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Ascorbic Acid Protects the Testis and Pituitary-Gonadal Axis from the Effects of Hyperthermia in Adult Male Albino Rats

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Abstract **Objective:** The effect of hyperthermia on pituitary – gonadal axis of adult male albino rats exposed to high temperature (40 ± 1°C) for 12 hours was investigated. The protective role of vit. C in hyperthermia exposed rats was also studied. **Animals and Methods:** Thirty adult male rats, about 3 months old, were used. They were divided into three equal groups and separated into two rooms. The rats in the first room (gp 1/ control) were maintained under 25 ± 5°C environmental temp. Rats of second room (gp 2 & 3) were subjected to 40 ± 1°C for 12 hours. Rats of gp 1 & 2 were fed on a normal commercial rat ration while, rats of gp3 were fed on the same ration supplemented with vit. C. Serum, testicular and pituitary levels of testosterone, LH, FSH and PRL were evaluated in all rat groups. Histopathological examination of testes were evaluated after heat exposure. **Results:** obtained revealed a significant lowered levels of testicular testosterone in gp 2 as compared with that of (gp 1 & 3). Serum levels of Testosterone, LH and FSH were significantly decreased and PRL level was significantly increased in gp 2 as compared with (gp 1 & 3). In addition, pituitary Levels of FSH or LH were significantly decreased and PRL level was significantly increased in gp 2. Histopathological examination revealed disorganization of semineferous tubules of the testes in gp 2. **Conclusion:** avoidance of hyperthermia is very important to maintaine a proper pituitary - gonadal axis. Vit.C was found to counteracts the adverse effects of hyperthermia. Also, rat model could be considered as a basis to investigate the effects of hyperthermia on human males working in areas of higher heat exposure.

Keywords: *hyperthermia, pituitary - gonadal axis ,testis, Vit.C, rats*

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1. Introduction

Hyperthermia is a condition of abnormal rise of core body temperature resulting from exceeded heat gain over heat loss. Increasing hyperthermia can lead to a series of systemic and cellular changes that increase risk for exhaustion, organ failure and death. Although hyperthermia can occur without severe dehydration, it exacerbates the condition. Hyperthermia is mostly achieved following prolonged exposure to high atmospheric temperature (Michel et al., 2007). This occurs usually in the tropic regions during summer. Heat generation is also occurred by injection of pyrogens or during exposure to hyperthermic agents as microwaves, ultrasonic and radiofrequency radiation.

Hyperthermia may be a consequence of environmental conditions, microbial infections and/or hyperthyroidism. Although regulation of body temperature and individual adaptation to environmental climatic changes is well documented, little is known about mechanisms and pathological aspects of hyperthermia (Sharma, 2007).

Researchers noted that when the body temperature rises beyond a critical temperature into the range of 40.5°C to 42.2°C, the individual is likely to develop heatstroke. They added that the hyperpyrexia itself is also exceedingly damaging to the body tissues and is responsible for most of the effects (Guyton and Hall, 2006)). In fact, even a few minutes of very high body temperature can sometimes be fatal. A complicating fact to achieve hyperthermia is the variety of normal body temperature between mammalian species, eg. 37°C in human, 38°C in rats, 39°C in pigs and 39°C in rabbits and sheep(Miller And Ziskin, 1989).

The testis is a complex organ that performs two crucial functions: a) synthesis and secretion of testosterone and other hormones through the process of steroidogenesis; and b) production of a sufficient number of competent spermatozoa through the process of spermatogenesis to attain fertility (Amann and Schanbacher 1983). Both processes are intimately coupled, in their essential requirement for adequate levels of testosterone for the normal production and maturation of spermatozoa.

Vit.C has long been associated with fertility and considered a major antioxidant in the testis. This vitamin is an essential water-soluble micronutrient required for an

array of biological functions. It is unstable, easily oxidized acid and can be destroyed by oxygen, alkali and high temperature (Iqbal et al., 2004). Oxidative stress in the testis was found to be one of the major factors that induces germ cell apoptosis, this organ has high concentrations of antioxidants such as ascorbic acid, to protect germ cells against oxidative damage (Linster and Van Schaftingen, 2007).

Consequently, there is an urgent need to identify antioxidants that can supplement the tissue's own antioxidant strategies to rescue the testes from the consequences of hyperthermia. Therefore, this study has been performed to clarify the effect of hyperthermia on pituitary-gonadal axis and testicular changes, including the endocrine function and control when adult male rats are exposed to high temperature (40°C) for 12 hours. The protective role of vit.C against the adverse effects of hyperthermia on testicular function was also investigated.

2. Material and Methods

2.1. Animals and Treatment

Thirty adult male albino rats, aged about 3 months, (120 ± 9 g) were used. Rats were acclimatized for 3 days at environmental temperature of (25 ± 5°C) with relative humidity (50 ± 10%) and 12 h. light/dark cycle. The animals were housed, one per cage in wire bottomed stainless steel cages in a temperature controlled room. During the acclimatization days rats were divided randomly into three groups each comprising 10 rats, a control group (gp 1) was fed on a commercial rat ration. The other experimental two groups (gp 2 & 3) were fed on a commercial rat ration (gp 2), while rats of (gp 3) were fed on a commercial ration supplemented with vit. C in a dose equal to 25 mg of ascorbic acid/kg of rat body weight per day dissolved in drinking water (Djurašević et al., 2008). Ascorbic acid was obtained as a 100% pure powder manufactured by "Wholesale Nutrition Company –USA" and purchased from "Al-Gomhouria Company for Chemicals – EGYPT". At the day of experiment, rats of gp 2 & gp 3 were subjected to high environmental temperature (40 ± 1°C) and relative humidity 90% for 12 hours in the temperature controlled room via adjustable automated heaters. During the time course of heat exposure rectal temperature was measured using a thermometer that was inserted through the anus to be in direct contact with the rectal mucosa to monitor the core temperature avoiding excessive hyperthermia. Water was offered to all rat groups ad libitum.

2.2. Blood, Testicular and Pituitary Gland Tissue Samples

After overnight fasting and light ether anesthesia, all animals were killed by decapitation, and the trunk blood was collected and allowed to clot for 2 h at 5°C. Sera, testis and pituitaries were obtained and used for determination of hormonal content. Serum levels of total testosterone, spermatogenic hormone (FSH), interstitial cells stimulating hormone (LH) and Prolactine (PRL) were measured by ELISA technique (*Jaffe* and *Behrman*, 1974). FSH, LH and PRL concentrations were measured in pituitary homogenates. Pituitaries were prepared by homogenizing each pituitary in 2 ml of ice-cold phosphate buffered saline (PBS). One of the testis was fixed in Bouin's fixative for histological evaluation, they were embedded in paraffin wax, sections were cut 5 µm thick and stained with hematoxylin and eosin. The other testes was used for preparation of testicular extract (El-Far, 1996) and used for evaluation of testicular testosterone (Xing et al., 1983).

2.3. Statistical Analysis

Data was expressed as means ± SE and statistically analysed by using analysis of variance (ANOVA) test. Means were tested at least significant difference (LSD). All statistical analysis was performed using SPSS (v.15.0) software.Results were considered significant only at $P < .05$ or less.

3. Results

Table 1. Levels of reproductive hormones in serum, testis and pituitary glands of male albino rats subjected to hyperthermia

Hormones	Groups			LSD
	Control	Hyperthermia	Hyperth. + vit c	
Serum				
Testosterone ng/ml	1.22 ± 0.23[a]	0.82 ± 0.15[b]	1.18 ± 0.24[a]	0.32
FSH / ng / ml	5.22 ± 0.6[a]	3.30 ± 0.40[b]	5.02 ± 0.40[a]	1.01
LH / ng / ml	0.92 ± 0.13[a]	0.52 ± 0.80[b]	0.95 ± 0.11[a]	0.36
PRL / ng / ml	3.86 ± 0.44[a]	6.60 ± 0.60[b]	4.10 ± 0.48[a]	1.95
Testis				
Testosterone/ng/gm	366 ± 42[a]	220 ± 31[b]	359 ± 50[a]	32.50
Pituatary Gland				
FSH / ug / g.pit	214 ± 18[a]	180 ± 12[b]	218 ± 20[a]	16.80
LH / ug / g.pit	832 ± 50[a]	610 ± 35[b]	818 ± 42[a]	82.60
PRL / ug / g.pit	650 ± 45[a]	835 ± 55[b]	642 ± 33[a]	66.90

Values are means ± SE. Means within the same raw having different letters are significantly different at $P \leq 0.05$. Number of rats / group: (10)

Data presented in Table 1 indicates that hyperthermia for 12 hours produced significant changes in the levels of reproductive hormones in serum, testis or pituitary glands of rats subjected to hyperthermia. Vit.C supplementation retained hormonal levels in rats subjected to hyperthermia within normal ranges. In rats subjected to hyperthermia, a significant decrease in srum levels of testosterone, FSH and LH associated with significant increase in PRL level were recorded as compared with control or Vit.C groups. In addition, testicular level of testosterone was significantly decreased comparable with control or Vit.C groups. Moreover, pituitary contents of FSH and LH were significantly decreased, while PRL content was significantly increased when compared with contents in control rats or Vit.C group.

Histopathological examination of testicular tissue revealed that semineferous tubules of rats subjected to hyperthermia showed disorganization, cell atrophy and desquamation of germinal cells (Figure 2) as compared with the histological picture of control or Vit.C groups (Figure 1).

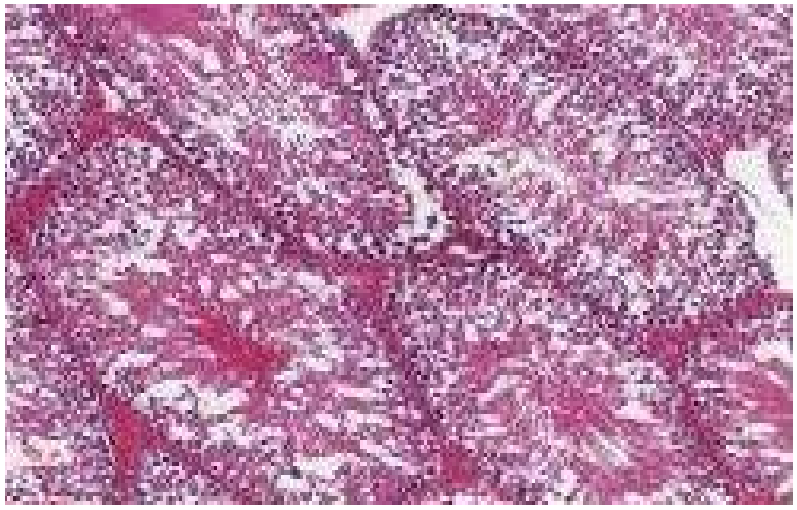

Figure 1. Transverse section in testis showing normal structure (H & EX100)

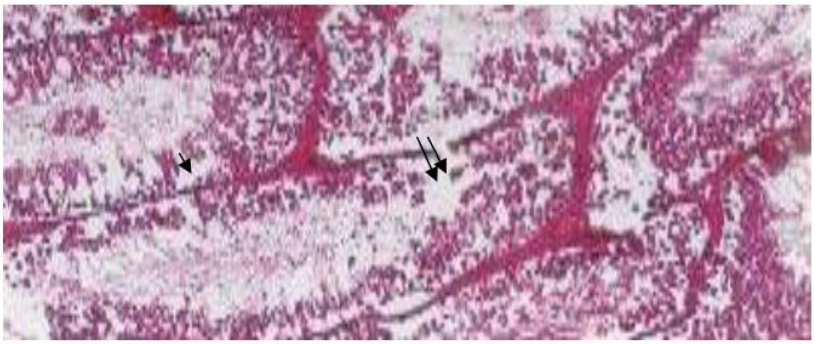

Figure 2. Degenerative changes (↓) and desquamation (↓↓) In seminiferous tubules

4. Discussion

Hyperthermia is an important stress factor and known to increase blood cortisol levels (Wright et al., 2010). This is expected since hypothalamo-pituitary-adrenocortical (HPA) axis is In the present study,hyperthermia appeared to disrupt the pituitary-gonadal axis in adult male rats subjected to hyperthermia (40°C) for 12 hours.This disruption was manifested by deviated hormonal patterns either on the level of pituitary gonadotropins, systemic blood or testicular tissue. A significant decrease in the levels of pituitary and serum FSH and LH was recorded. Serum and testicular contents of testosterone was also decreased. A higher levels of PRL were detected in serum and pituitary tissue. Other results found that rats with impaired HPA axis were less tolerant to heat stress exposure (Michel et al., 2007). Also, decreased androgen concentrations was reported in male subjects undergoing hot spring immersion (41°C) for 30 min (Wang et al., 2009).

Testicular function is highly dependent on temperature control and negatively influenced by hyperthermia (Khan and Brown, 2002). Long-term application of mild testicular hyperthermia induces stage-specific and germ cell-specific apoptosis in adult monkey testes (Lue et al., 2002). Similarly, exposure to heat for short period has been shown to trigger apoptosis in dividing cell in the testis (Michel et al., 2007). The disrupted series of spermatogenic cells recorded in the current study coincided with the previously detected alterations in the spermatogenic cell series of rats. rats (Michel (Michel et al., 2007). Also, it may reflects the deleterious effects of deviated hormonal pattern that required for the soundness of testicular architecture activated in response to stressors such as heat and inflammation (Schobitz et al., 1994).

There are several reports indicating that increased temperature inhibits spermatogenesis (Jung and Schuppe, 2007). Testosterone is secreted by the interstitial cells of Leydig in the testis, but only when they are stimulated by LH from the anterior pituitary gland. Furthermore, the quantity of testosterone secreted increases approximately in direct proportion to the amount of LH available (De Kretser, 2004). Hyperthermia can impair testosterone production that could later have secondary reproductive consequence such as the impairment of spermatogenesis, because testosterone is clearly essential for the maintenance of established spermatogenesis (Thoreux et al., 1995).The reduced LH concentration may affect leydig cell function decreasing testosterone secreation after exposure to hyperthermia (Kemprinas et al., 1990).

The decreased LH and FSH levels in the present investigation could be attributed to the suppressive effect of higher level of PRL induced by hyper secreation of cortisone as a consequence of stress stimulation mediated

through hyperthermia. The reduced LH concentration in the blood adversely affected the secrested testosterone level and consequently its testicular content. Intratesticular testosterone plays a pivotal role in protecting germ cells against heat-induced cell death (Lue et al., 1999). Thus, testes may be more susceptible to hyperthermia due to lack of protective effects of testosterone. This suggestion may be confirmed by the current histopathological findings in the form of disorganization and slight damage of germinal cells of the semineferous tubules.

However, Vit.C supplementation maintains the soundness of pituitary-gonadal axis in rats subjected to hyperthermia keeping the hormonal pattern within normal ranges and the testicular structure healthy. Dietary supplementation of Vit.C was found to increase total protein but decrease corticosterone, glucose and cholesterol concentrations (Sahin et al., 2003). Ascorbic acid supplementation was found to alleviate detrimental effects of heat stress (El-Shaieb et al., 2009).

The testis was found to be sensitive to a variety of stressors, such as hyperthermia, inflammation, radiation and exposure to agents that increase apoptosis of germ cells (Linster and Schaftingen, 2007). Vit.C is considered a major antioxidant in the testis (Augustine et al., 2005). It neutralizes ROS and prevents sperm agglutination (Aitken et al., 1999). In men who had decreased sperm count, motility or and increased abnormal sperm count, the Vit.C level was found to be decreased (Jedlinskaet al., 2006). A vitamin C deficient diet causes massive degeneration of the seminiferous epithelium with desquamation into the lumen of the testis tubules in guineapig (Lindsay and Medes, 1962).

5. Conclusion

In conclusion, our findings suggest that short term exposure to hyperthermia cause disruption in pituitary-gonadal axis which is associated with disconfiguration in testicular architecture. Vit.C supplementation was found to maintains pituitary-gonadal axis and testicular structure within normal. This meight be through its specific role as strong antioxidant or normalizing the cortisone secreation level, the suggestion that required further investigation. Individuals working in areas where high temperature is prevalent should probably be taking Vit.C regularly until the human situation is clarified by research.

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