Protective potential of Vitamin E against methylphenidate-induced male gonadal changes in albino rats

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ABSTRACT

Objective: Attention deficit hyperactivity disorder ranks among the top neuropsychiatric disorder of childhood and adolescents. Methylphenidate (MPH) is the most frequently used pharmacologic agent to treat this condition. Its long-term use has been associated with many unwanted and adverse effects on many organs including male gonads, but so far no study has been done to find out a protective agent. This study investigated the protective potential of Vitamin E (Vit E) against the microscopic and morphometric alterations in male gonads induced by MPH, using albino rats.

Methods: Adult male albino rats were assigned into three equal groups including one control and two experimental groups. Experimental groups administered with MPH (10 mg/kg) and MPH (10 mg/kg) + Vit E orally (50 mg/kg), daily for 40 days. Testes of the sacrificed animals were removed, processed, and stained with hematoxylin and eosin for examining the microscopic and morphometric alterations and protective potential of Vit E. Data were analyzed using ANOVA.

Results: Experimental animals treated with MPH showed a significant decrease in the diameter of seminiferous tubules (296.86 ± 14.70 µm) and height of germinal epithelium (51.73 ± 3.15 µm) with a corresponding gain in the thickness of the interstitium (47.05 ± 4.94 µm). Animals treated with MPH + Vit E did not reveal any significant testicular microscopic changes and seminiferous tubular alterations induced by MPH.

Conclusion: Vit E demonstrated a protective potential against the adverse changes induced by MPH in the male gonads in albino rats.

Keywords: Attention deficit hyperactive disorder, diameter of semineferous tubules, height of germinal epithelium, methylphenidate, reactive oxygen species, thickness of interstitial spaces, Vitamin E

Introduction

Attention deficit hyperactive disorder (ADHD) is a commonly reported neuropsychiatric disorder in recent years, characterized by age improper hyperactivity, impulsivity, and short attention span which can persist in adulthood.[2,3] ADHD, occurrence in children worldwide ranges from 2% to 10%[4] while in Pakistan, it is around 18.8%. ADHD has an huge effect on society in term of communication problems,[6] workplace behavioral issues which can affect their self-esteem and constant family worries related and financial cost.[7] Etiology of ADHD is still unclear, but largely it is believed that dysregulation of catecholamines is the root cause.[8] Methylphenidate (MPH) has shown very effective outcome in treating ADHD symptoms.[9,10] It blocks the synaptic reuptake of norepinephrine and dopamine and increases their availability into the extraneuronal space.[11] These neurotransmitters are known to influence spatial working memory in experimental animals.[12,13] MPH can be associated with some of the critical negative effects such as chest pain, convulsions, headache, visual disturbances, elevated serum alkaline phosphatase, bilirubin, and hepatic enzymes levels.[14] Some structural and functional alterations in tests have also been reported in literature.[15,16] Weight loss in children frequently leads to withdrawal of MPH.[14] Formation of reactive oxygen species (ROS) with emanating lipid peroxidation and alteration in the membrane structure and cellular integrity could be an underlying mechanism.[15,16] Vitamin E (Vit E) is a naturally occurring antioxidant that prevents the harmful outcomes of ROS.[17] It plays a crucial role in abolishing lipid peroxidation and stabilizing the cellular membranes by inactivating harmful free oxygen radicals.[17] Vit E has an essential role in normal spermatogenesis and its lack has been associated with dysfunction of germinal layer of the gonads in male albino rats amelioration against toxicity of various drugs by Vit E has been reported in literature.[19-21] A rising trend in
non-medical use of MPH for alertness by college and university students has been reported,[22,23] and it has become important to determine whether conjunt treatment with an antioxidant agent like Vit E can potentially be used to protect against male gonadal toxicity.

**Methods**

**Animals**

This study used albino rats as a mammalian model. 180–200 gms weighing, 8 weeks old, 24 healthy adult male albino rats were obtained from animal house of Liaquat National Hospital and Medical College, Karachi, Pakistan. All animals were housed in standard cages with free access to water and food and 12 h day and night cycle at 30°C. The animals were acclimatized 1 week before the commencement of the experiment. Experimental protocols were formally approved by the Institutional Ethics Review Committee.

**Experimental groups**

The rats (n = 24) were randomly divided into three groups with eight rats in each. Control, MPH and MPH + Vit E treated groups were designated as A, B, and C group, respectively.

**Dosage**

The drugs were administered using animal feeding intubation needle by gavage method[17] dosage of Vit E[24,25] and MPH used[10,26,27] in this study as prescribed by some earlier studies.

I. Group A (control group) animals received 2 ml of normal saline daily for 40 days[10]

II. Group B (MPH) animals received 10 mg/kg of MPH daily for 40 days.[10,26]

III. Group C (MPH + Vit E) animals received 50 mg/kg of Vit E + 10 mg/kg of MPH daily for 40 days.[17]

At the end of the experiment, animals were anesthetized using chloroform[17] and sacrificed. Abdominal cavities were opened through midline vertical incisions. Testes were removed and fixed in 10% formalin solution after washing with physiologic serum for at least 24 h.[17]

**Microscopy**

Fixed testes were dehydrated with ethanol and blocked in paraffin wax.[17] 5 µm thick sections were prepared and stained with hematoxylin and eosin for microscopy. Ten randomly selected sections from each testis were studied under a light microscope at power 10 x, and photomicrograph was captured. In each section, three parameters were studied:

1. Height of the germinal epithelium (HGE): Height of the epithelium was measured at four sites at right angles to one another in each tubule.
2. Diameter of the seminiferous tubules (DST): The diameter
3. Thickness of interstitial spaces (TIS) width of the interstitial tissue was measured at the narrowest point between two seminiferous tubules.

**Statistical analysis**

The data were analyzed (ANOVA) using software SPSS, version 21. Values were expressed as mean ± SD. P < 0.05 was considered statistically significant.

**Results**

**Morphometric finding**

Mean seminiferous tubular diameter (DST) of Group A; animals were 369.22 ± 4.39 µm while the mean height of germinal epithelium (HGE) and mean TIS were 174.29 ± 2.18 µm and 9.02 ± 3.17 µm, respectively [Graph 1-3].

Mean values of DST, HGE, and TIS of the Group B animals were 296.86 ± 14.70 µm, 51.73 ± 3.15 µm, and 47.05 ± 4.94 µm, respectively. These mean values were statistically significant lower than Groups A and C animals [Graph 1-3].
Mean values of DST, HGE, and TIS of group C animals were 394.32 ± 13.46 µm, 103.03 ± 4.37 µm, and 13.18 ± 2.54 µm, respectively. Mean DST of the Group C animals were statistically significant higher than Groups A and B animals [Graph 1-3] while the mean HGE and TIS were significantly higher than the MPH-treated animals and significantly lower than the control animals.

Microscopic finding

Seminiferous tubules were nearly rounded and lined with a stratified layer of germinal epithelium. Basement membranes were regular with several layers of spermatogenic cells lying over [Figure 1]. Most of the section fields contained seminiferous tubules with very narrow intervening interstitial spaces, having blood vessels and Leydig cells. Testicular sections from Group B animals showed distorted and narrowed seminiferous tubules [Figure 2]. Germinal epithelium showed a loss in height [Figure 2, Table 1] with reduced stratification and irregular vacuolations [Figure 2]. Widened interstitial spaces were present among the distorted seminiferous tubules all over the sections [Figure 2]. Testicular sections from Group C, animals showed seminiferous tubules similar in architecture to Group A control animals [Figure 3]. Germinal epithelium showed stratified epithelium with different layers of spermatogenic cells. No vacuoles were observed [Figure 3].

Discussion

This study finds a decrease in seminiferous tubular diameter, HGE and gain in TIS in the animals treated with MPH. Similar findings have been reported in literature. In contrast to that, no change in diameter has also been reported. This decline in diameter after chronic administration of MPH, could be due to its effect on body weight generally or on testicular weight particularly, that affects animal growth or could be because of the necrosis as suggested by Taghva et al., 2007.

Maintenance of germinal epithelium in males is a testosterone function which regulates the germinal cells division and thereby maintains the stratification and HGE. MPH administration in this study has shown a partial loss of
stratification and HGE. Many factors have been suggested as contributing factors toward this finding including, impaired maturation or loss of Leydig cells,[26] impairment of the pulsatile release of hypothalamic GnRH results in impaired release of the testosterone with either cessation of the germinal epithelial cell division or impaired progression of the cell cycle.[23] Apoptotic loss of developing germinal cells has also been suggested as a leading factor in the loss of HGE.[19] This findings favors the previous study by Fazelipour et al., in 2013,[10] who has reported a decrease in HGE, mainly involving the apoptotic decline in the number of spermatids or expressing protooncogene P53;[27] however, this study limited its focus on measuring the HGE only. Contrary to our finding, Adriani et al., in 2006, have reported a positive effect of MPH on testicular growth along with an increase in number of spermatogonium in mice using the same dose.[33]

Widening of interstitial spaces in the MPH-treated animals has also been observed in this study. This is in agreement with an earlier study done by Castells et al., 2018.[13] This widening is secondary to distortion and narrowing of the seminiferous tubules leaving a wider interstitial space. Montagnini et al., 2014,[29] have also reported a decline in the number of Leydig cells present within this space. Treatment with Vit E along with MPH has shown a mitigating effect on all the findings present in Group B animals. Vit E is an antioxidant agent that exterminate ROS and cellular injuries instigated by lipid peroxidation.[18] Vit E, naturally accumulates in the membranes of mitochondria and endoplasmic reticulum and protects testicular cells from lipid peroxidation.[14] It also scavenges free radicals to preserve cell membrane functions such as ion transport and membrane fluidity through maintaining the sulfhydryl groups of membrane proteins.[35]

Findings of this study have shown that Vit E treatment has not only have withstand the MPH-induced toxicity of the testicular tissue but surprisingly has also shown a significant surpassing effect with mean DST even higher than the control animals. The possible explanation to this could be that Vit E positively contributes to the growth of reproductive tissue.[18] This exogenous Vit E along with normal amounts present in the body possibly has resulted in this surpassing phenomenon.

Table 1: Comparision of mean values (µM) of the diameter of semineferous tubules, HGE and TIS thickness of interstitial spaces between experimental groups and their statistical significance

| Groups                      | A           | B           | C           | P value  |
|-----------------------------|-------------|-------------|-------------|----------|
| HGE                         | 174.29±2.18 | 51.73±3.15  | 103.03±4.37 | 0.0001***|
| Diameter of seminiferous tubules | 369.21±4.39 | 296.86±14.70 | 394.32±13.46 | 0.0001***|
| TIS                         | 9.02±3.17   | 47.05±4.94  | 13.18±2.54  | 0.0001***|

=standard deviation, ***highly significant, TIS: Thickness of interstitial space, HE: Height of the germinal epithelium

This exogenous Vit E along with normal amounts present in the body possibly has resulted in this surpassing phenomenon.

Conclusion

This study has demonstrated a protective potential of Vit E against MPH-induced testicular changes. Significant histo-pathological loss of the seminiferous tubules and spermatogenic cells was observed in animals treated with MPH alone. Simultaneous administration of Vit E along with MPH has shown a mitigating effect on the testicular changes and a significant recovery was possible.

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Conflicts of Interest

There were no conflicts of interest.

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