Original Article

Histopathological and Histomorphometric Studies on the Effects of Olanzapine on Testis: An Experimental Study in Albino Rats

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Abstract

Introduction: Toxic effects of an antipsychotic drug on male gonad were explored experimentally. Olanzapine, an antipsychotic drug, has affinity for a wide range of receptors posing danger to the large variety of organs. Since olanzapine can bind to these receptors, a direct effect of these drugs on spermatogenesis is possible. Therefore testis, an organ least attended, has been selected in the present study. **Material and Methods:** Two groups of animals of the equal number were designed in such a way that the first group acted as control and second group acted as experimental. After histopathological comparison in the tissue of the testis, histomorphometric analysis was also performed to support the former. Twelve albino male rats were divided into two groups of six animals each. Olanzapine was injected in experimental rats in a dose of 4 mg/kg body weight intraperitoneally for 6 weeks. Sections of both experimental and control testis were examined under light microscope for histopathological findings. Histomorphometric informations were also collected to support the former findings by using ocular and stage micrometers. Student's *t*-test was used to compare the control testis findings with the single variable experimental one. **Results:** Experimental testis showed intra and interlobular edema, compressed seminiferous tubules, thinning and fragmentation of basement membrane. Spermatogenic cells were smudgy and compressed by edema fluid. Blood vessels showed inflammatory cells in the lumen. **Discussion and Conclusion:** Olanzapine-induced testicular degeneration might lead to reduced libido, a fact of great social impact.

Keywords: *Albino rats, olanzapine, testis*

Introduction

Atypical antipsychotic agents are favored over traditional ones because of their lower incidence of extrapyramidal side effects, greater efficacy in improving negative systems of schizophrenia, and effectiveness in treating schizophrenic patients not responding to conventional neuroleptics.[1] However, side effects continue to pose a challenge to effective treatment.[2]

Olanzapine is a widely used atypical antipsychotic agent, approved by the U.S. Food and Drug Administration for bipolar disorder and schizophrenia.[3] It has a pharmacological profile very similar to that of clozapine.[4] It exhibits very high affinity for the H1 histamine receptors and 5-hydroxytryptamine (5-HT) 2A and 5-HT 2C receptors in human brain tissue.[5] It also shows affinity for D2 receptors, muscarinic and alpha 1 receptors with lower affinity for alpha 2, 5-HT 1D, and 5-HT1A receptors.[5] Such affinity poses danger to a wide range of organs in the body.

Soliman *et al*.[6] have done extensive study to show the effects of olanzapine on seminiferous tubules of adult male albino rats. Epithelial desquamation, vacuolization of Sertoli cells, apoptotic changes in germ cells, accumulation of lipid droplets, and dilatation of smooth endoplasmic retinaculum were histopathological findings by aforementioned scientists. Interestingly, they also did histomorphometric observations and found reduction of seminiferous tubule's diameter and epithelial height.

Few studies address sexual dysfunction associated with antipsychotic use, however, biological parameter like serum prolactin level was not included in them.[7-9] According to some authors, an increased level of prolactin resulting from olanzapine use[10-12] can lead to sexual dysfunction.[13-15]

Prolactin increase can induce hypogonadism because of inhibition

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of follicular-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and gonadotropin-releasing hormone (GnRH) which results in morphological changes in testis as well as a delay in spermatogenesis.[16] Others observed a reduction in plasma levels of testosterone in rats treated with olanzapine.[17] Several studies have shown that testosterone and FSH are important for the qualitative and quantitative maintenance of spermatogenesis[18,19] Konarzewska *et al*.[11] found that olanzapine decreases inhibin B level below normal, indicating Sertoli cell dysfunction. Serum inhibin B levels are positively correlated with sperm counts and testicular volume. In infertile patients, inhibin B decreases and FSH increases. There is a very good correlation with the degree of spermatogenetic damage and inhibin B levels.[20]

It has been shown in rats that dopamine receptors are present in germ cells of seminiferous tubules.[21,22] Considering that olanzapine can bind to these receptors, a direct effect of these drugs on spermatogenesis is possible.

Detailed histopathological and histomorphometric studies on testis are lacking in the literature. Such informations are directly related to the affinity of the drug to the testis, which might throw light in finding the mechanism of action.

Material and Methods

Twelve male albino rats (*Rattus norwegicus*) weighing around 180–200 g were divided into the equal number of experimental and control groups, i.e., six each. Rats have ready access to water *ad libitum* and standard pellet laboratory diet (Lipton India Ltd.). Olanzapine (inj. Oleanz, Sun Pharmaceuticals, Mumbai, India) was injected daily intraperitoneally in experimental rats at a dose of 4 mg/kg for 6 weeks. Control group received the same volume of normal saline, daily, intraperitoneally for the same period. After proposed experimental duration of 6 weeks exposure, the animals of both the experimental and control groups were anesthetized by giving injection Nembutal (30 mg/kg), intraperitoneally. The heart was exposed by thoracotomy. The needle of the blood transfusion set was introduced into the left ventricle (apex), and a nick was made in the right atrium. After saline wash, Karnovsky's fixative was infused till the body showed signs of fixation.

A longitudinal incision was given in the ventral wall of the scrotum to the right side of midline. The right half of scrotal sac was opened and testis dissected out. Similarly, a second longitudinal incision was given in ventral wall of the scrotum to the left of the midline and left testis was dissected out in the scrotal sac. Tissue was processed by the wax embedding technique. 10-µm thick section of both experimental and control testis were stained with hematoxylin and eosin.

Thorough histopathological observations and histomorphometry were done in the testicular tissue. The diameter of seminiferous tubules and thickness of tunica albuginea were measured during histomorphometry using ocular and stage micrometers. The latter was analyzed using Student's *t*-test.

This study was approved by IAEC meeting at JNMCH, AMU Aligarh, Uttar Pradesh, India.

Results

Photomicrograph of control testis shows seminiferous tubules with all stages of spermatogenesis and clusters of mature sperms in center of tubules [Figure 1]. Small groups of Leydig cells in intertubular connective tissue and all the stages of spermatogenesis and spermiogenesis are clearly visible [Figure 2]. Spermatogonia of Type A with small dark nuclei and clear cytoplasm and Type B with larger nuclei and more cytoplasm are visible. Mature sperms in the center of seminiferous tubules show sperm head with the blue nucleus and eosinophilic tail. Triangular Sertoli cells attached to basement membrane show clear cytoplasm and single oval nucleus with one nucleolus.

Experimental testis shows intra and intertubular edema. Tubules are compressed and separated by edema fluid [Figure 3] and show edematous swelling, focal thinning, and fragmentation of the basement membrane. Blood vessels show inflammatory cells in the lumen. The spermatogenic cells are also small in size with very little cytoplasm and separated by fluid [Figure 4]. Spermatogonia, spermatocytes, sperms and Sertoli cells are smudgy and compressed by edema fluid. Leydig cells in intertubular connective tissue are also compressed as elongated groups with highly eosinophilic cytoplasm and dark nuclei. Intratubular edema is causing disarray of the outer layer of cells consisting of spermatogonia, primary spermatocytes, and clumping of sperms in the center. Blood vessel contains a thrombus in its lumen made of fibrin, red blood cells, and white blood cells. Inflammatory cells are also seen in interstitial space [Figure 5].

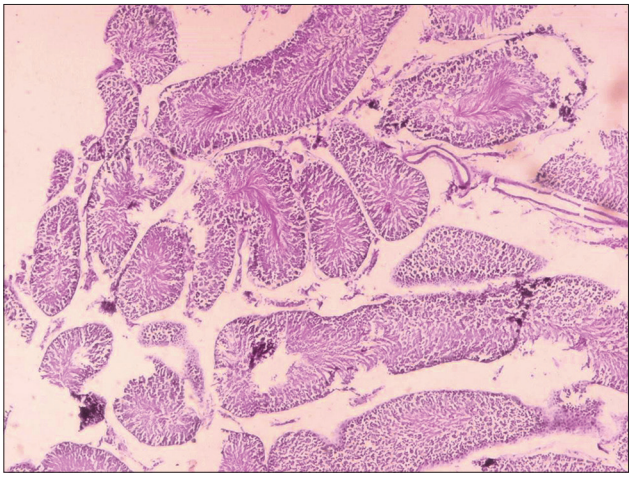

Figure 1: Photomicrograph of control testis: Section shows seminiferous tubules with all stages of spermatogenesis and clusters of mature sperms in center of tubules, H and E, ×4

Histomorphometric findings

Mean thickness of seminiferous tubules was reduced from 259.79 to 219.60 µm and this reduction was found to be highly significant ($P < 0.001$). Mean thickness of tunica albuginea was increased from 21.43 to 58.62 µm, and this increment was highly significant ($P < 0.001$). [Table 1]

Discussion

Edema is found to be a generalized feature in experimental testis leading to compression and disruption of both inter- and intra-seminiferous tubular cells, i.e., the Leydig cells and the cells undergoing spermatogenesis respectively. This is in conformity with our histomorphometric findings showing increase in the thickness of tunica albuginea and decrease in the diameter of seminiferous tubules. Degenerative changes are shown in the basement membrane of tubules in the form of thickening and fragmentation also seem to be due to edema. Soliman *et al*.[6] thoroughly explored seminiferous tubules at the ultramicroscopic level and confirmed our results. He found that olanzapine treated rats presented wide intercellular spaces and wavy thick basement membrane, which was discontinuous in some areas. They realized shrunken spermatogenic cells as well as irregularly fissured nuclei of the Sertoli cells and noticed wide intercellular spaces with cellular debris. The same scientists found shrunken spermatogonia with small heterochromatic nuclei. We also observed the spermatogenic cells very small in size with little cytoplasm. No one has earlier reported the effect of olanzapine on Leydig cells, which is responsible for the secretion of testosterone directly related to male libido. Therefore

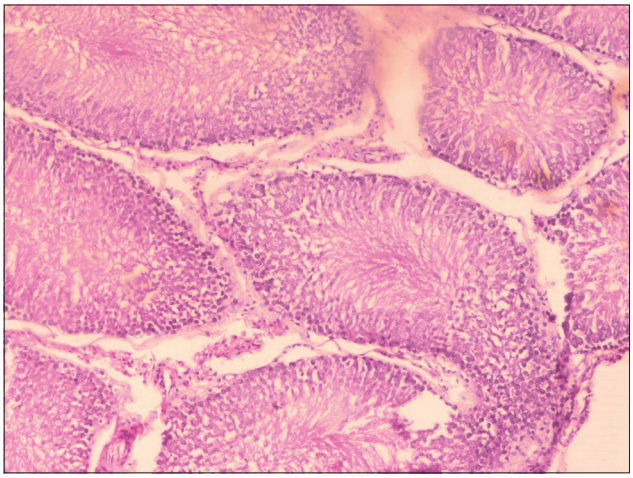

Figure 2: Photomicrograph of control testis: Seminiferous tubules are equal in size with thin basement membrane and small groups of Leydig cells in intertubular connective tissue. Stages of spermatogenesis and spermatogenesis with mature sperms in center are clearly visible, H and E, ×10

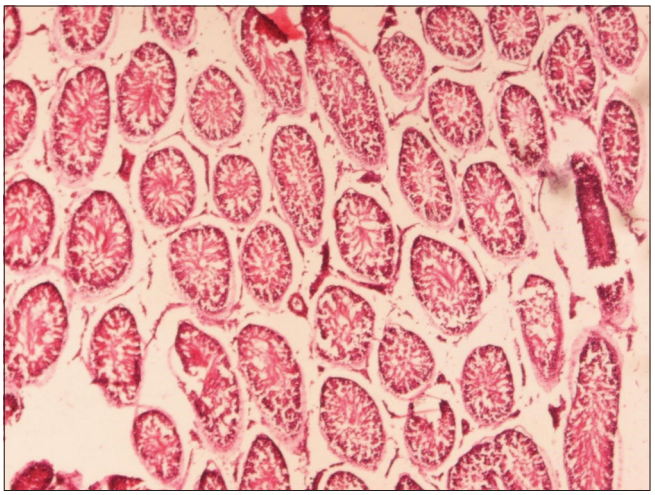

Figure 3: Photomicrograph of experimental testis: Section shows small compressed seminiferous tubules separated by edema fluid in spaces. Intratubular edema is also evident as separation of small sized spermatogenic cells, H and E, ×4

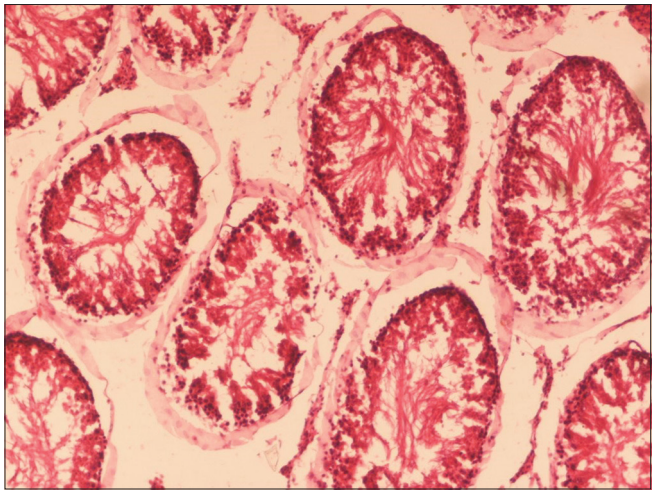

Figure 4: Photomicrograph of experimental testis: Inter and intratubular edema, compressed tubules, swollen and fragmented basement membrane are main features. There is mild grade inflammatory cell infiltrate and compressed Leydig cells, H and E, ×10

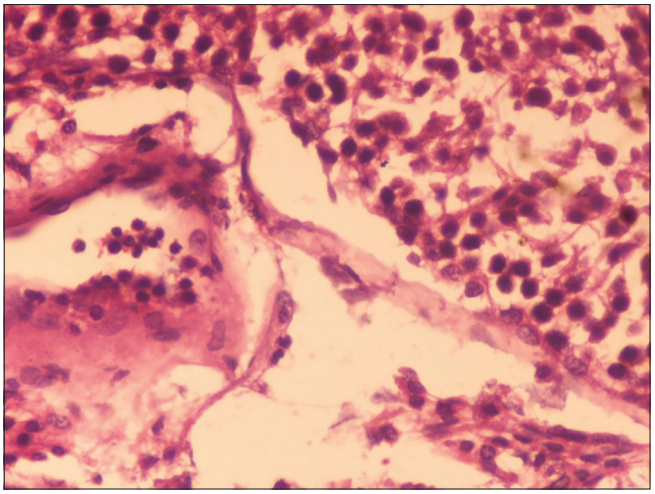

Figure 5: Photomicrograph of experimental testis: Seminiferous tubule shows swelling and focal thinning and breakage of basement membrane. Leydig cells appear as a group of compressed eosinophilic spindle cells in interstitial space. Inflammatory cells are also seen, H and E, ×40

Table 1: Different measurements in testicular tissue of control and experimental (olanzapine intoxicated) rats

Organs	Diameter/thickness (µm)	Mean±SD		Per cent change	*P*
		Control	Experimental		
Testis	Seminiferous tubules	259.79±24.11	219.60±28.60	↓15.47	<0.001
	Tunica albuginea	21.43±8.83	58.62±12.86	↑173.54	<0.001

SD=Standard deviation. ↓= Decreased, ↑= Increased

compressed elongated groups of Leydig cells with highly eosinophilic cytoplasm and dark nuclei in our study is of great social importance. Clumping of sperms in the center of seminiferous tubules is also explained by the edema in our findings. Presence of thrombus in blood vessels of the testis is another alarming feature in our experimental groups.

Olanzapine is known to show high affinity for many receptors such as H1 histamine, 5-HT 2A, 5-HT 2C, D2, muscarinic, and alpha 1. It exhibits lower affinity for alpha 2, 5-HT 1D and 5-HT1A receptors.[23-25] Aforementioned receptors are widely distributed throughout the body in different organs. Dopaminergic D2 receptors are known to occur in the central nervous system, anterior pituitary, stomach, small intestine, pulmonary artery, and smooth muscles.[26] H1 histamine receptor is present in smooth muscles, endothelium, and brain.[27] The presence of muscarinic receptors is well documented in nerves, heart, smooth muscles, glands, and endothelium.[28] Location of serotonergic receptors are as follows: 5-HT 2A in smooth muscles, platelet, and cerebral cortex; 5-HT 2C in the choroid, hippocampus and substantia nigra; 5-HT 1D in brain and 5-HT1A in raphe nucleus and hippocampus.[27]

Aforesaid facts may be indicative of the direct toxic effect of the drug on testis. At the same time, the damaging effects may also be an indirect expression due to its direct effects on other organs of the body.

Degeneration in spermatogenic cells and sperms might be due to damage of Sertoli cells leading to decreased inhibin B level.[11,20] Decreased testosterone level due to olanzapine may have also led to the adverse effects on spermatogenic cells.[20-22] This drug also leads to the increased level of prolactin,[10-12] which could be the reason of toxic effects as increased prolactin leads to a decreased level of testosterone, FSH, GnRH and LH.[16]

Formation of reactive oxygen species is induced by the use of olanzapine,[29] which may cause cellular damage and dysfunction.[30] It has been proved that using antioxidant can reduce the metabolic changes in rats receiving olanzapine.[31] Reactive oxygen species could also be the generalized reason for changes in organ under consideration. The vascular factor may be another generalized reason for the degenerative changes in the testis of experimental rats. However, this prediction needs further experimental studies for confirmation.

Conclusion

Olanzapine leads to histopathological and histomorphometric changes confirming degeneration in testis after prolonged use. Edema is a generalised feature of its toxic effects. Since olanzapine exhibits affinity for large number of receptors distributed among many organs in the body, the toxicity seems to be due to both direct and indirect effects. Olanzapine induced testicular degeneration might lead to reduced libido, a fact of great social impact.

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Conflicts of interest

There are no conflicts of interest.

References

1. Lieberman JA. Maximizing clozapine therapy: Managing side effects. J Clin Psychiatry 1998;59 Suppl 3:38-43.
2. Casey DE. Side effect profiles of new antipsychotic agents. J Clin Psychiatry 1996;57 Suppl 11:40-5.
3. Mitchell M, Riesenberg R, Bari MA, Marquez E, Kurtz D, Falk D, *et al.* A double-blind, randomized trial to evaluate the pharmacokinetics and tolerability of 30 or 40 mg/d oral olanzapine relative to 20 mg/d oral olanzapine in stable psychiatric subjects. Clin Ther 2006;28:881-92.
4. Bymaster FP, Calligaro DO, Falcone JF, Marsh RD, Moore NA, Tye NC, *et al.* Radioreceptor binding profile of the atypical antipsychotic olanzapine. Neuropsychopharmacology 1996;14:87-96.
5. Richelson E, Souder T. Binding of antipsychotic drugs to human brain receptors focus on newer generation compounds. Life Sci 2000;68:29-39.
6. Soliman HM, Wagih HM, Attia GM, Algaidi SA. Light and electron microscopic study on the effect of antischizophrenic drugs on the structure of seminiferous tubules of adult male albino rats. Folia Histochem Cytobiol 2014;52:335-49.
7. Nagaraj AK, Pai NB, Rao S. A comparative study of sexual dysfunction involving risperidone, quetiapine, and olanzapine. Indian J Psychiatry 2009;51:265-71.
8. Kandrakonda S, Reddy Jally M, Rani S, Reddy K, Miryala G. Prevalence of sexual dysfunction in patients with mental illness receiving psychotropic medication. AP J Psychol Med 2014;15:235-9.
9. Nebhinani N, Grover S, Avasthi A. Sexual dysfunction in male subjects receiving trifluoperazine, risperidone, or olanzapine: Rates vary with assessment questionnaire. Prim Care Companion CNS Disord 2012;14. pii: PCC.11m01199.
10. Haddad PM, Wieck A. Antipsychotic-induced hyperprolactinaemia: Mechanisms, clinical features and management. Drugs 2004;64:2291-314.
11. Konarzewska B, Wołczyński S, Szulc A, Galińska B,

Popławska R, Waszkiewicz N. Effect of risperidone and olanzapine on reproductive hormones, psychopathology and sexual functioning in male patients with schizophrenia. Psychoneuroendocrinology 2009;34:129-39.

12. Kelly DL, Conley RR. Sexuality and schizophrenia: A review. Schizophr Bull 2004;30:767-79.
13. Peuskens J, Pani L, Detraux J, De Hert M. The effects of novel and newly approved antipsychotics on serum prolactin levels: A comprehensive review. CNS Drugs 2014;28:421-53.
14. Schlechte JA. Clinical practice. Prolactinoma. N Engl J Med 2003;349:2035-41.
15. Chen YL, Cheng TS, Lung FW. Prolactin levels in olanzapine treatment correlate with positive symptoms of schizophrenia: Results from an open-label, flexible-dose study. Prim Care Companion J Clin Psychiatry 2009;11:16-20.
16. De Rosa M, Zarrilli S, Di Sarno A, Milano N, Gaccione M, Boggia B, *et al.* Hyperprolactinemia in men: Clinical and biochemical features and response to treatment. Endocrine 2003;20:75-82.
17. de Siqueira Bringel S, de Amorim Júnior AA, Amorim MJ, Brito LT, Morais RN, de Torres SM, *et al.* Endocrine and testicular changes induced by olanzapine in adult Wistar rats. J Appl Toxicol 2013;33:24-31.
18. França LR, Avelar GF, Almeida FF. Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs. Theriogenology 2005;63:300-18.
19. Walker WH, Cheng J. FSH and testosterone signaling in sertoli cells. Reproduction 2005;130:15-28.
20. Meachem SJ, Nieschlag E, Simoni M. Inhibin B in male reproduction: Pathophysiology and clinical relevance. Eur J Endocrinol 2001;145:561-71.
21. Hyun JS, Bivalacqua TJ, Baig MR, Yang DY, Leungwattanakij S, Abdel-Mageed A, *et al.* Localization of peripheral dopamine D1 and D2 receptors in rat corpus cavernosum. BJU Int 2002;90:105-12.
22. Otth C, Torres M, Ramírez A, Fernandez JC, Castro M, Rauch MC, *et al.* Novel identification of peripheral dopaminergic D2 receptor in male germ cells. J Cell Biochem 2007;100:141-50.
23. Kapur S, Zipursky RB, Remington G, Jones C, DaSilva J, Wilson AA, *et al.* 5-HT2 and D2 receptor occupancy of olanzapine in schizophrenia: A PET investigation. Am J Psychiatry 1998;155:921-8.
24. Raedler TJ, Knable MB, Jones DW, Lafargue T, Urbina RA, Egan MF, *et al. In vivo* olanzapine occupancy of muscarinic acetylcholine receptors in patients with schizophrenia. Neuropsychopharmacology 2000;23:56-68.
25. Tarazi FI, Zhang K, Baldessarini RJ. Long-term effects of olanzapine, risperidone, and quetiapine on dopamine receptor types in regions of rat brain: Implications for antipsychotic drug treatment. J Pharmacol Exp Ther 2001;297:711-7.
26. Ricci A, Mignini F, Tomassoni D, Amenta F. Dopamine receptor subtypes in the human pulmonary arterial tree. Auton Autacoid Pharmacol 2006;26:361-9.
27. Katzung BG. Histamine, serotonin and ergot alkaloids. In: Basic and Clinical Pharmacology. 13th ed. New York: Mc Graw Hill; 2015. p. 437-68.
28. Katzung BG. Cholinoceptor-blocking drugs. In: Basic and Clinical Pharmacology. 13th ed. New York: Mc Graw Hill; 2015. p. 209-27.
29. Heiser P, Sommer O, Schmidt AJ, Clement HW, Hoinkes A, Hopt UT, *et al.* Effects of antipsychotics and Vitamin C on the formation of reactive oxygen species. J Psychopharmacol 2010;24:1499-504.
30. Halici Z, Keles ON, Unal D, Albayrak M, Suleyman H, Cadirci E, *et al.* Chronically administered risperidone did not change the number of hepatocytes in rats: A stereological and histopathological study. Basic Clin Pharmacol Toxicol 2008;102:426-32.
31. Shertzer HG, Kendig EL, Nasrallah HA, Johansson E, Genter MB. Protection from olanzapine-induced metabolic toxicity in mice by acetaminophen and tetrahydroindenoindole. Int J Obes (Lond) 2010;34:970-9.