Original Article

Effect of Ascorbic Acid on Methylphenidate Induced Decreased Spermatogenesis in Albino Rats

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Abstract

Objectives: This study aimed to evaluate the anti-oxidative potential of ascorbic acid on methylphenidate induced decreased spermatogenesis and decreased testosterone levels in male Albino rats.

Methods: This experimental study was performed on twelve-week-old Albino male rats selected through randomized sampling technique divided into three groups. Only healthy male rats of the same age and weighing between 180 to 200 gms were included, and any rat was appearing sick or had less weight than 180gms and aged less than 12 weeks were excluded. Control group A received 2 ml of normal saline for 50 days. Group B was given 2mg/day methylphenidate per oral for 50 days, and Group C received 2mg /day methylphenidate for 50 days, then ascorbic acid 50mg/day for 10 days. After 60 days, rats were dissected, testes were removed and histologically examined for spermatogenesis by Johnson's scoring, and blood samples were collected to evaluate testosterone levels.

Results: On histological examination, the testes of animals of Group B showed a statistically significant decrease in Johnson's score (p-value < 0.001) with disruption of germinal epithelium and absence of mature sperms. These findings were associated with reduced levels of serum testosterone in this group. Administration of Ascorbic acid (AA) to group C showed statistically improved Johnson score and testosterone levels compared to group B.

Conclusion: It has been concluded that administering Ascorbic Acid can improve decreased spermatogenesis and serum testosterone levels induced by methylphenidate.

Keywords: Methylphenidate, spermatogenesis, Johnson's scoring, testosterone, Ascorbic acid, Attention deficit hyperactive disorder.

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Introduction

Testes are a pair of male external reproductive organs having a critical role in spermatogenesis and hormone synthesis and consider to be one of the most sensitive tissues that could be affected by various environmental risk factors[1]. Structural and functional alterations of testicular germ cells, in turn, can influence the spermatogenesis process. One of the drugs showing testicular toxicity is Methylphenidate (MPH). It is a central nervous system stimulant commonly prescribed for increasing attention span in Attention Deficit Hyperactive Disorder (ADHD).

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ADHD is a neuropsychological disorder that can affect both children and adults, and the common presenting symptom is decreased focus and attention[2]. This drug was originally used for several indications, such as fatigue, lethargy, depression (including those caused by sedatives), psychoneurosis and psychosis (associated with depression), geriatric behavioral disorders, narcolepsy. Its mechanism of action is to release the monoamines into the extraneuronal space by blocking the reuptake of norepinephrine and dopamine into the presynaptic neuron. MPH is one of the most common medications that is used for maintaining alertness and improvement of attention which may lead to an increase in the risk of substance abuse in some cases[3]. Although it is safe and effective at standard dose still, there are some known adverse effects on different organs because of its chronic use. A previous study showed that MPH could decrease serum testosterone levels by decreasing the number of Leydig cells[4].

Some relevant literature also showed decreased spermatogenesis due to the formation of reactive oxygen species. It is believed that male germ cells are more vulnerable to oxidative stress than other cells since they have higher polyunsaturated unsaturated fats[5]. Oxidative stress assumes an essential part in the etiology of damaged sperm development, function, sperm count, and male infertility[6]. For this reason, multiple studies used naturally occurring water-soluble ascorbic acid (AA) used to reverse the testicular damage done by oxidative stress[7]. AA is a potent antioxidant, and it scavenges free radicals by donating an electron and, in this way, decreases lipid peroxidation[8]. AA supplement improves the complete differentiation of testicular cells, ultimately increasing the cell range and stimulating sperm cell production. Moreover, AA could effectively improve the integrity of sperm DNA of human patients. Accumulating evidence indicates that AA treatment may benefit the male genital system (organs and cells) at different layers of action. AA could increase the concentration of glutathione (GSH), neutralize the reactive chemical element species (ROS), and regulate the activity of multiple enzymes concerned within the reaction pathway. AA has been found in testes which shows that its adequate concentration is required for protecting this organ from oxidative stress. Therefore, it supports normal spermatogenesis and serum testosterone levels by acting as a co-transmitter in regulating the release of LH, thus playing an important role in male fertility[9,10].

Due to the proven various side effects of methylphenidate on the reproductive system and the non-availability of data regarding its prevention, this study aimed to evaluate the effects of AA on the reproductive system through histo-morphological studies and testosterone levels damaged by MPH.

Subjects and Methods

The following study was performed at the Anatomy department, from 10th May 2017 to 10th July 2017. The accreditation for this research was taken from the Board of Advanced Research Studies (BASR) of the University before this experimental study. Ten to twelve weeks old, 30 male Albino rats about (weighing about 180-200 gms) Sprague Dawley species were taken from the animal house of Aga Khan University and Hospital Karachi with randomized sampling technique. After assessing the health of all Albino rats for one week, healthy animals with the same age and weight between 180-200 gms were considered for the study, and sick rats with weight less than 180gms and age less than 12 weeks were excluded. They were housed in standard cages in the animal house of the University for 60 days in the standard environment required for Albino rats.

MPH of Novartis Company has purchased in the form of tablets 10 mg, and before usage, it was dissolved in 10 ml distilled water, so 1ml solution contained 1 mg MPH. In the form of liquid preparation (cecon drops) of Abbott company, AA was purchased each ml containing100mg of this. Therefore, 0.5 ml was given daily, which made the dose of 50 mg[11]. After 60 days of the experiment, rats were weighed and dissected under anesthesia, blood samples were taken into a dry tube by intracardiac puncture and sent to a laboratory for serum testosterone levels, and both testes were removed completely. They were fixed in 10% formalin of all experimental animals. After processing, tissues were embedded in paraffin and cut into 5-6 micrometer sections on a microtome and stained with H & E (Hematoxylin and Eosin) for Johnson's scoring, which was used for assessing spermatogenesis[12,13]. For this, slides were observed at 40X magnification under the light microscope. Spherical seminiferous tubules were observed in each slide, and the mean was calculated. Each tubule was assigned a score from 1 – 10 using Johnson's scoring, and then the percentage was calculated (Table 1). The tubules with complete lack of activity scored as 1%, while those with maximum spermatogenic activity (5 or >5 spermatozoa) scored 100% and were stained with Periodic Acid Schiff[14]. It was done for mucopolysaccharides of the basement membrane to see the integrity of the basement membrane (Figure 1). Serum testosterone results were analyzed (ANOVA) using software SPSS, version 21.0.

Values were expressed as mean ± SD. $P< 0.05$ was considered statistically significant. There were three groups with 10 Albino rats in each group. Group A was of albino rats given 2 ml normal saline per oral once a day for 50 days. Group B was of albino rats given a single dose of 2 mg methylphenidate hydrochloride orally for 50 days. Group C was of albino rats that

received a single dose of 2 mg methylphenidate orally regularly for 50 days then treated with ascorbic acid (Abbott) 50 mg per oral once a day for ten days.

Results

Group A in PAS stained showed (Figure 1) the basal lamina as a thin layer, showing a strong positive reaction, i.e., magenta color. The Head of the spermatozoa was also stained by PAS and appeared to be facing the Sertoli cells, and all germ cells lineage is visible from spermatogonia to sperm. For group B in PAS-stained sections, there was overall in the PAS reaction reduction in the seminiferous tubules, with no magenta color due to arrest of germinal epithelium. However, basal lamina showed positive PAS reaction and it is observed that the germ cell from spermatogonia till spermatids are visible but reduced. No sperms are present the epithelium is disorganized and disrupted (Figure 1). Group C showed positive PAS reactions that were significant compared to both MPH treated and control groups. It is observed that all cells of germ lineage are visible from spermatogonia to sperms embedded in Sertoli cells (Figure 1).

Johnson's scoring was used to indicate spermatogenesis in groups, which comprises scores from 10 to 1, and results are observed in percentages. In control Group A all stages of spermatogenesis were observed with a score of 10. In Group B (MPH), scores were between 10 to 7. In Group C (MPH + AA), scores were between 10 to 9.

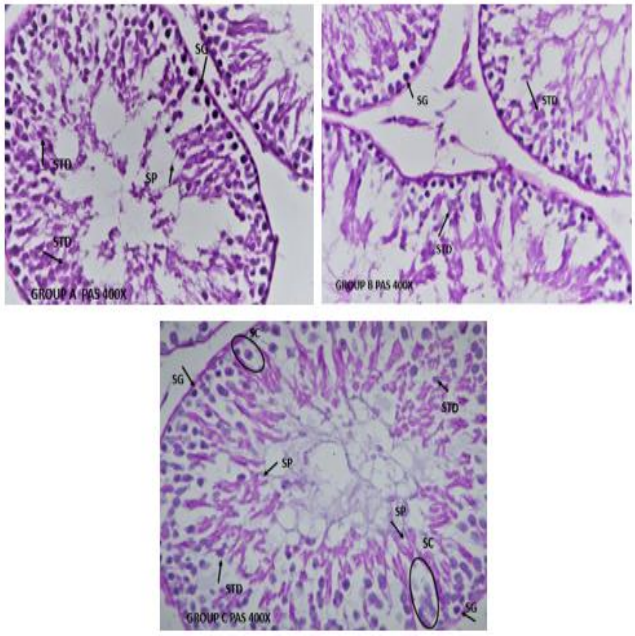

Figure 1: Photomicrograph of a section of seminiferous tubules from group a and experimental groups b and c showing spermatogonia (sg), Sertoli cells (sc), spermatids (std), and sperm (sp) (a comparison) pas 400x

Table 1: Johnson's scoring (percentage %).

Scoring For Spermatogenesis (1 to 10)	A (Control)	B (MPH)	C (MPH+AA)
All stages of spermatogenesis with intact germinal epithelium (score=10)	100%	10%	70%
Spermatozoa present with interrupted germinal epithelium (score=9)	0%	20%	30%
Scant spermatozoa(score=8)	0%	0%	0%
Azospermia>5 spermatids (score=7)	0%	60%	0%
Azospermia but < 5 spermatids (score=6)	0%	0%	0%
Spermatozoa or spermatids absent with Abundant spermatocytes with azoospermia and no spermatids (score=5)	0%	0%	0%
Spermatozoa absent or spermatids <5 spermayocytes (score=4)	0%	0%	0%
Spermatogonia onlypresent(score=3)	0%	10%	0%
Sertoli cells only(score=2)	0%	0%	0%
Tubules section showing no cells(score=1)	0%	0%	0%

Figure 2 summarizes the serum testosterone levels in groups A, B, and C. It showed that methylphenidate treated group B has a lower serum testosterone level (15.37 ng/dl) than control group A (20.26 ng/dl) and Group C (MPH +AA). Group C indicated increased serum testosterone levels (21.80 ng/dl). There was a significant difference among groups (p-value <0.05).

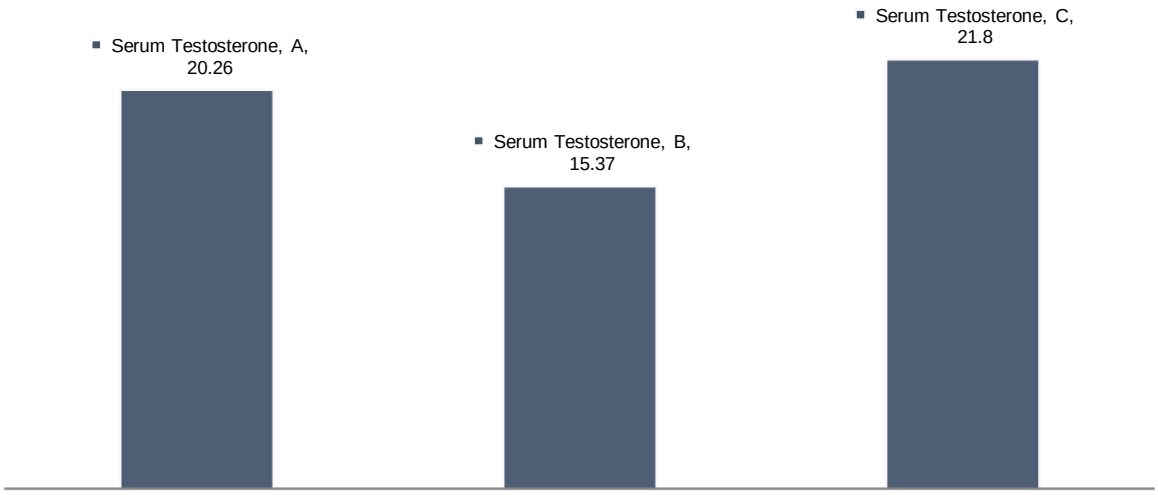

Figure 2: Mean Serum Level of Testosterone Between Control and Experimental Groups (ng/dl).

Discussion

This study was performed on Albino rats to see the effect of ascorbic acid on methylphenidate-induced decreased spermatogenesis. Spermatogenesis is a process in which, under the influence of steroidal hormone, i.e., testosterone, development and maturation of sperm, occur, and this spermatogenesis takes place in seminiferous tubules making the glandular part of the testes[15]. Inspite of the fact that spermatogenesis is always to create spermatozoa, all through the male reproductive life, the apoptosis of germ cell death has been detailed as a physiological phenomenon that guarantees the quality of sperm production[16].

The findings of the study, however, must be cautiously interpreted[17]. This study also showed negative effects on spermatogenesis, as proved by various authors previously[18]. We have observed a significant difference between groups with regard to hormonal and sperm germ cell lineage even with the low dose of 2 mg/kg of body weight of methylphenidate (MPH). As explained earlier, spermatogenesis was assessed by Johnson's scoring, and a score of Group A was 10 in 100% and Group B was 10% scored 10 20% scored 9, and 60% scored 7, so the score of the group was in between 10 to 7, MPH affected negatively on spermatogenesis in Group B by decreasing the number of spermatogonia, this finding was on the contrary with the findings of Zahra Tootian et al., in 2014[19]. Which showed an increase in the number of spermatogonia, and Ali Cansu et al., observed no increase or decrease in the number of spermatogonia. In contrast, observation of a decreased number of spermatids in methylphenidate treated group B in this study was similar to studies conducted by Ali Cansu et al. and Zahra Tootian et al., in 2014, this decrease could be due to increased apoptosis, decreased testosterone levels, and free radicals injury. Considering the explanation mentioned above, we treated Group C rats with ascorbic acid to find out whether the toxicity caused by methylphenidate would be reversed or not[20]. In Group C, 70% scored 10, 30% scored 9, so the score in Group C was between 10 to 9. It was noticed an improvement of spermatogenic lineage within seminiferous tubules confirming that this testicular toxicity was due to free radicals injury and AA being potential antioxidant restored testicular activity. Our finding was consistent with Das et al., in 2002[21] and M Sonmenz in 2005[22]. It is a known fact that testosterone plays an important role in spermatogenesis. It is required to convert round spermatids into elongated spermatids. It is produced

by Leydig cells and regulated by LH. In our study Group B (MPH only) showed a noticeable reduction in levels of serum testosterone with comparison to Group A and Group C (MPH + AA), which showed that the decrease in spermatogonia and spermatids could also be the result of a decrease in levels of testosterone, and this decrease could be the result of scattering and reduced Leydig count as explained by Walter Adriani et al., in 2006[23] and Siminfazelipour in 2012. On the other hand, this finding was contrary to the findings of Bruno et al., which showed no changes in serum testosterone levels[19]. As it is observed that Group C showed significantly increased serum testosterone compared with Group B and Group A, these findings were in approval with the findings of Biswas NM et al.[24], and this could be due to not only the antioxidant effect of AA but also it is acting as a transmitter that can cause the release of Luteinizing Hormone (LH) from the pituitary gland as it is presumed that it is stored in granules and co releases by exocytosis[25].

Conclusion

Ascorbic acid can ameliorate methylphenidate induced decreased spermatogenesis and decreased serum testosterone levels by releasing LH from the pituitary gland, which leads to increased testosterone from Leydig cells and also by scavenging free radicals.

Conflict of Interest

Authors have no conflict of interest and no grant/funding from any organization.

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