

Guarding Gonadal Health: Ascorbic Acid Attenuates Methylphenidate-Induced Toxicity in Rats

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ABSTRACT

Background: Methylphenidate (MPH), a prevalent treatment for Attention Deficit Hyperactivity Disorder (ADHD), offers therapeutic benefits but raises concerns about its impact on testicular health, potentially leading to reproductive complications. Ascorbic acid (Vitamin C) is a potent antioxidant renowned for its capacity to combat oxidative stress and enhance cellular resilience. This study aimed to evaluate the effects of ascorbic acid on methylphenidate-induced testicular toxicity in Albino rats by assessing histopathological changes in the testes.

Methods: Thirty Albino male rats were randomly assigned to three groups, each containing ten rats. The control group (Group A) received normal saline for 50 days. The two experimental groups received treatments as follows: Group B received 2 mg of MPH orally for 50 days, and Group C received 2 mg of MPH orally for 50 days, followed by 50 mg of Ascorbic acid orally for an additional 10 days. Testicular histopathology was performed 60 days after the procedure.

Results: Histological analysis revealed a significant reduction in germinal epithelium height in the MPH group ($57.52 \pm 5.59 \mu\text{m}$) compared with controls ($175.76 \pm 5.21 \mu\text{m}$). There was a partial recovery when treated with ascorbic acid ($107.22 \pm 8.47 \mu\text{m}$). Similarly, the seminiferous tubule diameter was decreased in the MPH group ($295.64 \pm 36.31 \mu\text{m}$) as compared to controls ($370.71 \pm 4.87 \mu\text{m}$), which was increased after ascorbic acid treatment ($401.83 \pm 11.87 \mu\text{m}$). Interstitial space thickness significantly increased after MPH exposure ($46.46 \pm 6.19 \mu\text{m}$) relative to controls ($8.08 \pm 4.25 \mu\text{m}$), which was reduced after ascorbic acid treatment ($14.94 \pm 3.71 \mu\text{m}$).

Conclusion: These findings suggest that ascorbic acid may effectively mitigate testicular toxicity associated with methylphenidate, preserving spermatogenesis and supporting overall reproductive health.

Keywords: Methylphenidate; Ascorbic Acid; Testicular Diseases; Oxidative Stress; Antioxidants; Attention Deficit Hyperactivity Disorder

INTRODUCTION

The cases of Psychiatric disorders are rising among children nowadays. Attention Deficit Hyperactivity Disorder (ADHD) is common and persists in adults as well.¹ The most common features observed in this disorder include lack of attention, indiscretion, and uncontrolled behavior.² The predominance for ADHD in Pakistan is found to make around 18.8%, the incidence of ADHD is increasing drastically, most commonly affecting young males, but ratio between boys and girls varies significantly from 2:1 to 9:1.^{3,4} The first-line of treatment ADHD, is methylphenidate

(MPH), acts as a CNS stimulant by inhibiting the reuptake of norepinephrine and dopamine, thereby increasing their release.^{5,6}

Despite its medical use, MPH is commonly misused by students in Pakistan as a cognitive enhancer without prescription, raising concerns about its off-label effects on various organ systems.^{7,8} A previous study showed that MPH decreases the number of Leydig cells and lowers serum testosterone levels, thereby affecting normal stages of spermatogenesis and testicular morphometry, which can lead to infertility.⁹ The utilization of natural supplements as reproductive-protective therapy has gained popularity recently. Ascorbic acid is a water-soluble vitamin; supplementation has been shown to increase sperm concentration, total sperm output, and improve testicular and erectile functions in both healthy and reproductive-compromised individuals.¹⁰

Various studies have proved that ascorbic acid has been utilized as a part of the management of male infertility on experimental grounds, particularly in conditions with oxidative stress. Given that MPH administration has been associated with increased oxidative stress and testicular toxicity in experimental models, ascorbic acid may mitigate these adverse effects

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by reducing reactive oxygen species (ROS) levels and protecting testicular tissue from oxidative damage.¹¹ In this context, the present study focused on exogenous supplementation with ascorbic acid in MPH-treated rats with affected morphometry of testes to ameliorate its effect.

SUBJECTS AND METHODS

This experimental study was conducted in the Department of Anatomy and the Animal House of Baqai Medical University (BMU), Karachi, Pakistan, with approval from the Ethical Review Committee. Thirty male Albino rats of the Sprague-Dawley strain, about 10 to 12 weeks old (180-200 g), were selected after 10 days of observation and assessment of their health status, based on feeding, elimination, and weight gain or loss. Weights were recorded at the beginning of the study. They were kept in a well-ventilated animal house at Baqai Medical University for 60 days.

After an acclimation period, 30 healthy adult male Albino rats were randomly assigned to three groups (n = 10 per group). The rats were housed in plastic cages with a 12-hour light/dark photo-period and had access to rat pellets and water ad libitum. The control group A received normal saline by mouth. Methylphenidate was purchased from the local pharmacy and administered daily at a dose of 2 mg/kg to groups B and C for 50 days per oral, while group C additionally received AA at a dose of 50 mg/day for 10 days per oral. Animals were sacrificed at day 60. In this study, the rats were anesthetized with isoflurane and dissected to remove the testes for tissue analysis. The testes were fixed in Bouin's solution for 48 hours, sectioned at 5 μ m, and stained with Hematoxylin and Eosin (H&E) for microscopic examination. Key measurements included:¹² Seminiferous tubule diameter (measured using a micrometer at 10X magnification, with two perpendicular diameters (D1 and D2) averaged), interstitial connective tissue thickness (measured at the narrowest point between seminiferous tubules using an ocular micrometer), and germinal epithelium height (measured at four points in each tubular cross-section, and the height of seminiferous epithelium was estimated from the basal membrane to the lumen). Two sections per animal (from the left and right testis) were analyzed.¹³ The study adhered to ethical guidelines for animal care and tissue disposal.

RESULTS

The present study was designed to assess the effects of MPH and its treatment with ascorbic acid on the histomorphometry of the testes of Albino rats. The observations of animals (during the experimental study, before and after sacrifice) and microscopic parameters were recorded. In Figure 1, the histological examination of

testicular sections in the control group (Group A) revealed that the seminiferous tubules were round or oval, with a basal lamina and stratified cuboidal germinal epithelium consisting of spermatogonia, primary and secondary spermatocytes, spermatids, and Sertoli cells. The seminiferous tubules were well-structured, with no vacuolization or detachment of the basement membrane. Interstitial spaces contained Leydig cells and small blood vessels. The MPH-only-treated group (Group B) revealed that the seminiferous tubules exhibited significant vacuolization, deformation of Sertoli cells (detached from the basal lamina), and a reduction in germ cells. Germinal epithelium was disorganized, and there was thinning, sloughing of germ cells, and pyknotic nuclei. This led to dilated interstitial spaces, few Leydig cells, and tubular atrophy. Spermatogenesis was arrested at the rounded spermatid stage, with the lumina of affected tubules containing sloughed germ cells. Finally, the MPH + Ascorbic Acid-treated group (Group C) showed seminiferous tubules that appeared nearly round, similar to those in the control group, with spermatogonia and elongated spermatids in the lumen. Vacuolization decreased, and the interstitial spaces were more compact, with a higher number of Leydig cells than in Group B, though still slightly fewer than in Group A. The thickness of the interstitial spaces was reduced, and no congested blood vessels were observed.

In summary, MPH treatment (Group B) caused significant damage to testicular structure, impairing spermatogenesis and reducing Leydig cell numbers. In contrast, the addition of ascorbic acid (Group C) partially reversed these effects, improving testicular morphology and increasing Leydig cell numbers. The Histo-morphometric parameters of the testes were analyzed quantitatively using SPSS, and p-values ≤ 0.05 were considered significant and summarized in Table 1.

DISCUSSION

The present study determined the histomorphometric and histopathological effects of methylphenidate hydrochloride (MPH) on the testes of Albino rats, focusing particularly on the potential mitigating effects of ascorbic acid. The findings revealed significant alterations in testicular morphology associated with MPH treatment and promising signs of recovery following ascorbic acid administration. While MPH is effective in improving attention and reducing hyperactivity, concerns have emerged regarding its impact on male reproductive health. Previous studies, including those in male mice, have shown that MPH can reduce fertility, as evidenced by alterations in sperm parameters and testicular structure.¹⁴ In this study, Group B (MPH-treated) exhibited significant histopathological changes, including increased vacuolization of seminiferous tubules, disorganization of

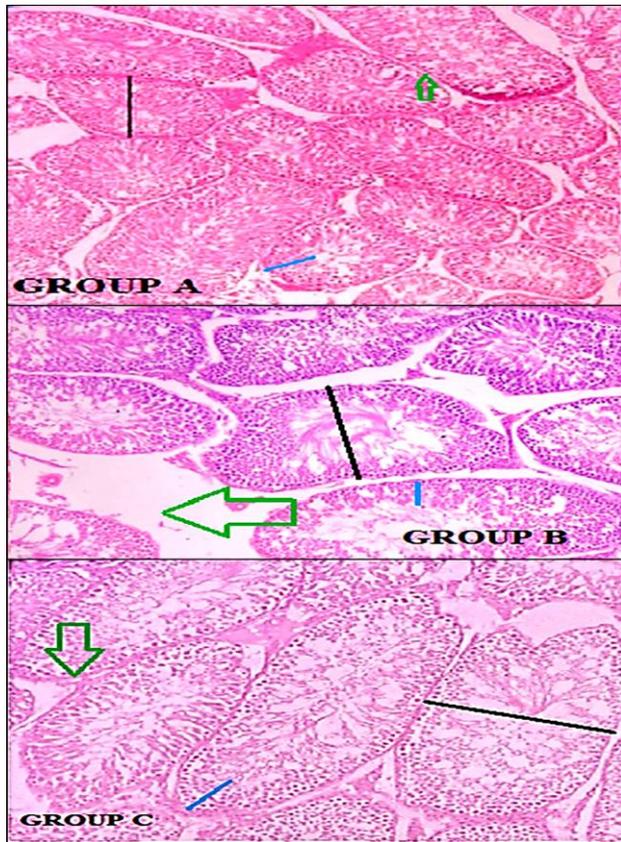


Figure 1: Histological sections of testes from three groups at different magnifications: Group A (Control) at 4X magnification shows normal architecture with well-defined seminiferous tubules and interstitial tissue. Group B at 20X magnification demonstrates seminiferous tubules with potential morphological changes, including disrupted cellular organization and degeneration of spermatogenic cells. Group C at 20X magnification demonstrates seminiferous tubules showing reversible distortion, with partial restoration of cellular organization and spermatogenic cells, indicating potential recovery from previous morphological changes. The blue line shows the height of the germinal epithelium, the black line shows the diameter of the seminiferous epithelium, and the green arrow shows towards the interstitial space.

the germinal epithelium, and a notable reduction in germ cell populations. The presence of pyknotic nuclei and luminal debris suggests severe cellular stress and apoptosis. These findings align with the existing literature, which indicates that MPH may impair spermatogenesis by affecting germ cell viability and disrupting normal developmental processes.¹⁵

Quantitative assessments (Table 1) revealed significant reductions in the height of the germinal epithelium and the diameter of seminiferous tubules in Group B compared to the control group. The decrease in tubular diameter is particularly concerning, as it reflects compromised testicular function and reduced spermatogenic capacity. This observation corroborates findings from previous research, which highlighted the detrimental effects of MPH on seminiferous tubule structure and function.¹⁶ Conversely, the administration of ascorbic acid in Group C resulted in a marked recovery in both the height of the germinal epithelium and the diameter of seminiferous tubules. These results suggest that ascorbic acid may possess protective properties against MPH-induced testicular damage, potentially mitigating cellular injury in the testes, and its protective role against various drugs' adverse effects on the testes is well documented.^{17,18}

In addition to changes in the seminiferous tubules, significant alterations were observed in the interstitial spaces within the testes. Group B demonstrated a pronounced increase in the thickness of interstitial connective tissue, likely in response to testicular stress. Leydig cells are critical for testosterone production, and their reduction could have profound implications for male fertility. In Group C, the recovery of interstitial space thickness and Leydig cell numbers after ascorbic acid treatment suggests that it may help to restore normal testicular architecture and function. The study highlights the potential long-term impact of MPH on male reproductive health, showing structural and functional impairments in the testes that could affect fertility. The results of this study highlight the potential for Ascorbic acid to serve as a therapeutic agent in mitigating the adverse effects of MPH on testicular histomorphology and function, which is in line with the published study showing its potential effect on reversing the adverse effects caused by various drugs and metals, and also alleviating the quality of sperm by promoting meiosis.¹⁹ Recent studies have also highlighted that ascorbic acid is responsible for preserving central nervous system function via multiple molecular pathways, which could be a beneficial or alternative treatment for ADHD.²⁰ Future research should explore the mechanisms by which ascorbic acid protects and optimize its dosing and timing. Longitudinal studies are needed to

Table 1: Comparison between the heights of germinal epithelium, the diameter of seminiferous tubules, and the thickness of interstitial space in distinct groups of rats among different treatment groups.

Parameters	Group A (Control)	Group B (MPH 2 mg/kg/day)	Group C (MPH 2 mg/kg/day + ascorbic acid 50 mg)	p-value*
Height of germinal epithelium (μm , [mean + SD])	175.76 \pm 5.21	57.52 \pm 5.59	107.22 \pm 8.47	0.001
Diameter of seminiferous tubules (μm , [mean + SD])	370.71 \pm 4.87	295.64 \pm 36.31	401.83 \pm 11.87	0.001
Thickness of interstitial spaces (μm , [mean + SD])	8.08 \pm 4.25	46.46 \pm 6.19	14.94 \pm 3.71	0.001

*A p-value less than 0.05 was taken as significant

assess MPH's long-term effects on male reproductive health and the role of ascorbic acid in preserving fertility.

The study's limitations include a small sample size and limited applicability to humans due to species differences. Future research should increase sample sizes, conduct human trials, explore molecular mechanisms, and use advanced models, such as organ-on-chip systems, to improve the relevance and validity of the findings.

CONCLUSION

MPH remains an important medication for managing ADHD; it is crucial to remain vigilant about its potential reproductive side effects. The findings from this study advocate for a balanced approach to treatment, considering both the psychological benefits of MPH and the potential risks to male fertility, with an eye toward protective strategies that can help mitigate these risks.

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