) vs. T2 (4.74 ± 0.65), and C1 (3.2 ± 0.45) vs. C2 (6.19 ± 0.78)) (P<0.05). Also, the number of fecal pellet output was significantly higher in all acute and chronic groups when compared to the normal group (1.79±0.6) (P<0.05).

The number of fecal pellets in controls was significantly higher when compared with WAS in acute group (C1 (3.2 ± 0.45) vs. T1 (2.12 ± 0.24), P<0.05). Also the higher fecal pellet output was observed in the control group when compared with WAS group at the end of day 6 in the chronic group (C2 (6.19 ± 0.78) vs. T2 (4.74 ± 0.65), P<0.05) (Fig2).
Histopathology of testis:
The effect of WAS on histomorphometric changes of testicular tissue is shown in Table 1. In morphometric measurements, the diameter of seminiferous tubules and germinal epithelium height was significantly lower in all acute and chronic groups when compared to the normal group. In both control groups (C1, C2), these parameters were lower when compared with the WAS groups (T1, T2). Also, the thickness of interstitial connective tissue was significantly higher in all treatment (T1, T2) and control (C1, C2) groups when compared with those measured in the normal group. Moreover, in the control groups, the thickness of interstitial connective tissue was higher than the treatment groups (Fig 3).

Table 1: Comparison the histomorphometric changes of testicular tissue in rat after WAS treatment.

<table>
<thead>
<tr>
<th>experimental groups</th>
<th>diameter of seminiferous duct (micrometer)</th>
<th>Germinal epithelium height</th>
<th>thickness of interstitial connective tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>288.18±1.99</td>
<td>79.78±1.74</td>
<td>31.86±1.01</td>
</tr>
<tr>
<td>C1</td>
<td>282.028±1.68*</td>
<td>72.31±1.36</td>
<td>34.47±1.46*</td>
</tr>
<tr>
<td>T2</td>
<td>274±2.33</td>
<td>71.93±2.24</td>
<td>35.6±0.96</td>
</tr>
<tr>
<td>C2</td>
<td>270.4±1.91*</td>
<td>64.78.4±1.79</td>
<td>38.67±6.25</td>
</tr>
<tr>
<td>N</td>
<td>328.3±4.98</td>
<td>85.15±2.04</td>
<td>29.67±0.96</td>
</tr>
</tbody>
</table>

Data are presented as mean ±SE, *significant different at p<0.05 level, (compared with the normal group)

Fig.3: Photomicrographs of seminiferous tubules in all groups (Normal, C1, T1, C2 and T2) (H&E ×40)

Discussion:
Different stress models have been investigated to describe the stress-induced effects in the body [19, 16, 20, 21]. Water avoidance stress, as a stress model, includes psychological stimuli and it may be a model of the daily stress in the human's life [29, 30, 23, 27].

In the present study, we used WAS to demonstrate its effect on The histomorphometric changes of testicular tissue including, seminiferous tubules diameter, seminiferous epithelial thickness and thickness of rat's interstitial tissue. According to the previous studies, WAS had effects on immune system cells [27] and it had many destructive effects on various tissues such as liver [16], bladder [31, 27], tracheal epithelial [14] and gastrointestinal tract [31-34].
Psychogenetic or somatic stress impairs the reproductive function in male primates or other animals [19, 16, 7, 17, 4, 6, 3, 20, 18, 35-37]. It is known that activation of the hypothalamic-pituitary-adrenal (HPA) axis in response to stress may corrupt reproductive functions of male through a depression of the hypothalamic-pituitary-testicular (HPT) axis [17, 38]. In the study conducted by Mingoti et al., exposing of adult male rats to 15 days of forced swimming stress, significantly decreased spermatide production [39].

HPA axis regulates the glucocorticoid (cortisol in primates, corticosterone in mice and rats) response to stressful stimuli [40-43]. Also, in the present study levels of corticosterone increased in all groups -except the normal group- during stress. It is known that during stress, enhanced corticosteroids suppress gonadotrophins [44, 45] and directly suppress reproductive function of testicular tissue [37]. On the other hand, it is suggested that cortisol affects directly on sertoli cells and/or on germ cells and impairs testicular development [46].

Fecal pellet output is a parameter that describes the stress-induced stimulation of the rat colon well [27]. This stimulation occurs by sacral parasympathetic outflow and it is observed into physiological anxiety-like behavior [47, 27]. In our study, the higher fecal pellets were recorded in all groups except normal group during the stress which was similar to the other reports [14, 30].

To the authors' knowledge, this is the first study reporting the effects of WAS on histomorphometric changes of testicular tissue in rat. Many forms of stress in animals such as social stress, high altitude, surgery, and immobilization stress affect body weight, testosterone levels, and mating behavior with variable effects on morphology of testicular tissue [48]. The results of the present study showed that in all groups, seminiferous tubules diameter and seminiferous epithelial thickness were lower when compared with the normal group. On the other hand, interstitial tissue thickness of testis was increased in all groups when compared with the normal group following stress.

There are Evidences that emotional stress suppresses testosterone and perhaps participates with spermatogenesis in male [48]. The stressful stimulus may interfere both endocrine and local factors and disturbs spermatogenesis [49-51]. These destructive effects induced by stress were described by the previous researchers. For example, Mingoti et al., reported that forced swimming stress caused a significant decrement in the count of spermatides in seminiferous tubules [39]. Nirupama et al. revealed that stress-induced loss of germ cells leading to decrease in the numbers of sperm may be due to oxidative damage caused by chronic stress [35]. The other research works on rats stated that chronic emotional stress led to spermatogenesis impairment [52].

Also, in a research work, leyding cells impairment was observed in the rat's testis during stress [53].

According to the results, in contrast to the most previous researches about the WAS [31, 54, 55, 33, 34], in our study, stress of all control groups (waterless) was significantly higher than the test groups. The evidences of this postulate are the higher levels of corticosterone and larger number of fecal pellet output and also, changes of histomorphometric parameters in the control groups. Thus, it is not unlikely that an other psychological stress factor except "water" such as "high altitude" could be more effective in stress-induced various damages in rat and it seems necessary to verify the WAS method.

Conclusion:

In conclusion, stress was observed in all tests and control groups. These results assigned that stress can enhance number of fecal pellets and corticosterone levels and it can develop histological inconvenience such as decrease of seminiferous tubules diameter, decline of seminiferous tubules epithelium thickness and increase of interstitial tissue thickness. In all groups, compared to normal group, stress of control groups was more severe than test groups. More studies are needed to clarify "high altitude" or other confounding factors which have effect on the results of WAS method.

ACKNOWLEDGEMENT

Thanks for financial Support from Young Researchers and Elite Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran.

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