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Original Article

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[1] 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%.[5] 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[18] 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 conjuvant 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 of seminiferous tubules with profiles that were round or nearly round were estimated for each animal and the mean ± standard deviation (SD) of the diameter was determined by taking the average of two diameters, D1 and D2 (perpendicular to one another)[28]
3. Thickness of interstitial spaces (TIS) width of the interstitial tissue was measured at the narrowest point between two seminiferous tubules.

These parameters were calibrated using manual micrometry.[17]

Statistical analysis

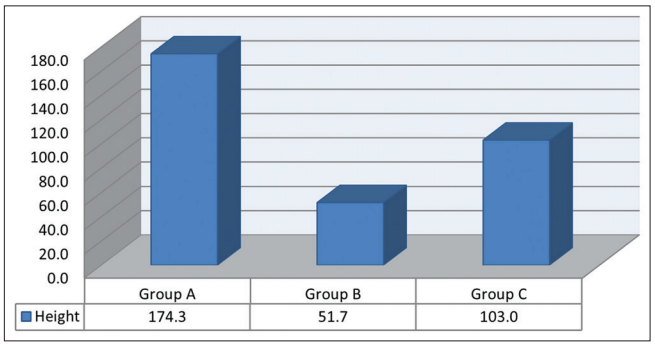
The data were analyzed (ANOVA) using software SPSS, version 21. Values were expressed as mean ± SD. $P < 0.05$ was considered statistically significant.

Results

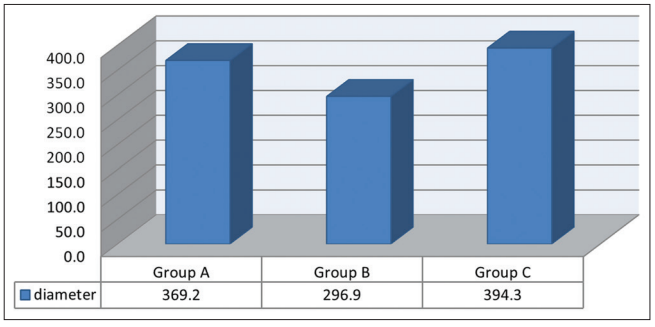
Morphometric finding

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].

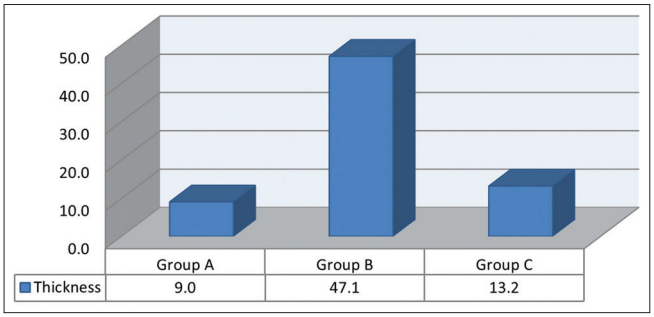
Mean values of DST, HGE, and TIS of the Group B animals were 296.86 ± 14.70 µm, 51.73 ± 3.15 µm, and 47.05 ± 4.94 µm, respectively. These mean values were statistically significant lower than Groups A and C animals [Graph 1-3].



Graph 1: Comparison of mean values of the height of germinal epithelium (um) between groups



Graph 2: Comparison of mean values of the diameter of seminiferous tubule (um) between groups



Graph 3: Comparison of mean values of the thickness of interstitial spaces (um) between groups

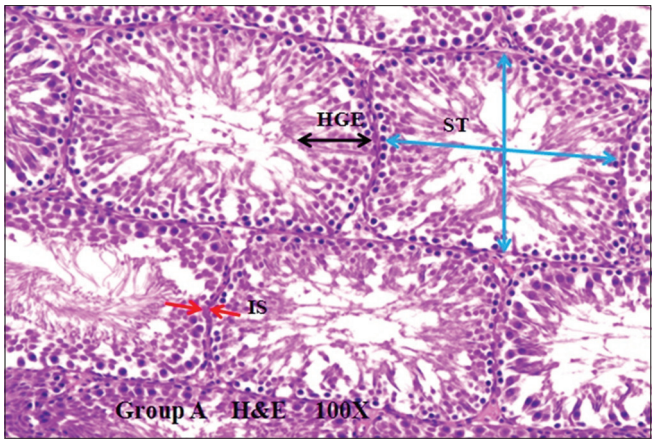


Figure 1: Group A. 5 u thick section of testis, stained with hematoxylin and eosin × 100, showing normal histomorphological diameter of seminiferous tubules (blue arrow), interstitial spaces (red arrow), and height of germinal epithelium (black arrow)

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

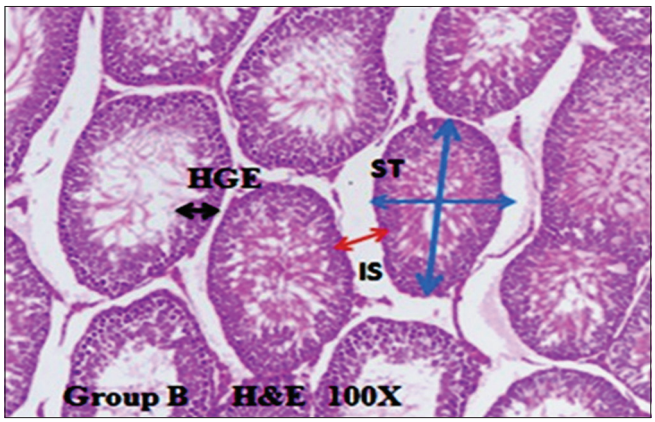


Figure 2: Group B. 5 u thick section of testis, stained with hematoxylin and eosin × 100, showing reduced diameter of semineferous tubule (blue arrow), and height of germinal epithelium (black arrow) with widening of interstitial spaces (red arrow)

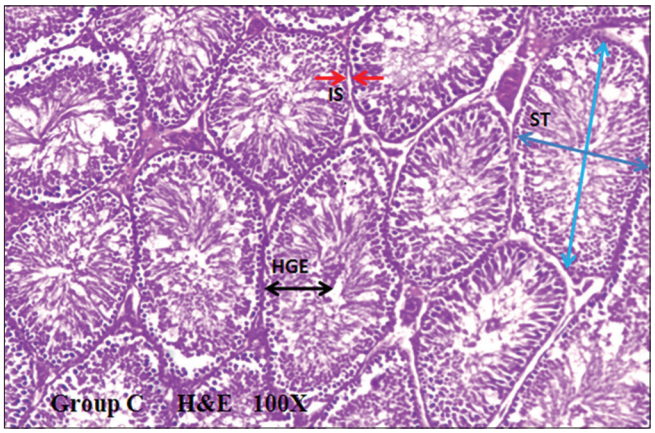


Figure 3: Group C. 5 u thick section of testis, stained with hematoxylin and eosin × 100, showing an increase in seminiferous tubules diameter (blue arrow) and height of germinal epithelium (black arrow) with narrowing of interstitial spaces (red arrow)

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.[29,26] In contrast to that, no change in diameter has also been reported.[10] 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.[30]

Maintenance of germinal epithelium in males is a testosterone function which regulates the germinal cells division[31] and thereby maintains the stratification and HGE. MPH administration in this study has shown a partial loss of

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

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.[32] Apoptotic loss of developing germinal cells has also been suggested as a leading factor in the loss of HGE.[10] 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 protoncogene 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.[3] 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.[16] Vit E, naturally accumulates in the membranes of mitochondria and endoplasmic reticulum and protects testicular cells from lipid peroxidation.[34] 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.

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.

References

1. Swanson JM, Volkow ND, Newcorn J, Casey BJ, Moyzis R, Grandy D, *et al*. Attention deficit hyperactivity disorder. In: Encyclopedia of Cognitive Science. London: Nature Publishing Group; 2006.
2. Faraone SV, Larsson H. Genetics of attention deficit hyperactivity disorder. Mol Psychiatry 2019;24:562-75.
3. Castells X, Blanco-Silvente L, Cunill R. Amphetamines for attention deficit hyperactivity disorder (ADHD) in adults. Cochrane Database Syst Rev 2018;8:CD007813.
4. Schweitzer JB, Cummins TK, Kant CA. Attention-deficit hyperactivity disorder. Med Clin North Am 2001;85:757-7.
5. Malik TA, Rooney M, Chronis-Tuscano A, Tariq N. Preliminary efficacy of a behavioral parent training program for children with ADHD in Pakistan. J Atten Disord 2017;21:390-404.
6. Mirza NM, Nisar N, Ikram Z. Knowledge, attitude and practices towards attention deficit hyperactivity disorder among private elementary school teachers of Karachi, Pakistan. J Dow Univ Health Sci 2017;11:11-7.
7. Du Plooy C, Coetzee H, Van Rensburg E. Psychological effects of multimedia-induced sexualisation of girls in middle childhood: A systematic literature review. J Child Adolesc Ment Health 2018;30:67-85.
8. Etiology of ADHD. Available from: https://www.lumhs.edu.pk/jlumhs/Vol04No01/pdfs/v4n1c01.pdf. [Last accessed on 2016 Nov 02].
9. Angyal N, Horvath EZ, Tarnok Z, Richman MJ, Bognar E, Lakatos K, *et al*. Association analysis of norepinephrine transporter polymorphisms and methylphenidate response in ADHD patients. Prog Neuropsychopharmacol Biol Psychiatry 2018;84:122-8.
10. Fazelipour S, Tootian Z, Saremi ZG, Shafii M, Sheibani MT, Kiaei SB, *et al*. Evaluation of histopathologic and histomorphometric changes of testicular tissue and gonadotropin levels following consumption of methylphenidate in male mice. Turk J Med Sci 2014;44:554-9.
11. Pride NA, Barton B, Hutchins P, Coghill DR, Korgaonkar MS,

Hearps SJ, *et al*. Effects of methylphenidate on cognition and behaviour in children with neurofibromatosis Type 1: A study protocol for a randomised placebo-controlled crossover trial. BMJ Open 2018;8:e021800.

12. Mehta MA, Owen AM, Sahakian BJ. Methylphenidate enhances working memory by modulating discrete frontal and parietal lobe regions in the human brain. Year B Psychiatry Appl Ment Health 2002;2002:87-8.
13. Ruocco LA, Treno C, Carnevale UA, Arra C, Mattern C, Huston JP, *et al*. Prepuberal intranasal dopamine treatment in an animal model of ADHD improves spatial attention, working memory, amino acid transmitters and synaptic markers in prefrontal cortex, ventral and dorsal striatum. Amino Acids 2014;46:2105-2.
14. StorebÃ OJ, Pedersen N, Ramstad E, Kielsholm ML, Nielsen SS, Krogh HB, *et al*. Methylphenidate for attention deficit hyperactivity disorder (ADHD) in children and adolescentsâ€ assessment of adverse events in nonâ€-randomised studies. Cochrane Database Syst Rev 2018;2018:CD012069.
15. Agarwal A, Virk G, Ong C, Du Plessis SS. Effect of oxidative stress on male reproduction. World J Mens Health 2017;32:1-7.
16. González CR, González B, Matzkin ME, Muñiz JA, Cadet JL, Garcia-Rill E, *et al*. Psychostimulant-induced testicular toxicity in mice: Evidence of cocaine and caffeine effects on the local dopaminergic system. PLoS One 2015;10:e0142713.
17. Mehranjani MS, Taefi R. The protective role of Vitamin E on the testicular tissue in rats exposed to sodium arsenite during the prenatal stage till sex maturity: A stereological analysis. Iran J Reprod Med 2012;10:571.
18. Oyeyemi WA, Shittu ST, Kolawole TA, Ubanecheand P, Akinola AO. Protective effect of Vitamin E on nicotine induced reproductive toxicity in male rats. Niger J Basic Appl Sci 2015;23:7-13.
19. Jalili T, Khaki A, Ghanbari Z, Imani AM, Hatefi F. A study of the therapeutic effects of Vitamin E on testicular tissue damage caused by fluoxetine. Crescent J Med Biol Sci 2014;1:37-41.
20. Mehranjani MS, Noorafshan A, Momeni HR, Abnosi MH, Mahmoodi M, Anvari M, *et al*. Stereological study of the effects of Vitamin E on testis structure in rats treated with para-nonylphenol. Asian J Androl 2009;11:508.
21. Fulia A, Chauhan PK, Sharma RK. Ameliorating effect of Vitamin E on testicular toxicity induced by endosulphan in *Capra hircus in vitro*. J Pharmacol Toxicol 2011;6:133-40.
22. Arria AM, Wish ED. Nonmedical use of prescription stimulants among students. Pediatr Ann 2006;35:565-71.
23. Imran N. Attention deficit hyperactivity syndrome: An update on assessment and management. Pak J Med Sci 2007;23:9.
24. Ourique GM, Saccol EM, PÃªs TS, Glanzner WG, Schiefelbein SH, Woehl VM, *et al*. Protective effect of Vitamin E on sperm motility and oxidative stress in valproic acid treated rats. Food Chem Toxicol 2016;95:159-67.
25. Astiz M, de Catalfo GE, GarcÃa MN, Galletti SM, Errecalde AL, de Alaniz MJ, *et al*. Pesticide-induced decrease in rat testicular steroidogenesis is differentially prevented by lipoate and tocopherol. Ecotoxicol Environ Saf 2013;91:129-38.
26. Fazelipour S, Jahromy MH, Tootian Z, Kiaei SB, Sheibani MT, Talaee N. The effect of chronic administration of methylphenidate on morphometric parameters of testes and fertility in male mice. J Reprod Infertil 2012;13:232-6.
27. Cansu A, Ekinci O, Ekinci O, Serdaroglu A, Erdogan D, Coskun ZK, *et al*. Methylphenidate has dose-dependent negative effects on rat spermatogenesis: Decreased round spermatids and testicular weight and increased p53 expression and apoptosis. Hum Exp Toxicol 2011;30:1592-600.
28. Saalu LC, Akunna GG, Oyewopo AO. The histo-morphometric evidences of *Vernonia amygdalina* leaf extract-induced testicular toxicity. Int J Morphol 2013;31:662-7.
29. Montagnini BG, Silva LS, dos Santos AH, Anselmo-Franci JA, Fernandes GS, Sde FM, *et al*. Effects of repeated administration of methylphenidate on reproductive parameters in male rats. Physiol Behav 2014;133:122-9.
30. Taghva M, Toutian Z, Fazelipour S. Effects of formaldehyde on morphometric structure of testis in BALB C mice. Med Sci J Islam Azad Univ Tehran Med Branch 2007;17:91-3.
31. Denk AG, Kempenaers B. Testosterone and testes size in mallards (*Anas platyrhynchos*). J Ornithol 2006;147:436-40.
32. Chatterjee-Chakrabarty S, Miller BT, Collins TJ, Nagamani M. Adverse effects of methylphenidate on the reproductive axis of adolescent female rats. Fertil Steril 2005;84:1131-8.
33. Adriani W, Leo D, Guarino M, Natoli A, Di Consiglio E, De Angelis G, *et al*. Shortâ€-term effects of adolescent methylphenidate exposure on brain striatal gene expression and sexual endocrine parameters in male rats. Ann N Y Acad Sci 2006;1074:52-73.
34. Gavazza M, CatalÃ A. The effect of Î±tocopherol on the lipid peroxidation of mitochondria and microsomes obtained from rat liver and testis. Mol Cell Biochem 2001;225:121-8.
35. Balakumar BS, Ramanathan K, Kumaresan S, Suresh R. DNA damage by sodium arsenite in experimental rats: Ameliorative effects of antioxidant Vitamins C and E. Indian J Sci Technol 2010;3:322-7.