

EFFECT OF VITAMIN E AGAINST HEAT STRESS INDUCED TESTICULAR DAMAGE IN WISTAR ALBINO RATS

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ABSTRACT

Background: Heat stress is one of the most important stressors especially in hot regions of the world. Spermatogenesis and normal testicular function are both temperature dependent. There is compelling evidence that there is a correlation between male infertility and testicular heat stress. Since oxidative stress is the major source of damage after testicular heat stress, it seems logical that antioxidant can prevent germ cell apoptosis and sperm damage. Therefore, the aim of the study was to observe the effect of vitamin E against heat stress induced testicular damage.

Materials and Methods: Thirty-two healthy Wistar Albino rats weighing 130-200gm were randomly divided into 4 groups i.e. group I (control) and group II (vitamin E), III (heat stress), IV (heat stress with vitamin E) each consisting of 8 rats. Rats belonging to group I and II were kept in controlled room temperature of (25±0.5 °C) and rats of group III and IV were kept in controlled room temperature of (37 ±0.5 °C) for two weeks. In addition, rats of group II and IV were injected 200mg/kg of vitamin E intraperitoneally. On 15th day all rats were sacrificed and testes was removed and processed for slide preparation and were observed for histological changes. Paired t-test and one way ANOVA were used for data analysis at 95% confidence interval.

Results: Heat stress caused decrease in body weight, testicular weight and size of testes. Histological study showed decrease in diameter and necrosis of epithelial lining of seminiferous tubule in rats under heat stress. Vitamin E showed partially protective effect against heat stress.

Conclusion: Heat stress caused both morphological and histological changes in testes and Vitamin E was partially effective in protecting the testicular damaging effect of heat stress.

KEY WORDS: Testes, Heat Stress, Vitamin E, Rats, Light Microscopy and Histology.

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INTRODUCTION

Stress denotes the magnitude of forces external to the body system which tends to displace that system from its resting state [1].

It is an unfavorable ambient condition that bring about changes in the body system [2]. Stress includes environmental conditions like noise,

over crowdies, atmospheric pollution and heat [3]. Heat stress is one of the most important stressors especially in hot regions of the world. Adaptation to heat stress requires the physiological integration of many organs and systems like endocrine, cardio respiratory and immune system [4].

Spermatozoa are generated in the testis by a process named spermatogenesis [5]. It is well established that spermatogenesis and normal testicular function are both temperature dependent, and in most mammals the testes are kept between 2 and 8°C below core body temperature by virtue of being situated in the scrotum outside the main body cavity [6]. The scrotal skin has a great number of sweat glands to keep the temperature down by evaporative heat loss [7]. There is compelling evidence that there is a correlation between male infertility and testicular heat stress [8]. Experimental studies have suggested that a mild increase in testicular temperature could be a potential contraceptive method for men [9]. Genital heat stress is detrimental to spermatogenesis and results in spermatozoa of inferior quality. Both epididymal sperm and testicular germ cells are sensitive to damage by heat stress [10]. Spermatozoa resulting from sperm cells exposed to hyperthermia in mice undergo apoptosis [11] and contain DNA [12], leading to poor fertilizing capacity in vivo and in vitro. The severity of damage to sperm cells subjected to heat stress varies with the intensity, frequency and duration of heat exposure [13,14].

Several rodent models have been used to study the impact of heat stress on the testis including transient exposure of the testes to elevated temperatures (typically greater than 40°C), surgical induction of cryptorchidism resulting in long-term exposure of the testes to core body temperature (37 °C) or housing of males at elevated temperatures (e.g. 35–36 °C) for several hours. In these studies, the common features of disturbances in testicular function that have been recorded include decreased testicular weights, germ cell loss and increased rates of apoptosis [15,16]. During heat stress at 37°C somatic cells do not undergo apoptosis but germ cells undergo apoptosis which indicates that heat stress activate the apoptotic pathway only in germ cells [17]. Heat stress increases lipid peroxidation which is associated with production of large number of free radicals which are capable of initiating peroxidation of polyunsaturated fatty acids [18].

Testicular temperatures are reported to be higher in men of certain occupations (bakers,

welders and professional drivers) and in those with poor posture or tight clothing [19]. Since oxidative stress is the major source of damage after testicular heat stress, it seems logical that antioxidant supplement have been chosen, has the primary treatment in order to prevent germ cell apoptosis and sperm damage in several animal model [20]. Vitamin E is a fat soluble antioxidant that stops the production of Reactive Oxygen Species formed when fat undergoes oxidation. Vitamin E is expected to show the palliative effect in damage caused by heat stress in testis which this study is aimed to find out.

MATERIALS AND METHODS

Thirty-two healthy Wistar Albino rats weighing 130-200gm were randomly divided into 4 groups i.e. group I (control) and group II (vitamin E), III (heat stress), IV (heat stress with vitamin E) each consisting of 8 rats. Rats belonging to group I and II were kept in controlled room temperature of (25±0.5 °C) and rats of group III and IV were kept in controlled room temperature of (37 ±0.5 °C) for two weeks in a well-ventilated room with a 12 hours alternating light-dark cycle. In addition to this; Rats of group I was injected 1mL of saline intraperitoneally (i.p.) per day whereas Rats of group II and IV were injected (i.p) 200mg/kg of vitamin E. All rats were fed standard pellet diet, Bengal gram and tap water ad libitum. On 15th day of experiment after weighing the rats, they were anesthetized and testes were removed. Weight of testes was measured by electronic balance and size by volume by water displacement method. After tissue processing slides were prepared by H&E staining and were observed for histological changes. The diameters of seminiferous tubules were measured by oculo-micrometer. Paired t-test and one way ANOVA were used for data analysis and the data were considered statistically significant at 95% confidence interval. All the experimental works were carried out as per ethical guidelines of Nepal Health Research Council (NHRC) and ethical clearance was obtained from Institutional review board, BP Koirala Institute of Health Science.

RESULTS AND DISCUSSION

Body weight of rats: The details of the body

weight of rats before and after experiment is shown in the Table 1. Weight of the rats were found to be increased significantly ($P < 0.01$) in group I (normal control) and group II (Vitamin E group) after experimental period. In group III (heat stress group); heat stress caused the significant ($P < 0.01$) decrease in body weight. Use of Vitamin E against heat stress (group IV) somewhat checked the decrease in body weight due to heat stress but not significantly ($P > 0.05$) when compared to group III. Reduction in the body weight after heat stress was observed in similar study by Gonzalez Alonso et al. [21] This reduction in body weight might be due to decrease in anabolic activity and the increase in tissue catabolism caused by high elevated temperature.

Table 1: Comparison of body weight before and after experiment.

Groups (N=8)	Body weight before experiment (gm.)	Body weight after experiment (gm.)	Mean difference (gm.)	P Value
Group I (Normal control)	142.50±2.25	160.00±3.57	17.50±1.32	$P < 0.01$
Group II (Vitamin E)	142.50±2.02	163.75±2.26	21.25±0.24	$P < 0.01$
Group III (Heat stress)	143.50±2.70	140.62±3.70	-2.87±1.00	$P < 0.01$
Group IV (Heat stress) with vitamin E)	138.62±2.53	140.75±3.44	2.12±0.91	$P > 0.05$

Weight of testes: After the experimental period the weight of testes were found as shown in the Table 2. No significant difference ($P > 0.05$) in the weight was found in between group I (normal control) and group II (Vitamin E group); suggesting that Vitamin E has safe effect on testicular weight. However, compared with the normal (group I), weight of the testes was decreased significantly ($P < 0.01$) in the heat stress treated rats (group III), depicting that heat stress caused the decrease in testicular weight. Use of Vitamin E against the heat stress (group IV) resisted the decrease in testicular weight by heat stress but not significantly ($P > 0.05$) when compared to compared to group III, suggesting that Vitamin E is not potent enough to counter the effect of heat stress in testicular weight. The decrease in testicular weight due to heat stress has been reported in earlier studies as well [9,15].

Size of testes: The size of the testes was determined by the volume displacement method. There was no significant difference in the

volume of the water displaced by the testes in between group I rats (normal control group) and group II rats (vitamin E group) suggesting that Vitamin E has safe effect on testes size. Whereas, size of the testes was significantly reduced ($P < 0.01$) in group III rats (heat stress group) compared to control group. Use of vitamin E against heat stress (group IV) showed some protective effect however it was not significant ($P > 0.05$) when compared to group III, suggesting that Vitamin E couldn't counter the shrinking effect of heat stress on testes.

Table 2: Weight of testes in different groups.

Groups (N=8)	Mean± SD (gm.)
Group I (Normal control)	2.19±0.23
Group II (Vitamin E)	2.18±0.22
Group III (Heat stress)	1.59±0.20
Group IV (Heat stress with vitamin E)	1.64±0.21

Table 3: Size of testes in different groups.

Groups (N=8)	Mean± SD (ml)
Group I (Normal control)	13.75±1.03
Group II (Vitamin E)	13.38±0.92
Group III (Heat stress)	11.75±1.39
Group IV (Heat stress with vitamin E)	12.38±1.51

Diameter of Seminiferous tubule: The diameter of seminiferous tubule in group I (normal control) was $55.61 \pm 7.18 \mu\text{m}$, in group II (Vitamin E group) was $56.53 \pm 6.83 \mu\text{m}$, whereas in group III (Heat stress group) was $40.78 \pm 7.45 \mu\text{m}$ and in group IV (Heat stress with vitamin E group) was $47.52 \pm 6.70 \mu\text{m}$. There was no significant difference ($P > 0.05$) in the diameter of seminiferous tube between normal and vitamin E treated group, suggesting that Vitamin E has safe effect in seminiferous tubular diameter. However, there was significant difference ($P < 0.01$) in the diameter of seminiferous tube in between normal and heat stress group, depicting that heat stress caused the constriction of seminiferous tubule. Use of Vitamin E against the heat stress (group IV) resisted the constriction of tubule by heat stress but not significantly ($P > 0.05$) when compared to group III; suggesting that Vitamin E is not potent

enough to counter the constricting effect of heat stress in seminiferous tubule.

Histological architecture of Seminiferous Tubule: In the observation of histological section, the control group (group I) showed numerous cut sections of seminiferous tubules. Each seminiferous tubule was lined by a basement membrane. Internal to the basement membrane was a complex stratified layer of spermatogenic and supportive cells. The lumen of the tubule was filled with spermatozoa. Space between the seminiferous tubule was filled with vascular loose connective tissue, containing groups of interstitial cells of Leydig as shown in the Figure 1. The architecture of the histological section of the Vitamin E treated rats (group II) were similar to the control which is shown in Figure 2.

Fig. 1: Photomicrograph of rat testes section showing normal histology in control group [X400]. Section of Seminiferous tubule lined by a basement membrane, resting where is stratified layer of spermatogenic and supportive cells. The lumen of the tubule is filled with spermatozoa. Space between the seminiferous tubule is containing interstitial cells of Leydig.

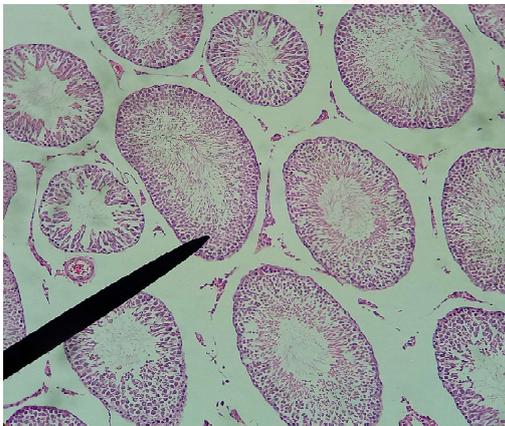


Fig. 2: Photomicrograph of rat testes section showing normal histology in vitamin E treated group [X400]. Characteristics features are similar to Normal histology.



Fig. 3: Photomicrograph of rat testes section showing disrupted histology in Heat stress treated group [X400]. Spermatogenic and supportive cells have undergone necrosis. Lumen is infiltrated with necrotic materials.

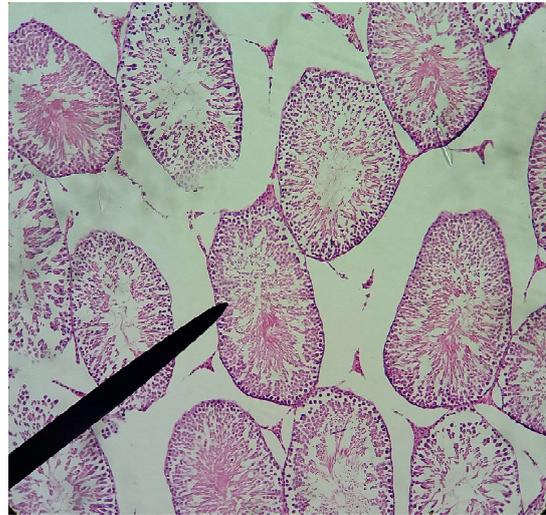


Fig. 4: Photomicrograph of rat testes section showing partially disrupted histology in Heat stress with vitamin E treated group [X400]. Spermatogenic and supportive cells have undergone necrosis in some seminiferous tubules whereas some tubules have normal architecture.



The histological section of the testes of heat stress treated rats (group III) showed thickened basement membrane. The stratified layer of spermatogenic and supportive cells internal to the basement were found to be necrotized. The lumen was found to be infiltrated with necrotized cells. Number of interstitial cells of Leydig were found to be reduced which is shown in Figure 3. Similar was reported by Ikeda M. et al [22].

As observed in the histological section, use of Vitamin E against heat stress (group IV) showed somewhat protective effect but was not much effective. Some of the sections of the seminiferous tubule were normal whereas some of the sections showed partially necrotized stratified layer of spermatogenic and supportive cells. The

lumen of the normal tubule were filled with spermatozoa whereas section of the necrotized tubule showed empty lumen. Thus, the histological observation suggest that Vitamin E was partially protective against heat stress induced testicular damage. This was in contrary to the study of Lucesoli F et al. [23] and Gavazza MB et al [24] where they had demonstrated that Vitamin E suppresses the lipid peroxidation in testicular microsomes and mitochondria and to reverse the detrimental effects of oxidative stress on testicular function.

CONCLUSION

The present study showed that heat stress caused both morphological and histological changes in testes. Reduction in body weight, testes weight, testes volume, diameter of seminiferous tubule were observed in heat stress treated group. Heat stress caused loss of normal architecture of seminiferous tubule. Hemorrhagic and necrotic areas were also seen in the medulla. This alterations were partially improved when treated with Vitamin E, suggesting that Vitamin E partially effect agent to counter the effect of heat stress.

Conflicts of Interests: None

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