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Zinc sulphate alleviates olanzapine-induced testicular oxidative stress and alters trace elements in male rats

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Abstract

Male infertility is a complex and multifactorial clinical condition affecting a large population attributed to various factors, including perturbation in oxidative stress and the level of essential trace elements. Oxidative insults exert multiple issues related to reproductive health, including male infertility, decreased sperm motility, sperm DNA damage, and an increased susceptibility to genetic disorders. Besides chemical toxins and food allergens in junk food items, many drugs can also lead to male fertility. Olanzapine, a general antipsychotic drug, has also been reported to induce male fertility. A great deal of literature entails that zinc can alleviate oxidative stress-related clinical complications including male fertility. This study investigates the potential protective effects of zinc sulphate on olanzapine-induced male infertility. In the present study, there were four treatment groups (n = 6) Group 1 as control (without any treatment); Group 2 treated with olanzapine (10 mg/kg) orally daily for six weeks; Group 3 and 4 treated with 50mg/kg and 100 mg/kg of zinc sulphate respectively in pre-exposed olanzapine (10mg/kg) orally daily for six weeks. After completion of the treatment, the biochemical analysis of serum and tissue samples demonstrated that group 2 had compromised levels of antioxidant parameters (SOD, CAT and GSH) as well as elevated levels of stress parameters (GSSG, MDA and NO). However, ZnSO4 corrected all these parameters in a dose-dependent way in groups 3 and 4. Other health indicators, like taurine, enzyme Q10, phosphatidylcholine, ascorbic acid, and vitamin E, were also observed to improve with the supplementation of ZnSO4. Intriguingly, all the fertility parameters (sperm motility, count and level of testosterone) were also found to significantly enhanced with decreased sperm abnormalities in both the combination groups. The histological evaluation of the testis section also agreed with the biochemical analysis. Therefore, the study strongly advocates that supplementation of Zn can ameliorate olanzapine-induced male infertility to a significant extent.

Introduction

Male infertility is a complicated and multifaceted disease that may be affected by a variety of circumstances, including oxidative stress and changes in trace elements (1). Oxidative stress has been associated with male infertility, decreased sperm motility, sperm DNA damage, and an increased risk of genetic disorders (2). Moreover, exposure to environmental pollutants and toxic substances has been found to induce testicular oxidative stress, emphasizing the detrimental effects of external factors on male reproductive function (3–5). Additionally, the protective effects of antioxidants such as zinc against oxidative stress-induced testicular damage have been investigated, highlighting the potential role of this compound in mitigating oxidative stress (3; 6).

Olanzapine is a commonly given atypical antipsychotic drug that has been shown to help treat several psychological diseases (7). Olanzapine has been linked to producing reactive oxygen species (ROS) and disrupting antioxidant defense systems. This can cause oxidative damage in many tissues, including the testes (8; 9). Additionally, evidence suggests that olanzapine may influence trace element metabolism, further contributing to testicular dysfunction (10; 11). However, olanzapine has been associated with side effects when used for medical purposes, such as problems with sexual health (12). It might cause

reactive stress in the testicles and changes in the balance of trace elements, which could hurt a man's ability to reproduce (13). Nevertheless, the medicinal use of olanzapine has been linked to detrimental consequences, such as disruptions in reproductive well-being (14). An issue that arises is the initiation of oxidative stress in the testicles and changes in the balance of trace elements, which may harm male reproductive function (15–18).

Zinc, an essential trace element, is vital in maintaining testicular health by acting as a co-factor for antioxidant enzymes and regulating DNA synthesis and repair (19; 20). Alterations in trace elements, such as zinc (21; 22) and copper (23; 24), have been associated with metabolic imbalances and oxidative stress, indicating the intricate relationship between zinc and male reproductive health. Given its antioxidant properties, zinc supplementation has been investigated as a potential therapeutic strategy to mitigate the adverse effects of olanzapine-induced oxidative stress (25; 26). Previous studies have shown that zinc sulphate can defend against testicular oxidative stress caused by different substances, indicating its effectiveness in reducing such stress (27–30).

Understanding the interplay between olanzapine, zinc sulphate, and testicular health is paramount in elucidating potential interventions to safeguard male reproductive function in individuals undergoing olanzapine treatment. This study aims to identify the potential protective effects of zinc sulphate on testicular oxidative stress and provides a foundation for developing targeted therapeutic approaches to mitigate these adverse effects.

Material and methods

Materials

Zinc sulphate was obtained from Merk. Olanzapine was brought from the Global Napi, Pharmaceutical (Brand name: Integrol), Egypt.

Animals

Twenty-four Wistar adult male rats at 12 weeks weighing 150–170 g (Animal house of National Research Center, Egypt) have stayed at stable room temperature (25 Co) with a 12 h light/dark cycle and free access to food and water. The animals were left for a week for adaptation.

Experimental design

The rats were distributed into 4 groups:

Group 1: Normal rats served as control

Group 2: Rats treated with olanzapine 10mg/Kg (8) orally daily for six weeks

Group 3: Rats administered 50mg/Kg zinc sulphate + OLZ 10mg/Kg orally daily for six weeks

Group 4: Rats administered 100mg/Kg zinc sulphate + OLZ 10mg/Kg orally daily for six weeks.

Animal handling was carried out in according to recommendations and under the regulations of Animal Care and Use of National Research Centre in Egypt with ethical approval No.01430124. All surgery was performed under anesthesia and all efforts were made to reduce suffering.

Samples

At the end of the treatment period, the rats of all groups were under anesthesia by ketamine. Blood samples were collected from a retero-orbitatreal vein in heparinized tubes and separated plasma was stored in Eppendorf tubes at -80˚ for biochemical analysis. The weight of body, testis and accessory reproductive organs were recorded. The relative weight is calculated, which equals organ /Bodyweight x 100.

Immediately after blood sampling, animals were sacrificed by cervical dislocation testis was separated. A part of testis was homogenized with ice-cooled saline (0.9% NaCl) to prepare homogenate. The homogenate was then centrifuged at 3000 rpm for 10 min. at 5°C using a cooling centrifuge “Laborzentrifugen, Sigma, Germany”. The supernatant was used for various analyses. A portion of testis was fixed immediately in 10% neutral buffered formalin, processed for light microscopy through a graded series of alcohol, cleared in xylene and embedded in paraffin wax to get (5μm) paraffin sections that stained with Hematoxylin & Eosin (H &E).

Sperm analysis

Sperm motility, count and abnormality

Spermatozoa were secured from the left epididymis of adult rats. Epididymis was cut into small pieces with a sharp razor blade and dispersed in 3 ml of phosphate-buffered saline (pH 7.2) to obtain a suspension kept in an incubator of $37C^{O}$.10 μL of supernatant containing the sperm was placed between the warm slide and cover slip and observed at 400x in a light microscope with a warmed stage. The count from each rat sample continued until the total number of spermatozoa was noticed to be about 200, and the percentage of motile spermatozoa was calculated. We take different slides for each sample. The sperm number was evaluated by using a hemocytometer (31). The sperm abnormality was carried out by staining a portion of suspension with 1% eosin and head or tail abnormality was recorded. The sperm abnormality was expressed as a percentage (Number of sperm abnormality/total number of sperm x 100).

Testicular oxidative stress parameters

Testicular oxidative parameters were evaluated by the Agilent HP 1200 series (USA) HPLC system consisting of a quaternary pump, a column oven, Rheodine injector with 20 μL loop, and variable-wavelength ultraviolet detector. The resulting chromatogram identified the sample concentration compared to the standard purchased from Sigma Aldrich.

Testosterone assay

Plasma testosterone was determined by enzyme immunoassay kits from Diagnostic products Co. (Los Angeles, CA USA).

Determination of zinc and iron in testicular tissue

Iron and zinc were determined as stated by (32). Briefly, tissue (1 g) was treated with 65% nitric acid for 30 minutes and then with 60% perchloric acid. After centrifugation (3000 g for 12 minutes), the supernatant was diluted with deionized water, and non-heme iron was determined by graphite furnace atomic absorption spectrophotometer and expressed as mg non-heme iron/g of tissue weight."

Statistical Analysis

Values were given as the mean standard error. The significance of the differences between the groups' variances was examined using Analysis of Variance (ANOVA), then the LSD comparison test, calculated using SPSS software, version 21. $P \leq 0.05$ was the statistical significance threshold.

Results

The present investigation addressed the impact of the application of olanzapine on the physical and chemical conditions of sperm as a functional unit of the male reproductive system. Data analysis showed a significant variation among the experimental groups in sperm motility, total sperm abnormalities, sperm count by epididymis, and testosterone concentration. Besides, it enlightens the influence of olanzapine on the body weight and relative weight of male reproductive organs such as testis, epididymis, seminal vesicles, and prostate and epididymal fats.

Weight of reproductive organs

The present analysis clarified the significant increase in the relative body weight (290.83 ± 30.13 gm) in olanzapine rats. Also, relative weight of the testis, epididymis, seminal vesicle, and prostate of olanzapine rats expressed significant decline (0.45 ± 0.04 gm), (0.28 ± 0.03 gm), (0.51 ± 0.05 gm), and (0.15 ± 0.02 gm) respectively, in comparison to the control rats. Conversely, the epididymis gland showed more relative adipose tissue (0.97 ± 0.1 gm) than normal epididymis (Table 1).

Table 1
Absolute weight of reproductive organs among the different four rat groups.

Groups	Body	Testis	Epididymal fat	Epididymis	Seminal vesicle	Prostate
Control	277.33 ± 20.53	1.77 ± 0.04	1.89 ± 0.49	0.91 ± 0.14	1.82 ± 0.09	0.66 ± 0.09
Olanzapine	290.83 ± 30.13	1.51 ± 0.17	2.98 ± 0.62 *	0.74 ± 0.1	1.22 ± 0.31 *	0.37 ± 0.06 *
Olanz + ZnSO4 (50mg/kg)	242.66 ± 25.61 #	1.56 ± 0.12	2.18 ± 0.41	0.73 ± 0.08 *	1.03 ± 0.12 #	0.38 ± 0.04 #
Olanz + ZnSO4 (100 mg/kg)	249.83 ± 22.86	1.71 ± 0.22	2.04 ± 0.41 #	0.76 ± 0.01	1.17 ± 0.11 #	0.44 ± 0.04 #

Zinc sulfate in two doses was found to recover the relative weight of the male reproductive organs to the normal in olanzapine rats. Whereas the seminal vesicle, prostate and body weight were restored (242.66 ± 25.61 gm) by administration of zinc sulfated in olanzapine rats (50µg/kg). Both doses of zinc sulfate signaled non-significant variations in the relative weight of the testis, seminal vesicle, and prostate. While the epididymis of in zinc sulfate-treated rats restored its normal relative weight (0.32 ± 0.03 gm) significantly (Table 2).

Table 2
Relative weight of reproductive organs to the body weight among the different four rat groups.

Groups	Body	Testis	Epididymal fat	Epididymis	Seminal vesicle	Prostate
Control	277.33 ± 20.53	0.64 ± 0.04	0.81 ± 0.06	0.39 ± 0.03	0.66 ± 0.05	0.19 ± 0.01
Olanzapine	290.83 ± 30.13	0.45 ± 0.04 *	0.97 ± 0.1 *	0.28 ± 0.03 *	0.51 ± 0.05 *	0.15 ± 0.02 *
Olanz + ZnSO4 (50mg/kg)	242.66 ± 25.61 #	0.72 ± 0.08 #	0.82 ± 0.08 #	0.32 ± 0.03 #	0.47 ± 0.05 *	0.18 ± 0.02 #
Olanz + ZnSO4 (100 mg/kg)	249.83 ± 22.86	0.56 ± 0.05 #	0.62 ± 0.05 *#	0.26 ± 0.02 *	0.46 ± 0.04 *	0.18 ± 0.02 #

* Indicates statistically different from the control at $p \leq 0.5$, while # indicates statistically different from the Olanzapine group.

The present investigation addressed the impact of olanzapine on the physical and chemical conditions of sperm as a functional unit of the male reproductive system. Data analysis showed a significant

variation among the experimental groups in sperm motility; total sperm abnormalities; sperm count by epididymis, and testosterone concentration.

Here, olanzapine resulted in long oxidative stress with a significant reduction in the vital antioxidant enzymes. Statistics exhibited a significant decline in the levels of antioxidant parameters in olanzapine rats compared to prooxidant levels, which had a considerable increase. Particularly, the SOD activity of olanzapine rats recorded a significant deficiency (40.86 ± 1.89 U/g tissue) in comparison to the control rats. Conversely, the olanzapine rats supplemented with 100µg of zinc sulfate significantly restored the control value (48.80 ± 4.42 U/g tissue) (Fig. 1a).

Catalase significantly decreased in olanzapine rats (12.89 ± 0.48 U/g tissue) compared to the control animals. On the contrary, the higher dose of zinc sulfate of 100µg showed a significant recovery rate of catalase (15.61 ± 0.97U/g tissue) (Fig. 1b).

The vital nonenzymatic glutathione demonstrated a significant decrease in olanzapine rats in comparison to the control one. Besides, the two groups of zinc sulfate significantly improved glutathione concentration in the olanzapine rats (Fig. 1c).

Vitamin C and vitamin E work together to improve the functional quality of sperm, including its motility, counting, and morphology. The concentration of ascorbic acid was significantly reduced (174 ± 13.79µmol/g tissue) in olanzapine rats (Fig. 2a) in comparison with the control animals. Interestingly, either the high or the low zinc sulfate doses significantly restore the ascorbic acid level to the control values. Similarly, vitamin E concentration was significantly decreased by olanzapine as an antischyopatic drug (5.01 ± 0.19 µg/g tissue) in comparison to the control group. On the contrary, the two therapeutic doses of zinc sulfate exhibited a significant recovery (Fig. 2b).

Due to the vital benefits of Coenzyme Q10 for the fertility and sperm motility of males, it was estimated here. Data revealed that olanzapine significantly reduced the level of Coenzyme 10 (175.16 ± 6.43µmol/g tissue) compared to the control. Either the high or the low zinc sulfate doses significantly restore the Coenzyme 10 level to the control values (Fig. 2c).

For sperm vitality and efficiency of sperm motility, the concentration of phosphatidylcholine must be preserved. Here, we found that olanzapine caused a significant deficiency in the phosphatidylcholine concentration (237.83 ± 20.23 µmol/g tissue) compared to the control. Zinc sulfate, either the high or the low dose, was found to significantly restore the control levels of phosphatidylcholine in olanzapine in comparison to control animals (Fig. 2d).

Taurine is a potential factor responsible for promoting spermatogenesis and improving sperm quality in the testis by enhancing testosterone concentration. Therefore, our study explored the deficiency impact of olanzapine on the concentration of taurine (2.02 ± 0.13µg/g tissue) compared to the control. The 50 &100µg doses of zinc sulfate displayed a significant recovery of taurine in olanzapine rats (Fig. 2e).

Notably, olanzapine as an antipsychotic drug caused the elevation of oxidative stress rate. Consequently, the prooxidant levels were significantly increased due to the elevation rate of oxidative stress. Certainly, malondialdehyde (MDA) is considered the end product of cellular lipid peroxidation, which results from increasing the density of free radicals and is regarded as a marker of oxidative stress. Analysis exhibited the highest level of MDA in olanzapine rats in all experimental groups (47.86 ± 3.26n mol/g tissue) (Fig. 3a). On the contrary, the 50 or 100µg of zinc sulfate was found to partially and non-significantly improve the concentration of the MDA in the olanzapine rats in comparison of the control rats. Similarly, the olanzapine rats treated with 50 or 100µg of zinc sulfate revealed a partial significant recovery of the concentration of the reduced glutathione (GSSG) (Figs. 3b) in comparison to the control animals in a dose-dependent manner. Similarly, the olanzapine rats treated with 50 or 100µg of zinc sulfate revealed a partial non-significant recovery of the concentration of the NO in comparison to the control animals (Figs. 3c).

Olanzapine indirectly influenced ion concentrations such as zinc and iron and energy storage indicator (ATP). The current study displayed a significant decrease in zinc, iron, and ATP levels in olanzapine rats. The data analysis indicates a substantial restoration in zinc, iron, and ATP levels between two administrated groups with zinc sulfate (Fig. 4).

Olanzapine showed a significant decrease (77.33 ± 2.46%) in sperm motility in comparison to the normal rats. Data exhibited a non-significance variation in sperm motility between olanzapine administrated 50µg of zinc sulfate in comparison to the nontreated olanzapine rats. Interestingly, 100 µg of zinc sulfate was found to significantly restore the motility in olanzapine rats to the control value (84.16 ± 5.67%) (Fig. 5a).

Regarding Fig. 5b, the total sperm abnormalities displayed a higher significant increase in olanzapine rats (8.33 ± 0.67%) more than the control ones. Although it was not completely recovered, the total sperm abnormalities were significantly improved by either the 50µg or 100 µg of zinc sulfate in comparison to the nontreated olanzapine rats.

Testosterone concentration significantly decreased (2.27 ± 0.47 ng/ml) in olanzapine rats compared with the other experimental groups. The 100 µg of zinc sulfate significantly recovered the testosterone concentration in olanzapine rats (3.34 ± 0.33 ng/ml) to a value close to the control one (Fig. 5c).

Similarly, the sperm count in the olanzapine rats showed a nonsignificance deficiency (18.83 ± 1.47 x_{106}) in comparison to the control rats. Conversely, the olanzapine rats that were exposed to the zinc sulfate of 50µg or 100 µg exhibited a significance (21.83 ± 1.94), (25.83 ± 1.72), respectively, in comparison to the control rats, in a dose-dependent manner (Fig. 5d).

Histopathological results

Examination of H&E stained-sections of control rats' testis revealed parenchyma was formed of rounded seminiferous tubules surrounded by tunica albuginea. Seminiferous tubules were lined by stratified germinal epithelium (spermatogonia, spermatocytes and spermatids) resting on a regular basement

membrane. Most of them had narrow lumina. Leydig cells were found in the narrow interstitial (Fig. 1A & B).

Histopathological examination of testis of Olanzapine group showed disturbed architecture of seminiferous tubules, degenerated and exfoliation germinal epithelium of a seminiferous tubule (spermatogonia, spermatocytes and spermatids) with moderately thickened basement membrane. Note wide interstitial tissue with few Leydig cells and vacuoles (Fig. C & D).

The groups treated with Olz + 50mg/Kg $ZnSO_4$ showed moderate improvement of testicular architecture- seminiferous tubules lined with germinal epithelium; spermatogonia primary spermatocytes, spermatids resting on thin basement membrane. Other seminiferous tubule is seen with more or less normal spermatogenic cell lines. Note mild wide interstitial tissue with few Leydig cells and few vacuoles (Fig. 1E & F).

The rats treated with Olz + 100mg/Kg $ZnSO_4$ showed nearly normal testicular architecture- seminiferous tubules lined with germinal epithelium; (spermatogonia spermatocytes, spermatids) resting on thin basement membrane. Most of them had narrow lumina. Clusters of Leydig cells were found in the narrow interstitial (Fig. 1G & H).

Discussion

Olanzapine was a typical antipsychotic drug that proved its efficiency against schizophrenia (33). Little research stated the harmful impacts of this drug on the male reproductive system. This study highlights the toxic impact of the olanzapine and the efficiency of zinc sulfate in treating these side effects. Correspondingly, zinc is considered a corrector element for vital processes, such as adjusting the secretion of hormones and immune response. Here, zinc was supplied for feeding as zinc sulfate. We applied two doses of zinc sulfate to explore the therapeutic efficiency of zinc elements on olanzapine disorders. Studies investigating the effects of zinc oxide nanoparticles on male rats' reproductive performance, testicular antioxidants, gene expression, and histology provide further evidence for the possible influence of zinc on testicular health and reproductive function (34; 35). Furthermore, zinc has shown its vital significance in immune system function and the maintenance of reproductive characteristics (36; 37). Moreover, the addition of zinc has been shown to influence hormonal factors and sperm attributes in males, suggesting its potential influence on reproductive well-being (22; 38).

The current investigation pointed to the influence of olanzapine for schizophrenia and bipolar disorders on the relative weight of the body and male reproductive organs. Our findings revealed that the olanzapine rats suffered from a gain in body weight, although the testis, epididymis, seminal vesicle, and prostate recorded significant atrophy in their relative weight.(39; 40) explained the weight gain of olanzapine rats and the presence of epididymal fats due to metabolic function disorders. As long, (41) demonstrated significant shrinkage in the testicular structure relative weight due to the generalized edema distributed internally in the sexual structures. They recorded that olanzapine had a degenerative impact on seminiferous tubules, spermatogonia cells, and Sertoli cells. (8) referred this to sexual atrophy

occurred by the cellular damage of increasing ROS. Zinc sulfate recovered all relative weights of the body, testis, epididymis, seminal vesicle, and prostate to their normal range of the control rats in olanzapine rats. (42) confirmed our findings and stated that zinc had an improved impact on the antipsychotic disorders of olanzapine and risperidone. (43) shared the importance of zinc in removing the depression symptoms of antipsychotic medications.

Here, we revealed that the group of olanzapine expressed a significant decrease in the rate of antioxidant enzymes such as SOD, CAT, and GSH less than the normal rats. Similarly (44) announced a reduction in antioxidant levels in cardiac-arrested rats in their hippocampus region. However, zinc sulfate was given to olanzapine rats in two doses, and zinc acted as a protective element against free radicals. Similar observations were recorded by (45) in the intestinal regions of young boilers.

Also, (46) revealed the importance of ascorbic acid in many vital roles concerning the male reproductive system. (47) referring to the administration of olanzapine and clozapine-induced generation of ROS that in turn influence reduction of testicular antioxidant levels such as vitamin C. The accumulating data exhibited a similar reduction in the ascorbic acid level that led to genotoxicity in comparison to the control animals. (48) explained the priority of vitamin E in removing oxidative stress from the rat reproductive organs. Here, a significant deficiency of the vitamin E in olanzapine rats was found in comparison to rats that received saline only. Likewise (49) reported a similar deficiency in vitamin E rate by olanzapine. In other words, our findings showed that the zinc sulfate intake of 50 and 100μg was an effective therapy for olanzapine-caused disorders. (50) confirmed that the supplementation with zinc sulfate elevated the ascorbic acid and vitamin E.

Coenzyme Q10 is a bioactive nonenzymatic antioxidant that counts the sperm and its motility. Therefore, it is shared in the fertility of male rats (51). Our investigation recorded a decline of Coenzyme Q10 in the seminal plasma of olanzapine rats. (52) clarified similar registration of Coenzyme Q10 deficiency in the depression phase of bipolar disorders. Supplementation with zinc sulfate led to a significant restoration of coenzyme Q10 in olanzapine rats. Zinc sulfate improved Coenzyme Q10 and spermatogenesis.

Phosphatidylcholine has a role in the male reproductive system in supporting the fluidity and integrity of cell membranes that result in sperm motility and integrity. (53) reported a significant decline in the level of phosphatidylcholine in female rats with Schizophrenia treated with olanzapine. Additionally, it was found that the administrative of zinc sulfate promoted phosphatidylcholine secretion in the seminal serum at a rate that reached to sterile dose. (54) stated similar observations in treated rats with several antipsychotic drugs.

Taurine is a cellular protective agent, an amino acid with sulfur, and a nutritional factor (55). Taurine deficiency is involved in aging features of testicular cellular structures and neuro diseases. The current analysis showed a regression of the taurine level during in olanzapine rats. Olanzapine influences the nutrition absorption responsible for taurine uptake and oxidative damage status (56). We aimed at enhancing the performance of antipsychotic drugs as well as promoting the secretion of enzymatic and nonenzymatic antioxidants such as taurine to stop the oxidative damage caused by olanzapine. So, by

administering zinc sulfate, the taurine concentration recovered and approached the normal level in olanzapine rats. (57; 58) displayed the vital role of taurine in inhibiting inflammatory and apoptotic processes and raising the antioxidant and sexual capacity.

One of the side impacts of olanzapine is the formation of ROS (59). The oxidative damage induced significant lipid peroxidation products. (60) indicated that haloperidol-induced more peroxidation in the lipid membrane than olanzapine. (26) referred to this as a significant increase in the MDA concentration. Zinc sulfate displayed a substantial decrease in the MDA concentration in olanzapine rats. GSSG and NO of olanzapine rats exhibited higher rates. A similar increase in the GSSG rate was announced (61) during hepatic oxidative stress. Additionally, (62) confirmed the essential role of olanzapine in the elevation of NO during the neurodevelopmental disorder. Regardless, (63) suggested that a diet with zinc is considered an effective treatment for decreasing the level of NO and GSSG in elderly rats.

Zinc is crucial in preserving the continuity of spermatogenesis and testosterone concentration while maintaining sperm maturation and motility (64). Our investigation recorded a deficiency in the concentration of zinc in the serum of olanzapine rats. Analogous observations were registered (65) during administration with clozapine. (66) explained the role of zinc as a cofactor for many antioxidants such as SOD and CAT that neutralized the free. The instant investigation used zinc supplementation as a co-factor in advancing the therapeutic impact of olanzapine by raising the level of zinc concentration to the normal physiological rate in seminal serum. (67) confirmed the role of zinc in the refinement of fertility capacity by removing oxidative stress and increasing the ejaculation capacity and movement of the sperm. On this side, the concentration of iron was assumed a significant indicator of oxygen supply and regulation of hormones in male sexual organs (68). The iron was recorded a lower rate in olanzapine animals in the current study. (69) explained this deficiency by the irregular metabolism of iron due to the excessive oxidative stress that resulted from using olanzapine and chronic inflammatory signs that led to a deficiency in iron absorption. An identical decrease in iron concentration in the presence of olanzapine was recorded (70) in brain disorders. Otherwise, it was found that the zinc sulfate treatment enhanced the iron concentration levels. ATP was considered a mitochondrial product indicating storage energy (71). Due to cellular destruction by olanzapine, there was a disturbance in the synthesis of ATP. Consequently, the present data analysis showed a significant decline in ATP levels in the olanzapine group. Comparatively, the identical reduction in ATP synthesis was registered by aripiprazole injection in liver destruction (72).

The present study considers the physical impact of olanzapine on sperm characterization as a functional unit of the male reproductive system. Olanzapine causes multiple sexual dysfunctions in the male reproductive system. The result of a deficiency in potential and metabolic parameters such as ascorbic acid; vitamin E; Coenzyme Q10; phosphatidylcholine, and taurine reflected on the sperm motility diminishing when the rats were administrated by olanzapine. Furthermore, the current analysis revealed that the testosterone concentration and sperm count per epididymis (spermatogenesis activity) suffered from depletion during the treatment. (73) proved that the elevation of the prolactin hormone inhibited the

activity of spermatogenesis, reduced testosterone, and raised the probability of abnormalities in the morphological features of the sperm besides the negative impact on sperm motility.

Conclusion

Our findings presented that olanzapine has a degenerative influence on the testicular structures, leading to a significant decrease in the relative weight of the testis, prostate, and epididymis gland. We found vigorous oxidative damage in the male reproductive system of olanzapine rats that was measured by the lower concentration of enzymatic antioxidants like SOD, CAT, and GPX, besides the nonenzymatic antioxidants vitamin C and E, taurine, and coenzyme Q10. In addition, high level of prooxidants such as MDA, GSSG, and nitric oxide with sperm abnormalities were detected. Our findings exhibited the vital role of zinc in treating repro-toxicity caused by olanzapine.

Declarations

Author Contribution

Authors' contribution:H.E and SAEB conceived of the research idea, study design and experimental set up. JAT SAH, TIM, EAO, YSB conducted the experiments as per their respective expertise.HE, SAEB and IH analyzed the results of the experiments. HE. arranged the funding and required setup in the laboratory. Z.H. was responsible for type setting and formatting. HE and IH drafted the manuscript. All authors have read and agreed to the published version of the manuscript. EMA supervised and communicated the manuscript.Concept and Methods, Analysis, Data preparation and curation : HE, SAEBMethods, Data analysis, Graphs: JAT SAH, TIM, EAO, YSBMethods, Analysis, Write up Darfting :HE, IHSupervision, Draft: EMA

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References

1. Liew FF, Dutta S, Sengupta P (2024) Fertility treatment-induced oxidative stress and reproductive disorders. J Integr Sci Technol 12:756
2. Alahmar AT (2019) Role of oxidative stress in male infertility: an updated review. J Hum reproductive Sci 12:4
3. Paramanik A, Das T, Ghosh R, Choudhury SM, Pradhan A (2018) Zinc and α-lipoic acid ameliorates testicular damage and oxidative stress in cypermethrin-intoxicated rat. Int J Adv Res 6:821–832

4. Othman AI, Edrees GM, El-Missiry MA, Ali DA, Aboel-Nour M, Dabdoub BR (2016) Melatonin controlled apoptosis and protected the testes and sperm quality against bisphenol A-induced oxidative toxicity. Toxicol Ind Health 32:1537–1549
5. Olukole SG, Ola-Davies EO, Lanipekun DO, Oke BO (2020) Chronic exposure of adult male Wistar rats to bisphenol A causes testicular oxidative stress: Role of gallic acid. Endocr Regul 54:14–21
6. El-Beshbishy HA, Aly HA, El-Shafey M (2013) Lipoic acid mitigates bisphenol A-induced testicular mitochondrial toxicity in rats. Toxicol Ind Health 29:875–887
7. Liu-Seifert H, Osuntokun OO, Feldman PD (2012) Factors associated with adherence to treatment with olanzapine and other atypical antipsychotic medications in patients with schizophrenia. Compr Psychiatr 53:107–115
8. Ardıç CM, Ilgın S, Baysal M, Karaduman AB, Kılıç V et al (2021) Olanzapine induced reproductive toxicity in male rats. Sci Rep 11:4739
9. Nikolić-Kokić A, Tatalović N, Brkljačić J, Mijović M, Nestorović V et al (2022) Antipsychotic Drug-Mediated Adverse Effects on Rat Testicles May Be Caused by Altered Redox and Hormonal Homeostasis. Int J Mol Sci 23:13698
10. Baj J, Forma A, Sitarz E, Karakuła K, Flieger W et al (2020) Beyond the mind—serum trace element levels in schizophrenic patients: A systematic review. Int J Mol Sci 21:9566
11. Chang G-R, Liu H-Y, Yang W-C, Wang C-M, Wu C-F et al (2021) Clozapine worsens glucose intolerance, nonalcoholic fatty liver disease, kidney damage, and retinal injury and increases renal reactive oxygen species production and chromium loss in obese mice. Int J Mol Sci 22:6680
12. Montejo AL, Montejo L, Baldwin DS (2018) The impact of severe mental disorders and psychotropic medications on sexual health and its implications for clinical management. World psychiatry 17:3–11
13. Meftah AM, Deckler E, Citrome L, Kantrowitz JT (2020) New discoveries for an old drug: a review of recent olanzapine research. Postgrad Med 132:80–90
14. Bhuvaneswar CG, Baldessarini RJ, Harsh VL, Alpert JE (2009) Adverse endocrine and metabolic effects of psychotropic drugs: selective clinical review. CNS Drugs 23:1003–1021
15. La Torre A, Conca A, Duffy D, Giupponi G, Pompili M, Grözinger M (2013) Sexual dysfunction related to psychotropic drugs: a critical review part II: antipsychotics. Pharmacopsychiatry 46:201–208
16. Boz Z, Hu M, Yu Y, Huang X-F (2020) N-acetylcysteine prevents olanzapine-induced oxidative stress in mHypoA-59 hypothalamic neurons. Sci Rep 10:19185
17. YILDIRIM S, Sengul E, Aksu EH, Cinar İ, Gelen V et al (2022) Effects of Selenium on Some Reproductive Parameters in Acrylamide-. Induced Testis Toxicity in Rats
18. El-Tabaa EFM, Rabah H, Basha E, Eid A, Tahoon D et al (2023) Silent Information Regulator 1 Mediates Quercetin Protective Effect on Cadmium-induced Testicular Damage in Adult Male Rats. Bull Egypt Soc Physiological Sci 43:47–63

19. Fallah A, Mohammad-Hasani A, Colagar AH (2018) Zinc is an essential element for male fertility: a review of Zn roles in men's health, germination, sperm quality, and fertilization. J Reprod infertility 19:69
20. Mirnamniha M, Faroughi F, Tahmasbpour E, Ebrahimi P, Beigi Harchegani A (2019) An overview on role of some trace elements in human reproductive health, sperm function and fertilization process. Rev Environ Health 34:339–348
21. Ma J, Tan H, Bi J, Sun B, Zhen Y et al (2023) Zinc Ameliorates Tripterygium Glycosides–Induced Reproductive Impairment in Male Rats by Regulating Zinc Homeostasis and Expression of Oxidative Stress–Related Genes. Biol Trace Elem Res :1–13
22. Chasapis CT, Ntoupa P-SA, Spiliopoulou CA, Stefanidou ME (2020) Recent aspects of the effects of zinc on human health. Arch Toxicol 94:1443–1460
23. Hassan I, Al-Tamimi J, Ebaid H, Habila MA, Alhazza IM, Rady AM (2023) Silver Nanoparticles Decorated with Curcumin Enhance the Efficacy of Metformin in Diabetic Rats via Suppression of Hepatotoxicity. Toxics 11:867
24. Alhazza IM, Hassan I, Ebaid H, Al-Tamimi J, Hasan Z (2023) Zinc Oxide Nanoparticles Blunt Potassium-Bromate-Induced Renal Toxicity by Reinforcing the Redox System. Molecules 28:5084
25. Mani S, Tyagi S, Pal KV, Jaiswal H, Jain A et al (2022) Drug-Induced Oxidative Stress and Cellular Toxicity. In *Free Radical Biology and Environmental Toxicity*:73–113: Springer. Number of 73–113 pp
26. De Simone G, Mazza B, Vellucci L, Barone A, Ciccarelli M, de Bartolomeis A (2023) Schizophrenia Synaptic Pathology and Antipsychotic Treatment in the Framework of Oxidative and Mitochondrial Dysfunction: Translational Highlights for the Clinics and Treatment. *Antioxidants* 12:975
27. Rawi SM, Seif Al Nassr FM (2015) Zinc sulphate and vitamin E alleviate reproductive toxicity caused by aluminium sulphate in male albino rats. Toxicol Ind Health 31:221–234
28. Salem S, Coward W, Lunn P, Hudson G (1984) Response of the reproductive system of male rats to protein and zinc deficiency during puberty. Annals Nutr metabolism 28:44–51
29. Emojevwe V, Nwangwa EK, Naiho AO, Oyovwi MO, Igiehon O et al (2022) Therapeutic efficacy of N-acetylcysteine and zinc sulphate against di-(2-ethylhexyl) phthalate-induced testicular oxido-nitrergic stress in male Wistar rat. Andrologia 54:e14508
30. Dissanayake D, Wijesinghe P, Ratnasooriya W, Wimalasena S (2009) Effects of zinc supplementation on sexual behavior of male rats. J Hum reproductive Sci 2:57
31. Narayana K, Prashanthi N, Nayanatara A, Kumar HHC, Abhilash K, Bairy K (2005) Effects of methyl parathion (o, o-dimethyl o-4-nitrophenyl phosphorothioate) on rat sperm morphology and sperm count, but not fertility, are associated with decreased ascorbic acid level in the testis. Mutat Research/Genetic Toxicol Environ Mutagen 588:28–34
32. Imeryuz N, Tahan V, Sonsuz A, Eren F, Uraz S et al (2007) Iron preloading aggravates nutritional steatohepatitis in rats by increasing apoptotic cell death. J Hepatol 47:851–859
33. de Bartolomeis A, Barone A, Begni V, Riva MA (2022) Present and future antipsychotic drugs: A systematic review of the putative mechanisms of action for efficacy and a critical appraisal under a

translational perspective. Pharmacol Res 176:106078

34. Goma AA, Tohamy HG, El-Kazaz SE, Soliman MM, Shukry M et al (2020) Insight study on the comparison between zinc oxide nanoparticles and its bulk impact on reproductive performance, antioxidant levels, gene expression, and histopathology of testes in male rats. Antioxidants 10:41
35. El-Kossi DM, Ibrahim SS, Hassanin KM, Hamad N, Rashed NA, Abdel-Wahab A (2023) Ameliorative effects of zinc oxide, in either conventional or nanoformulation, against bisphenol A toxicity on reproductive performance, oxidative status, gene expression and histopathology in adult male rats. Biol Trace Elem Res :1–15
36. Hojyo S, Fukada T (2016) Roles of zinc signaling in the immune system. *Journal of immunology research* 2016
37. Wessels I, Fischer HJ, Rink L (2021) Dietary and physiological effects of zinc on the immune system. Annu Rev Nutr 41:133–175
38. Bold J, Swinburne D (2022) Pre-conceptual guidelines for men: A review of male infertility experience, including nutrition and lifestyle factors. Dietetics 1:164–181
39. Davey KJ, O'Mahony SM, Schellekens H, O'Sullivan O, Bienenstock J et al (2012) Gender-dependent consequences of chronic olanzapine in the rat: effects on body weight, inflammatory, metabolic and microbiota parameters. Psychopharmacology 221:155–169
40. Socała K, Doboszewska U, Szopa A, Serefko A, Włodarczyk M et al (2021) The role of microbiota-gut-brain axis in neuropsychiatric and neurological disorders. Pharmacol Res 172:105840
41. Akram W, Syed M, Faruqi NA (2019) Histopathological and Histomorphometric studies on the effects of Olanzapine on Testis: An experimental study in Albino Rats. J Anat Soc India 68:196–200
42. Behrouzian F, Nazarinasab M, Sadegh AM, Abdi L, Sabzevarizadeh M (2022) Effects of zinc sulfate on schizophrenia symptoms in patients undergoing atypical antipsychotic pharmacotherapy. J Family Med Prim Care 11:7795–7799
43. Fanelli G, Franke B, Fabbri C, Werme J, Erdogan I et al (2024) Local patterns of genetic sharing challenge the boundaries between neuropsychiatric and insulin resistance-related conditions. *medRxiv*:2024.03. 07.24303921
44. Park B-Y, Yoo Y-J, Kim R-H, Jang Y-J, Lee JH et al (2023) The Effect of Olanzapine through Antioxidant and Anti-Inflammation on the Hippocampus in the Asphyxial Cardiac Arrest Rat Model
45. De Grande A, Leleu S, Delezie E, Rapp C, De Smet S et al (2020) Dietary zinc source impacts intestinal morphology and oxidative stress in young broilers. Poult Sci 99:441–453
46. Baloglu FK, Tas DG, Yilmaz O, Severcan F (2023) The recovery effect of Vitamin C on structural alterations due to Streptozotocin-Induced diabetes in rat testicular tissues. Spectrochim Acta Part A Mol Biomol Spectrosc 288:122149
47. Madireddy S, Madireddy S (2020) Regulation of reactive oxygen species-mediated damage in the pathogenesis of schizophrenia. Brain Sci 10:742

48. Zubair M (2017) Effects of dietary vitamin E on male reproductive system. Asian Pac J Reprod 6:145–150
49. Zerin Khan F, Sultana SP, Akhter N, Mosaddek ASM (2018) Effect of olanzapine and risperidone on oxidative stress in schizophrenia patients. Int Biol Biomedical J 4:89–97
50. Hadwan MH, Almashhedy LA, Alsalman ARS (2014) Study of the effects of oral zinc supplementation on peroxynitrite levels, arginase activity and NO synthase activity in seminal plasma of Iraqi asthenospermic patients. Reproductive Biology Endocrinol 12:1–8
51. Alahmar AT, Calogero AE, Singh R, Cannarella R, Sengupta P, Dutta S (2021) Coenzyme Q10, oxidative stress, and male infertility: A review. Clin experimental reproductive Med 48:97
52. Jahangard L, Yasrebifar F, Haghighi M, Ranjbar A, Mehrpooya M (2019) Influence of adjuvant Coenzyme Q10 on inflammatory and oxidative stress biomarkers in patients with bipolar disorders during the depressive episode. Mol Biol Rep 46:5333–5343
53. Liu J, Xiu M (2021) Plasma lysophosphatidylcholine and lysophosphatidylethanolamine levels were associated with the therapeutic response to olanzapine in female antipsychotics-naïve first-episode patients with schizophrenia. Front Pharmacol 12:735196
54. Molina Martín JD, Ávila S, Rubio G, López Muñoz F (2022) Metabolomic Connections between Schizophrenia. A Variety of Players, Antipsychotic Drugs and Metabolic Syndrome
55. Ben-Azu B, Adebayo OG, Jarikre TA, Oyovwi MO, Edje KE et al (2022) Taurine, an essential β-amino acid insulates against ketamine-induced experimental psychosis by enhancement of cholinergic neurotransmission, inhibition of oxidative/nitrergic imbalances, and suppression of COX-2/iNOS immunoreactions in mice. Metab Brain Dis 37:2807–2826
56. Franscescon F, Souza TP, Müller TE, Michelotti P, Canzian J et al (2021) Taurine prevents MK-801-induced shoal dispersion and altered cortisol responses in zebrafish. Prog Neuropsychopharmacol Biol Psychiatry 111:110399
57. Meem LA (2021) Effect of the nutritional supplements in mental disorders–a review. Brac University
58. Li Y, Peng Q, Shang J, Dong W, Wu S et al (2023) The role of taurine in male reproduction: Physiology, pathology and toxicology. Front Endocrinol 14:1017886
59. Yang M, Wang C, Zhao G, Kong D, Liu L et al (2023) Comparative analysis of the pre-and post-medication effects of antipsychotic agents on the blood-based oxidative stress biomarkers in patients with schizophrenia: a meta-analysis. Curr Neuropharmacol 21:340
60. Phelps TI, Bondi CO, Mattiola VV, Kline AE (2017) Relative to typical antipsychotic drugs, aripiprazole is a safer alternative for alleviating behavioral disturbances after experimental brain trauma. Neurorehabilit Neural Repair 31:25–33
61. Todorović Vukotić N, Đorđević J, Pejić S, Đorđević N, Pajović SB (2021) Antidepressants-and antipsychotics-induced hepatotoxicity. Arch Toxicol 95:767–789
62. Sharma K (2023) Antipsychotics Prevent Psychosine Induced Toxicity in Glial Cells. School of Medicine, Trinity College Dublin, Ireland

63. Weyh C, Krüger K, Peeling P, Castell L (2022) The role of minerals in the optimal functioning of the immune system. Nutrients 14:644
64. Xia LZ, Liu LL, Yue JZ, Lu ZY, Deng RY et al (2024) Ameliorative effects of zinc and vitamin E against phthalates-induced reproductive toxicity in male rats. Environ Toxicol
65. Joe P, Getz M, Redman S, Petrilli M, Kranz TM et al (2018) Serum zinc levels in acute psychiatric patients: A case series. Psychiatry Res 261:344–350
66. Hekmat A, Saboury AA, Saso L (2024) Superoxide dismutases inhibitors. *Metalloenzymes*:523 – 31: Elsevier. Number of 523 – 31 pp
67. Pascua AM, Barbisan G, Nikoloff N, Carranza-Martín AC, Fabra MC et al (2024) Effect of estrogen and progesterone on intracellular free zinc and zinc transporter expression in bovine oviduct epithelial cells. *Theriogenology*
68. Szabo R, Bodolea C, Mocan T (2021) Iron, copper, and zinc homeostasis: Physiology, physiopathology, and nanomediated applications. Nanomaterials 11:2958
69. Yan F, Li K, Xing W, Dong M, Yi M, Zhang H (2022) Role of iron-related oxidative stress and mitochondrial dysfunction in cardiovascular diseases. *Oxidative Medicine and Cellular Longevity* 2022
70. Iman T, Akram R, Javed MS, Rasul A, Sajid F et al (2023) Available Treatment Modules for Brain Disorders. *The Role of Natural Antioxidants in Brain Disorders*:111 – 48: Springer. Number of 111 – 48 pp
71. Mortimer KR, Katshu MZUH, Chakrabarti L (2023) Second-generation antipsychotics and metabolic syndrome: a role for mitochondria. Front Psychiatry 14:1257460
72. Pirc Marolt T, Kramar B, Vovk A, Podgornik H, Šuput D, Milisav I (2023) Therapeutic Dosage of Antipsychotic Drug Aripiprazole Induces Persistent Mitochondrial Hyperpolarisation, Moderate Oxidative Stress in Liver Cells, and Haemolysis. *Antioxidants* 12:1930
73. Soares MA, Costa ALA, Silva NL, Martins AF, Matias DO et al (2023) Atypical antipsychotics olanzapine and clozapine increase bone loss in female rats with experimental periodontitis. J Periodontal Res 58:283–295

Figures

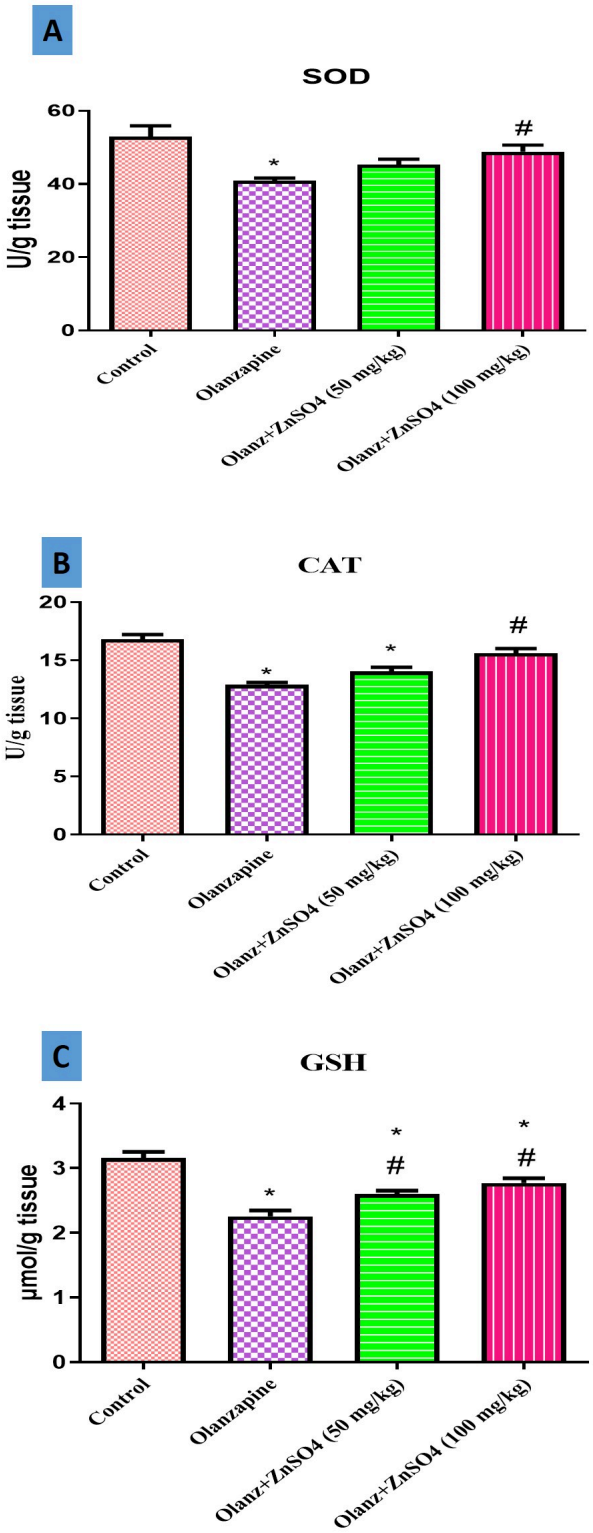


Figure 1

Bar charts showing the antioxidant enzyme concentration which a: concentration of (SOD), b: concentration of (CAT) and c: concentration of GSH, among four groups: Control; Olanzapine; Olanzapine treated with zinc sulfate (50 µg/body weight); and Olanzapine treated with zinc sulfate (100 µg/body weight). Values are represented as Mean ± STDV & n = 10 animals. Means within the same parameter

and not sharing a common superscript symbol(s) differ significantly at $p < 0.05$, and values that are recorded with non-significance difference (n.s).

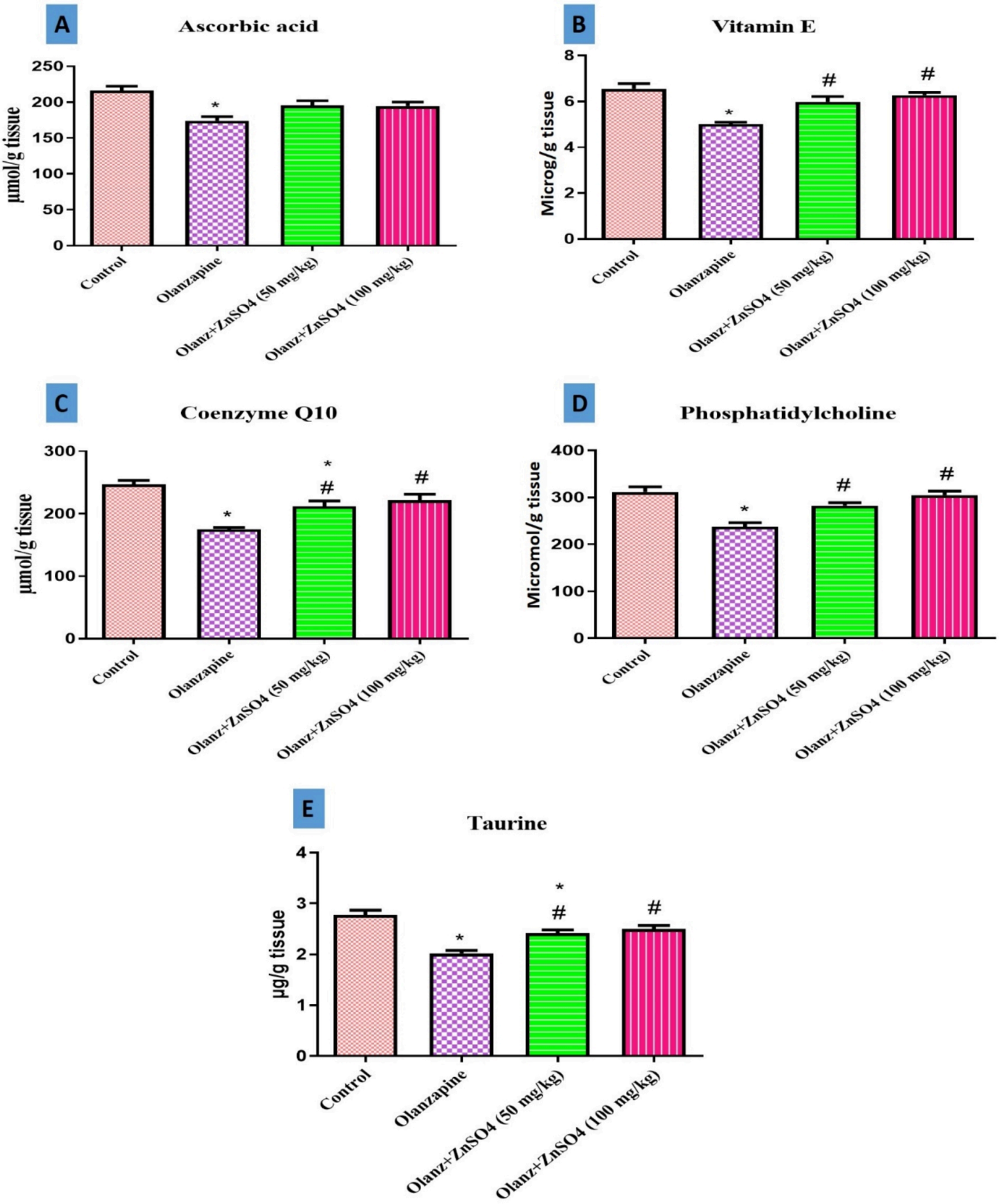


Figure 2

Bar charts showing a: ascorbic acid, b: Vitamin E, c: concentration of Coenzyme Q10, d: Phosphatidylcholine concentration and f: Concentration of Taurine among four the groups, namely, Control; Olanzapine among four groups: Control; Olanzapine; Olanzapine treated with zinc sulfate (50

µg/body weight); and Olanzapine treated with zinc sulfate (100 µg/body weight). Values are represented as Mean ± STDV & n = 10 animals. Means within the same parameter and not sharing a common superscript symbol(s) differ significantly at $p < 0.05$, and values that are recorded with non-significance difference (n.s).

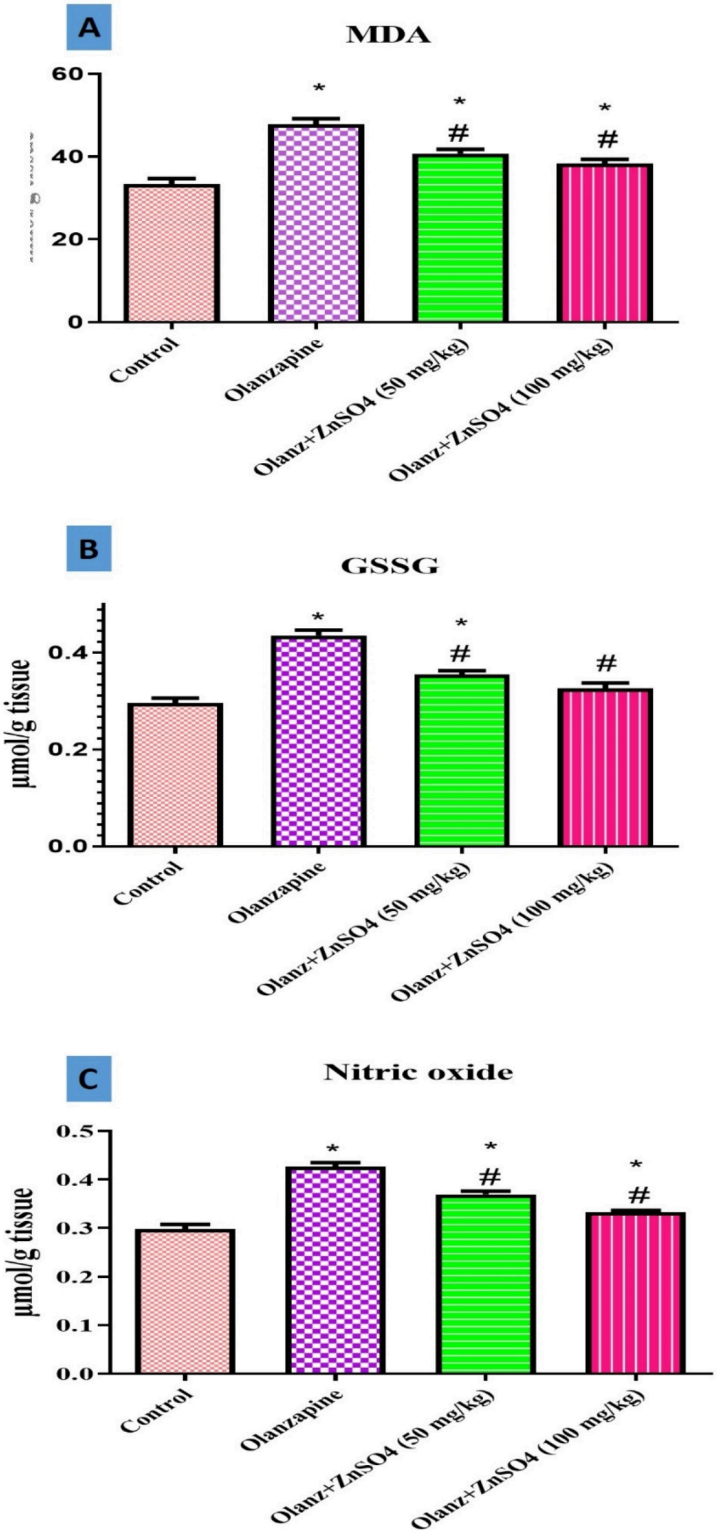


Figure 3

Bar charts showing the prooxidant concentration from where a: concentration of Malondialdehyde (MDA), b: concentration of GSSG, c: concentration of NO in Control, Olanzapine, Olanzapine treated with zinc sulfate (50 µg/body weight) or zinc sulfate (100 µg/body weight). Values are represented as Mean ± STDV & n = 10 animals. Means within the same parameter and not sharing a common superscript symbol(s) differ significantly at $p < 0.05$, and values that are recorded with non-significance difference (n.s).

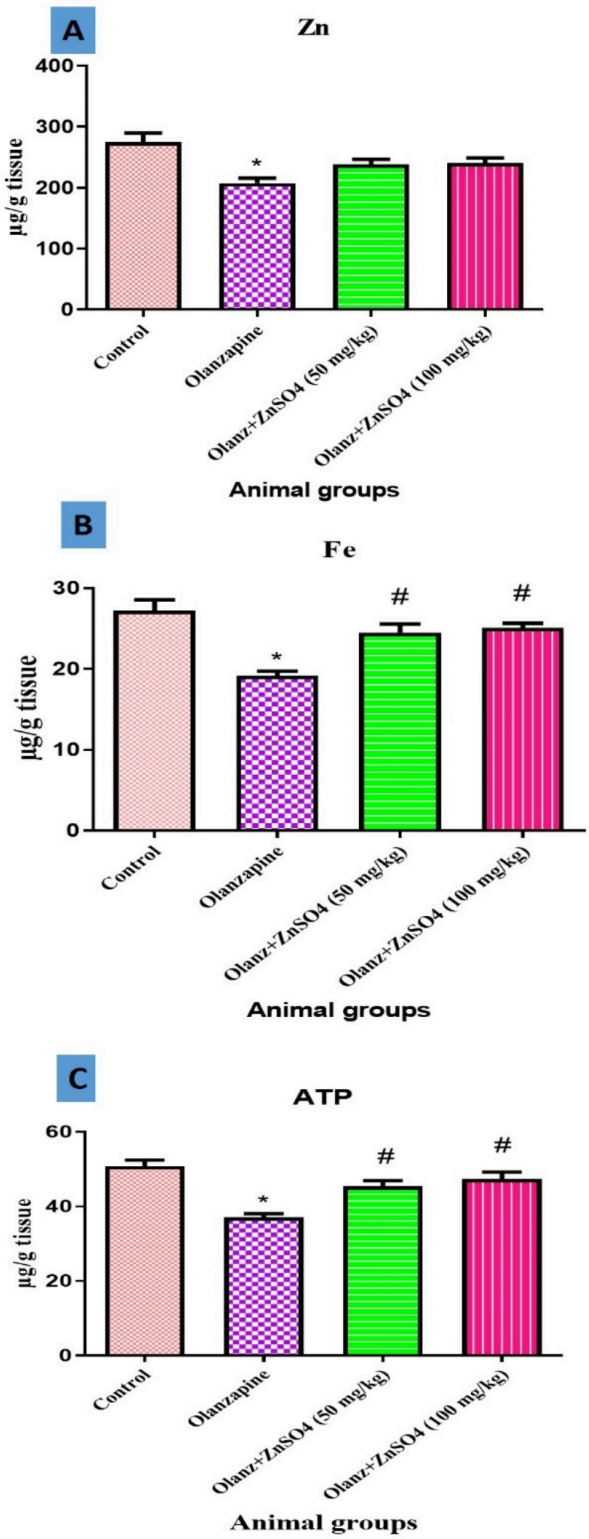


Figure 4

Bar charts showing the sperm analysis from where a: concentration of zinc, b: concentration of iron, c: concentration of ATP, in Control, Olanzapine, Olanzapine treated with zinc sulfate (50 µg/body weight) or zinc sulfate (100 µg/body weight). Values are represented as Mean ± STDV & n = 10 animals. Means within the same parameter and not sharing a common superscript symbol(s) differ significantly at p < 0.05, and values that are recorded with non-significance difference (n.s).

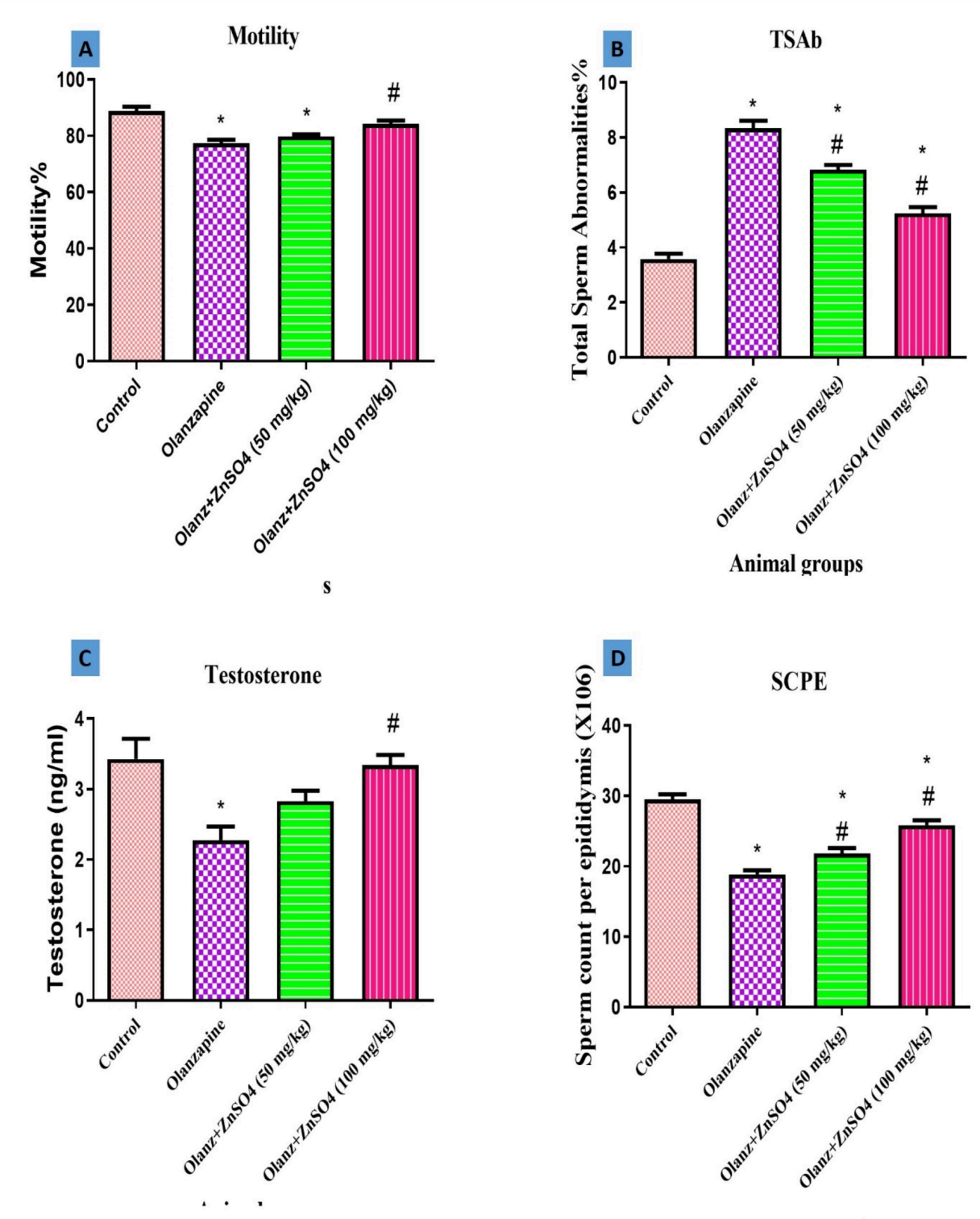


Figure 5

Bar charts showing the sperm analysis such as a: percentage of motility, b: percentage of total sperm abnormalities, c: concentration of testosterone, d: sperm count epididymis in Control, Olanzapine, Olanzapine treated with zinc sulfate (50 µg/body weight) or zinc sulfate (100 µg/body weight). Values are represented as Mean ± STDV & n = 10 animals. Means within the same parameter and not sharing a common superscript symbol(s) differ significantly at $p < 0.05$, and values that are recorded with non-significance difference (n.s).

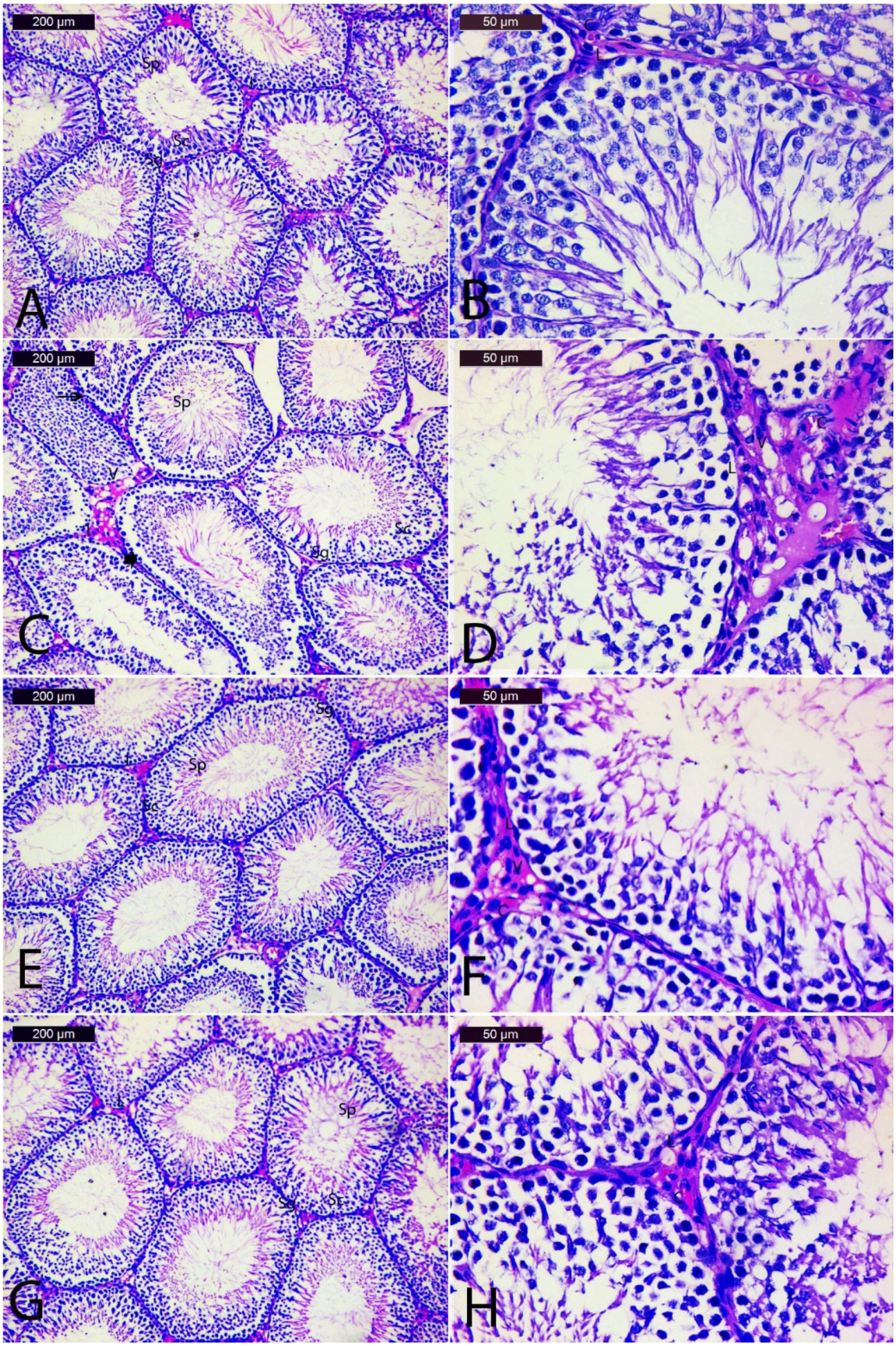


Figure 6

A: A photomicrograph of a testicular section of a control group showing a seminiferous tubule demonstrating different types of germinal epithelial cells; spermatogonia (Sg), primary spermatocytes (SC), rounded spermatids (RS), surrounded by thin basement membrane. **B:** A photomicrograph of a testicular section of a control group showing narrow lumina with normally Leydig cells (L). **C:** A photomicrograph of a testicular section from Olz group showing disturbed architecture of seminiferous tubules, degenerated and exfoliation (arrowhead) germinal epithelium of a seminiferous tubule (Sg, Sc & Sp), with moderately thickened basement membrane (arrow). **D:** A photomicrograph of a testicular section of from Olz group showing wide interstitial tissue with few Leydig cells (L) and vacuoles (V). **E:** Testicular section of Olz+ 50mg/Kg $ZnSO_4$ group showed moderate improvement apparently of testicular architecture- seminiferous tubules lined with germinal epithelium; spermatogonia (SG) primary spermatocytes (SC), rounded spermatids (RS) resting on thin basement membrane. Other seminiferous tubule is seen with more or less normal spermatogenic cell line. Note mild wide interstitial tissue with few Leydig cells. **F:** A photomicrograph of a testicular section of from Olz+ 50mg/Kg $ZnSO_4$ group showing mild wide interstitial tissue with few Leydig cells and few vacuoles (V). **G,H:** A photomicrograph of a testicular section from Olz+ 100mg/Kg $ZnSO_4$ group showing apparently nearly normal testicular architecture- seminiferous tubules lined with germinal epithelium; spermatogonia (SG) primary spermatocytes (SC), rounded spermatids (RS) resting on thin basement.