Egyptian Journal of Medical Research

Print ISSN: 2682-4396 / Online ISSN: 2682-440X

Evaluation Of The Reproductive Toxicity Of Olanzapine And The Possible Protective Effect Of Vitamin E On Male Albino Rats

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Article Info

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Keywords
Olanzapine
Reproductive Toxicity
Vitamin E
Oxidative stress
testosterone hormone.

Abstract:

Atypical antipsychotics like olanzapine (OLZ) are standard treatments for schizophrenia and bipolar disorder. Atypical antipsychotics, or OLZ, have been associated with toxicity to the male reproductive system and sexual dysfunction over the long run. This study aims to examine the potential adverse effects of olanzapine on male albino rats' reproductive systems and possible preventive effect of vitamin E. The study included seven groups of ten adult male albino rats, one of which served as a control group that received nothing more than distilled water. For a duration of eight weeks, the remaining six groups of rats were given oral doses of OLZ (2.5, 5, or 10 mg/kg/d) or OLZ with vitamin E (200 mg/kg/d). We measured the testicles' and the body's mass in addition to sperm count and motility. As well serum levels of luteinizing hormone, follicle-stimulating hormone, and testosterone. Oxidative stress markers such as reduced glutathione (GSH), superoxide dismutase (SOD), and

malondialdehyde (MDA) were evaluated in testicular tissue. Testicular tissue was examined histopathologically. The current investigation demonstrated that OLZ reduced sperm count and motility. It was shown that OLZ decreased blood levels of testosterone, FSH, and LH. While OLZ increased MDA levels, it also reduced the activities of SOD, CAT, and GSH in testicular tissue. Further examination using histopathology indicated that the seminiferous tubules had also deteriorated. These alterations were improved when vitamin E and OLZ were administered together. **Conclusion:** The male reproductive toxicity caused by OLZ seems to be mitigated by vitamin E.

1. Introduction:

For the acute treatment of psychosis, bipolar disorder, and schizophrenia, physicians use olanzapine (OLZ), an atypical (second-generation) antipsychotic medication. (1). Olanzapine acts as an antagonist for dopamine and serotonin and also has anticholinergic and antihistaminic properties (2). People with psychiatric disorders and/or who use psychotropic drugs are just as likely as the general population to have sexual dysfunction (3).

Low testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and gonadotropin-releasing hormone (GnRH) are all caused by high prolactin levels, which can cause hypogonadism. This can lead to alterations to the testis's morphology, a postponement of spermatogenesis, and impaired sperm motility and quality of semen (4).

Prior studies have shown a connection between antipsychotic drug side effects and oxidative stress, as well as the concurrent production of reactive oxygen species (ROS) (5,6).

Oxidative stress is One of the most common pathogenic processes in male infertility which damages sperm (7). The extensive presence of polyunsaturated fatty acids (PUFA) in the plasma membrane of sperm makes it susceptible to oxidation. (8).

Vitamin E (alpha-tocopherol) is a lipophilic antioxidant which prevents damage to phospholipid (PUFA), lipoprotein (protein), cellular membrane, and intracellular membranes (9). Vitamin E's antioxidant properties make it useful in preventing lipid peroxidation and oxygen free radical damage to vital cellular components. (10).

This study aims to examine the potential adverse effects of olanzapine on male albino rats' reproductive systems and possible preventive effect of vitamin E.

2. Materials and Methods:

A- Materials

Drugs and chemicals used

1- **Olanzapine** (olapex 5mg & 10mg, Apex pharma, Egypt).
2- **Vitamin E** (1000mg capsules, Pharco pharmaceuticals, Alexandria, Egypt).
3- **Bouin′s solution**
4- **Diethyl ether solution.**

Animals used

Seventy adult male albino rats weighing (230-260 gm) and matched for age (3.5- 4 months) were used. Breeding rats from the University of Science Beni-Suef's animal home. Adaptation was accomplished by keeping the animals for 14 days prior to conducting laboratory studies. The experimental procedures involving the administration of drugs and the handling of animals were conducted in compliance with the regulations established by the local ethical committee at the Faculty of Medicine at Beni-Suef University. Following these guidelines is consistent with National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals (Approval No.021-196), which is put out by the National Institutes of Health. The animals were kept at a constant temperature of 26°C in separate cages with five individuals per pen. They were also treated to a 12-hour light-dark cycle. Food and water are available to everyone at all times.

B- Methods:

Experimental Design:

Each of the seven groups, consisting of 10 rats each, was assigned animals at random.

Group (1) control group: received distilled water 5 ml/kg, orally for 8 weeks.

Group (2) rats received 2.5 mg/kg/day olanzapine (11).

Group (3) rats received 5 mg/kg/ day olanzapine.

Group (4) rats received 10 mg/kg/day olanzapine.

Group (5) rats received 2.5 mg/kg/d olanzapine plus 200 mg/kg/d vitamin E (12).

Group (6) rats received 5 mg/kg/d olanzapine &200 mg/kg/d vitamin E.

Group (7) rats received 10 mg/kg/d olanzapine &200 mg/kg/d vitamin E.

- All administered drugs were given daily by oral syringe for 8 weeks.

-Determination of the body weight and testicle weight of each rat:

At the conclusion of the study, the body weight of each rat in the various groups was recorded. Subsequent to scarification, the mass of the testes was evaluated.

-Collection of serum blood samples

Following intravenous sedation with Diethyl ether solution, venous blood samples were collected from the retro-orbital plexus of each animal using heparinized micro-tubes at the conclusion of the experiment. For the purpose of measuring, sera were separated by centrifuging blood at 3000 Xg for 20 minutes and then kept at -80° C (13) until needed for the following measurements

A-Serum Follicle-stimulating hormone (FSH)

B-Luteinizing hormone (LH)

C-Testosterone hormone

-Measurement of oxidative stress biomarkers

The testis and epididymis were surgically removed after blood samples were taken. After homogenizing the left testis, the following biochemical parameters were measured in the resulting homogenate:

1-Glutathione (GSH)

2-Catalase (CAT)

3-Superoxide dismutase (SOD)

4-Malondialdhyde (MDA)

Which were assessed using readily accessible commercial assays

-Sperm count & motility

The motility and concentration of sperm were evaluated using a hemocytometer and a light microscope (14).

- Histopathological examination of the testis.

Histopathological examination of right testis tissue samples was conducted using hematoxylin and eosin (H&E) staining following fast fixation in Bouin's solution (15).

Statistical analysis

The SPSS statistical programs were used for all statistical calculations. The data was statistically characterized using mean and standard deviation (SD). In order to characterize the quantitative data, descriptive statistics such as standard deviation and mean were used. When three or more sets of quantitative data are examined using the analysis of variance (ANOVA) test, inferences regarding the relationships among the groups may be derived. We employed Tukey Post hoc HSD, version 27, to compare

the two groups. Results were deemed significant if their p-values were below 0.05.

3. Results:

A. Body weight

The mean body weight of OLZ (5mg/kg) & OLZ (10mg/kg) showed significant lower value compared to that of the control group. Also, body weight was decreased in higher doses of OLZ (5mg/kg) & (10mg/kg) compared to OLZ (2.5 mg /kg) (Table: 1). Administration of vitamin E with OLZ in group 6 showed significantly higher values compared to OLZ alone in group 3. (Table: 1).

Table (1) Comparison between the studied groups regarding the body weight:

Body weight (gm) / Groups	Mean±SD
Group 1 Control group	**268.6±13.3**
Group 2 (Olanzapine (2.5 mg/kg)	**250.8±10.9**
Group 3 (Olanzapine (5mg/kg)	**215.0±15.8 ɤ ¥**
Group 4 (Olanzapine (10mg/kg)	**222.6±15.9 ɤ ¥**
Group 5 (Olanzapine (2.5mg/kg)+vitamin E)	**266.0±17.1**
Group 6 (Olanzapine (5mg/kg)+vitamin E)	**250.2±10.6 ¶**
Group 7 (Olanzapine (10mg/kg)+vitamin E)	**250.0±10.9**

Values are expressed as mean ± SD, n= 10 rats
ɤ: significant difference with controls at p <0.05
¥: significant difference with group 2 at p <0.05
¶: significant difference with group 3 at p <0.05

B- Testes weight

The mean testicular weight in OLZ (10mg/kg) showed significant lower value compared to that of the control group & OLZ (2.5mg/kg) (Table: 2).

Table (2) Comparison between the studied groups regarding the testes weight:

Testes weight (gm) / Groups	Mean±SD
Group 1 Control group	**6.5±0.3**
Group 2 (Olanzapine (2.5 mg/kg)	**6.2±0.5**
Group 3 (Olanzapine (5mg/kg)	**5.5±0.3**
Group 4 (Olanzapine (10mg/kg)	**4.9±0.5 ɤ ¥**
Group 5 (Olanzapine (2.5mg/kg)+vitamin E)	**5.7±0.9**
Group 6 (Olanzapine (5mg/kg)+vitamin E)	**5.4±0.3 ɤ**
Group 7 (Olanzapine (10mg/kg)+vitamin E)	**4.8±0.6 ɤ**

C- Sperm Count

The mean sperm count in groups No. 2, 3, 4, 5, 6 and 7 showed significant lower values compared to that of the control group. The mean sperm count was significantly decreased with increasing doses of OLZ. (Table: 3).

Coadministration of Vit E with OLZ resulted in a significant improvement in the mean sperm count compared to that in the groups which received OLZ alone (Table: 3)

Table (3) Comparison between the studied groups regarding the Sperm Count (X106/ml):

Sperm Count ($X10^6$/ml) / Groups	Mean±SD
Group 1 Control group	**51.0±3.4**
Group 2 (Olanzapine (2.5 mg/kg)	**31.8±2.3 ɤ**
Group 3 (Olanzapine (5mg/kg)	**24.6±3.6 ɤ ¥**
Group 4 (Olanzapine (10mg/kg)	**14.8±2.6 ɤ ¥ ¶**
Group 5 (Olanzapine (2.5mg/kg)+vitamin E)	**43.2±1.9 ɤ ¥**
Group 6 (Olanzapine (5mg/kg)+vitamin E)	**38.6±2.9 ɤ ¶**
Group 7 (Olanzapine (10mg/kg)+vitamin E)	**32.4±3.0 ɤ £**

£: significant difference with group 4 at p <0.05

D- Sperm motility

The results showed a significant decrease in mean sperm motility in OLZ (2.5mg/kg, 5 mg/kg& 10 mg/kg) compared to that of the control group (Table: 4).

The mean sperm motility in OLZ 10mg/kg showed significant lower value compared to that of OLZ 2.5mg/kg and OLZ 5 mg/kg (Table: 4). Administration of Vit E with OLZ showed a significant improvement in mean sperm motility compared to that of the groups which received OLZ alone (Table: 4).

Table (4) Comparison between the studied groups regarding the Sperm Motility (%):

Sperm motility (%) / Groups	Mean±SD
Group 1 Control group	**76.2±5.1**
Group 2 (Olanzapine (2.5 mg/kg)	**47.4±4.0 ɤ**
Group 3 (Olanzapine (5mg/kg)	**36.8±5.1 ɤ**
Group 4 (Olanzapine (10mg/kg)	**22.4±4.0 ɤ ¥ ¶**
Group 5 (Olanzapine (2.5mg/kg)+vitamin E)	**65.8±3.4 ¥**
Group 6 (Olanzapine (5mg/kg)+vitamin E)	**57.2±7.9 ɤ ¶**
Group 7 (Olanzapine (10mg/kg)+vitamin E)	**43.0±8.7 ɤ £**

E-Results of Hormonal Assay

I. Testosterone hormone level

The current results showed a significant decrease in mean serum testosterone hormone level in OLZ administered groups compared to that of the control group. The current results showed that mean serum testosterone level was significantly decreased in higher doses of OLZ (10mg/kg & 5mg/kg) in comparison with OLZ 2.5mg/kg (Table: 5).

Administration of Vit E with OLZ had improved serum testosterone level when compared to that which received OLZ alone (Table: 5).

Table (5) Comparison between the studied groups regarding the serum testosterone (ng/ml):

Serum testosterone (ng/ml): / Groups	Mean±SD
Group 1 Control group	**1.798±0.041**
Group 2 (Olanzapine (2.5 mg/kg)	**0.714±0.039 ɤ**
Group 3 (Olanzapine (5mg/kg)	**0.584±0.041 ɤ ¥**
Group 4 (Olanzapine (10mg/kg)	**0.438±0.038 ɤ ¥ ¶**
Group 5 (Olanzapine (2.5mg/kg)+vitamin E)	**1.516±0.098 ɤ ¥**
Group 6 (Olanzapine (5mg/kg)+vitamin E)	**1.396±0.103 ɤ ¶**
Group 7 (Olanzapine (10mg/kg)+vitamin E)	**1.392±0.037 ɤ £**

II. Serum Follicle-stimulating hormone (FSH) level

The current results showed a significant decrease in mean serum FSH hormone level in OLZ administered groups compared to that of the control group. The present results showed that mean serum FSH level was significantly decreased in higher doses of OLZ (5mg/kg& OLZ 10mg/kg compared to OLZ 2.5mg/kg) (Table: 6).

The present study showed that there was improvement in mean serum FSH level on administration of Vit E with OLZ (Table: 6).

Table (6) Comparison between the studied groups regarding the serum FSH (mIU/ml):

FSH (mIU/ml) / Groups	Mean±SD
Group 1 Control group	**6.058±0.067**
Group 2 (Olanzapine (2.5 mg/kg)	**4.590±0.476 ɤ**
Group 3 (Olanzapine (5mg/kg)	**3.474±0.247 ɤ ¥**
Group 4 (Olanzapine (10mg/kg)	**2.800±0.144 ɤ ¥**
Group 5 (Olanzapine (2.5mg/kg)+vitamin E)	**5.448±0.926**
Group 6 (Olanzapine (5mg/kg)+vitamin E)	**4.466±0.331 ɤ ¶**
Group 7 (Olanzapine (10mg/kg)+vitamin E)	**4.454±0.118 ɤ £**

III. Serum Luteinizing hormone (LH)

The current results showed a significant decrease in mean serum LH hormone level in OLZ administered groups compared to that of the control group. (Table: 7). The current results showed that mean serum LH level was significantly decreased in higher doses of OLZ (Table: 7).

The present study showed that there was improvement in mean serum LH level on administration of Vit E with OLZ (Table: 7).

Table (7) Comparison between the studied groups regarding the serum LH (mIU/ml):

LH (mIU/ml) / Groups	Mean±SD
Group 1 Control group	**2.128±0.037**
Group 2 (Olanzapine (2.5 mg/kg)	**1.640±0.170 ɤ**
Group 3 (Olanzapine (5mg/kg)	**1.242±0.091 ɤ ¥**
Group 4 (Olanzapine (10mg/kg)	**0.754±0.054 ɤ ¥ ¶**
Group 5 (Olanzapine (2.5mg/kg)+vitamin E)	**1.950±0.330 ¥**
Group 6 (Olanzapine (5mg/kg)+vitamin E)	**1.598±0.116 ɤ ¶**
Group 7 (Olanzapine (10mg/kg)+vitamin E)	**1.592±0.044 ɤ £**

F-Results of oxidative stress biomarkers

1) Superoxide dismutase (SOD) in testicular tissue.

The current study showed a significant decrease in the mean testicular SOD in all groups received OLZ compared to that of the control group. Comparing the mean testicular SOD level in (5mg/kg&10mg/kg) OLZ administered groups to that of OLZ 2.5mg/kg showed significant lower values (Table: 8).

The present study showed that coadministration of Vit E with OLZ had improved the mean testicular SOD level (Table:8)

Table (8) Comparison between the studied groups regarding the testicular SOD level (U/g):

SOD level (U/g) / Groups	Mean±SD
Group 1 Control group	**2.618±0.133**
Group 2 (Olanzapine (2.5 mg/kg)	**1.272±0.058 ɤ**
Group 3 (Olanzapine (5mg/kg)	**1.016±0.059 ɤ ¥**
Group 4 (Olanzapine (10mg/kg)	**0.790±0.071 ɤ ¥**
Group 5 (Olanzapine (2.5mg/kg)+vitamin E)	**2.262±0.249 ɤ ¥**
Group 6 (Olanzapine (5mg/kg)+vitamin E)	**1.986±0.108 ɤ ¶**
Group 7 (Olanzapine (10mg/kg)+vitamin E)	**1.856±0.067 ɤ £**

2) Catalase (CAT) in testicular tissue.

The present study showed a significant decrease in the mean testicular CAT level in all groups received OLZ compared to that of the control group. The current results showed that mean testicular CAT level was significantly decreased in higher doses of OLZ (10mg/kg & 5mg/kg) compared to that of OLZ 2.5mg/kg (Table: 9).

The present study showed that coadministration of Vit E with OLZ had improved the mean testicular CAT level (Table:9).

Table (9) Comparison between the studied groups regarding the testicular CAT level (U/g):

CAT level (U/g) / Groups	Mean±SD
Group 1 Control group	**2.520±0.096**
Group 2 (Olanzapine (2.5 mg/kg)	**1.186±0.098 ɤ**
Group 3 (Olanzapine (5mg/kg)	**0.948±0.084 ɤ ¥**
Group 4 (Olanzapine (10mg/kg)	**0.686±0.051 ɤ ¥ ¶**
Group 5 (Olanzapine (2.5mg/kg)+vitamin E)	**2.120±0.208 ɤ ¥**
Group 6 (Olanzapine (5mg/kg)+vitamin E)	**1.866±0.078 ɤ ¶**
Group 7 (Olanzapine (10mg/kg)+vitamin E)	**1.760±0.099 ɤ £**

3) Malondialdhyde (MDA) level in testicular tissue.

The current study showed that the mean MDA level in testicular tissue in OLZ (2.5mg/kg, 5mg/kg &10mg/kg) showed a significant increase compared to that of the control group. The present results showed that mean testicular MDA level was significantly increased in higher doses of OLZ (10mg/kg &5mg/kg) compared to that of OLZ administered group 2.5mg/kg. coadministration of Vit E with OLZ had decreased the mean testicular MDA level (Table: 10).

Table (10) Comparison between the studied groups regarding the testicular MDA level (nmol/g):

MDA level (nmol/g) / Groups	Mean / Std. Deviation
Group 1 Control group	**0.864±0.071**
Group 2 (Olanzapine (2.5 mg/kg)	**1.876±0.132 ɤ**
Group 3 (Olanzapine (5mg/kg)	**2.366±0.127 ɤ ¥**
Group 4 (Olanzapine (10mg/kg)	**2.722±0.084 ɤ ¥ ¶**
Group 5 (Olanzapine (2.5mg/kg)+vitamin E)	**0.828±0.167 ¥**
Group 6 (Olanzapine (5mg/kg)+vitamin E)	**0.866±0.061 ¶**
Group 7 (Olanzapine (10mg/kg)+vitamin E)	**1.060±0.190 £**

4) Reduced glutathione (GSH) level in testicular tissue.

The mean tissue level of GSH in OLZ administered groups showed a significant decrease in the tissue level of GSH compared to that of control group values. The highest dose of OLZ resulted in significant lower value in mean testicular GSH level when compared to the lower ones (Table: 11).

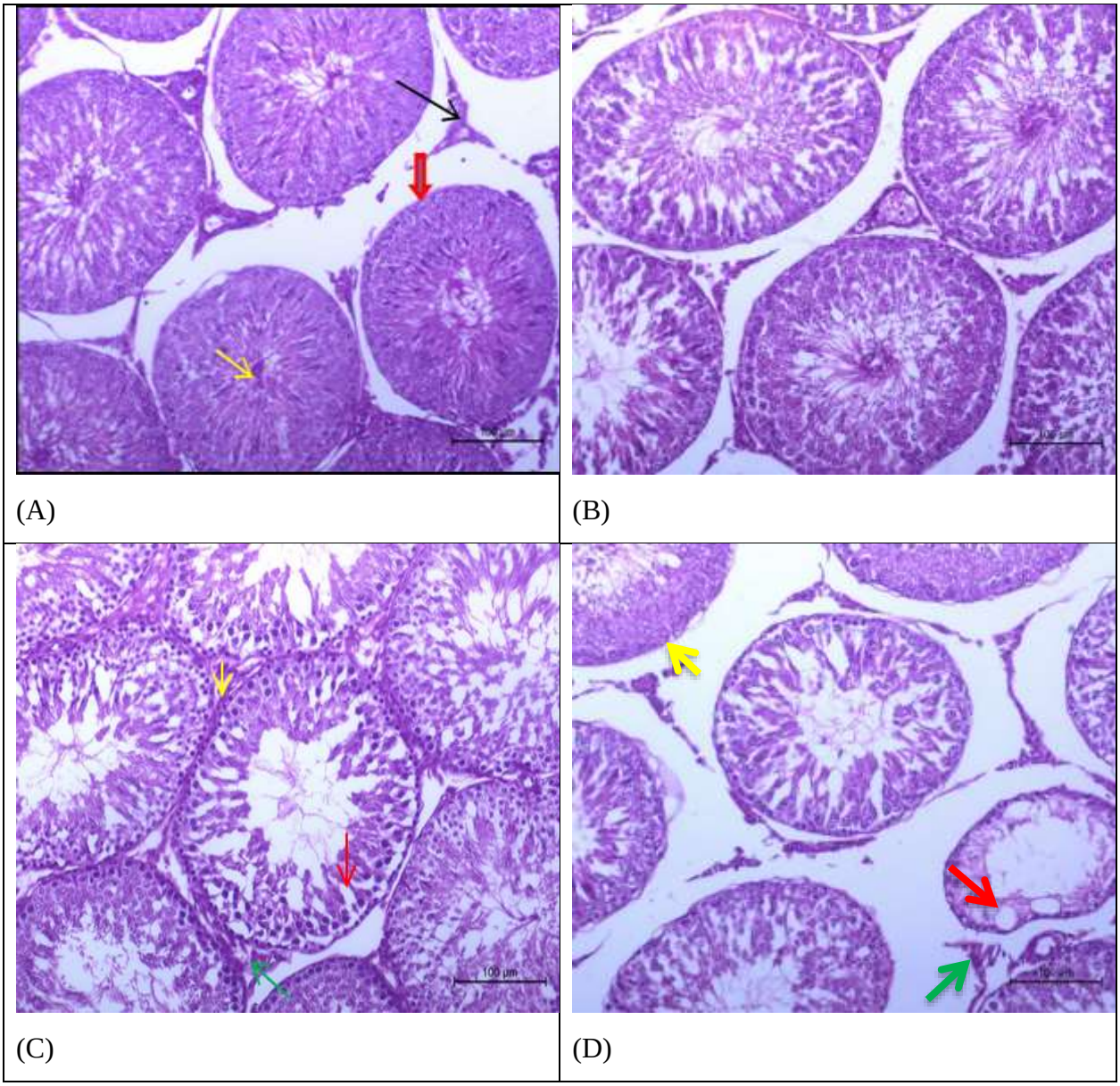
Administration of Vit E with OLZ had improved testicular GSH level (Table: 11).

Table (11) Comparison between the studied groups regarding the testicular GSH (mmol/g):

GSH (nmol/g) / Groups	Mean / Std. Deviation
Group 1 Control group	**2.364±0.063**
Group 2 (Olanzapine (2.5 mg/kg)	**1.080±0.111 ɤ**
Group 3 (Olanzapine (5mg/kg)	**0.898±0.0788 ɤ**
Group 4 (Olanzapine (10mg/kg)	**0.658±0.051 ɤ ¶ ¥**
Group 5 (Olanzapine (2.5mg/kg)+vitamin E)	**2.030±0.181 ¥**
Group 6 (Olanzapine (5mg/kg)+vitamin E)	**1.798±0.066 ¶**
Group 7 (Olanzapine (10mg/kg)+vitamin E)	**1.704±0.1085 £**

G- Results of histopathological study

Histological analysis was performed on tissue samples obtained from both the control and experimental groups of rats. The following data was logged.



(A)

(B)

(C)

(D)

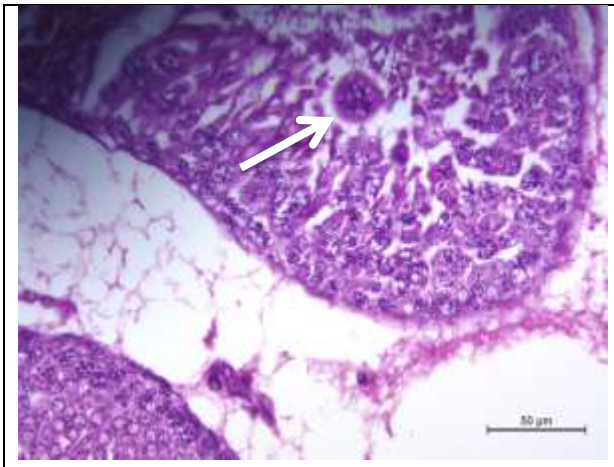
(A): Section of testicular tissues of adult male albino rats of the Control group

Seminiferous tubules (red arrow) lined with spermatogenic cells and sertoli cells and packed with a high quantity of sperm were seen in slices of typical testicular tissues (yellow arrow). The Leydig interstitial cells (black arrow) seemed healthy. (H&E X200).

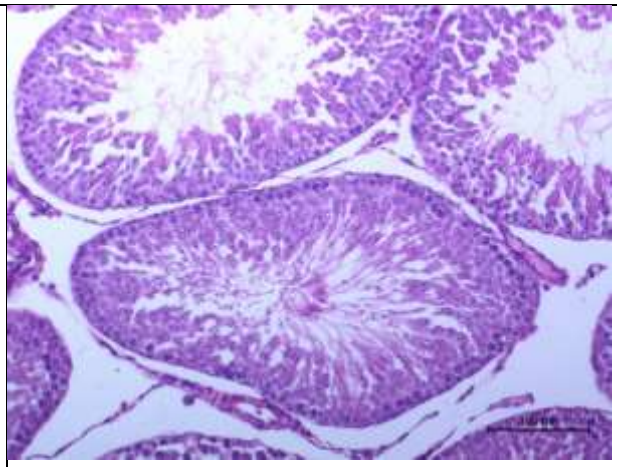
(B): Testicular tissue from OLZ 2.5 mg/kg showed very minor vacuolar degenerative alterations of the tubular epithelia in H and E staining.

(C): Testicular tissue from OLZ 5 mg/kg showed Moderate testicular degeneration (vacuolation (red arrow), basement membrane thickening (yellow arrow), and necrotic alterations (green arrow) (H&E X200).

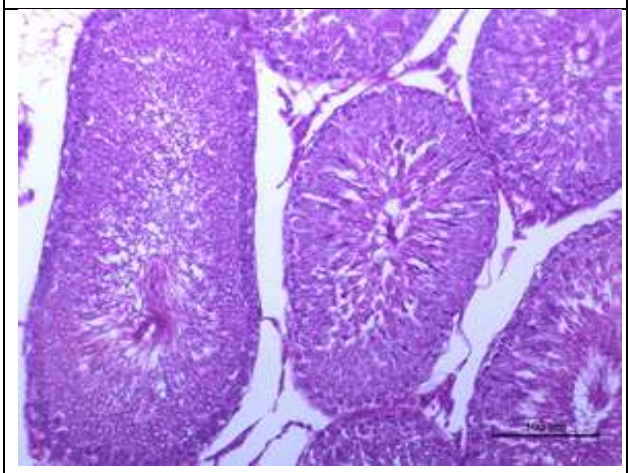
(D): Testicular degeneration, vacuolation (red arrow), basement membrane thickening (yellow arrow), and necrotic alterations (green arrow) OLZ 10 mg (H&E X200).



(E)



(G)



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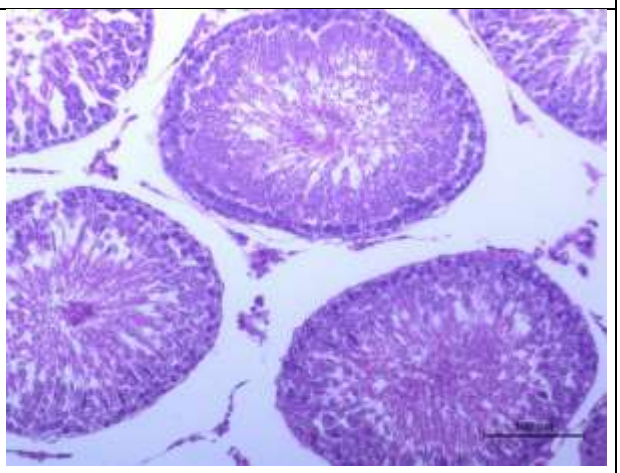

(H)

(E): Multinucleated giant cells (White arrow) are seen in a tissue slice of the testicles of OLZ 10 mg/kg-treated rats (H&E X400).

(F): Testicular tissue sections stained with H and E from rats given 2.5 mg OLZ with Vit E showed improvement compared to those from animals in group 2 (H&E X200).

(G): Sections of testicular tissue from rats given 5 mg OLZ with vitamin E revealed a significant reduction in testicular pathology compared to animals given 5 mg OLZ alone. The amelioration involved the tubular germinal epithelia and the interstitial cells of Leydig) (H&E X200).

(H): Testicular pathology improved significantly in rats given 10 mg/kg OLZ with vitamin E, compared to rats given 10 mg/kg OLZ alone. The amelioration involved the tubular germinal epithelia and the interstitial cells of Leydig) (H&E X200).

4. Discussion:

One of the most troubling side effects of antipsychotic medication is an increased risk of sexual dysfunction in patients on these medications. (16,17)

Psychological disorders such as schizophrenia and bipolar disorder may be treated with the atypical antipsychotic olanzapine (OLZ) (18). Although its binding for alpha 2, 5-HT 1D, and 5-HT1A receptors is low, OLZ has a strong affinity for muscarinic, alpha 1, and D2 receptors. (19).

The purpose of this study is to demonstrate the reproductive system-harming effects of olanzapine and the protective impact of vitamin E in male albino rats.

The present study indicated that the experimental animal groups had weight loss after the administration of OLZ. The group given high doses of OLZ (5 & 10 mg/kg) exhibited a substantial drop in weight compared to the groups receiving lesser dosages of OLZ.

The findings corroborate those of the research of De Siqueira Bringel et al. 2013(20), which also discovered that therapy with high doses of OLZ resulted in weight reduction. Consistent with this, research found that OLZ therapy caused sleepiness and somnolence, which may be related to the sedative effects seen in humans and result in less frequent eating. For quite some time, changes in the weight of organs and tissues have been acknowledged as a sign of organ-related toxicity.

França and Russell (1998) (21) state that testicular weight is an important indicator of a man's reproductive health because of the positive correlation between the two. Testes in the OLZ (10 mg/kg) group were found to be much lighter than those in the control group and those given OLZ at a dose of 2.5 mg/kg in this study.

The findings align with a research by De Siqueira Bringel et al. (2013) (20), which demonstrated that adult rats administered 10 mg/kg OLZ for 45 days had reduced testosterone levels, perhaps explaining the smaller than anticipated size of their testicles and epididymis.

The results of this study showed that the groups given OLZ (2.5, 5, and 10 mg/kg) or vitamin E (2.5, 5, and 10 mg/kg) had a significantly lower mean sperm count compared to the control group. The results demonstrated that, relative to the control group, all groups receiving OLZ (2.5, 5, 10 mg/kg and OLZ 5 mg/kg and 10 mg/kg with Vitamin E) exhibited significantly decreased mean sperm motility, with the reduction

being more pronounced at elevated OLZ dosages.

These findings corroborate those of earlier researchers (Ardç et al., 2021) (11) who discovered a lower sperm concentration in groups given OLZ compared to the control group.

The current findings further demonstrate that supplementing OLZ with vitamin E significantly increases sperm count compared to OLZ alone. Khorramabadi et al. (2019) (22) shown that the antioxidant capabilities of vitamin E may safeguard sperm DNA from oxidative degradation in the rat testis.

The present study found that compared to the control group, all OLZ treatment groups, particularly those given the highest dosage, and all OLZ groups given vitamin E had significantly lower blood testosterone levels. The known drops in testosterone levels in adult rats could be explained by the deterioration of the Leydig cells, which produce the hormone testosterone (23). The levels of the hormone testosterone were significantly higher in the groups who received both OLZ and vitamin E compared to the groups that received only OLZ.

The current findings show that serum follicle-stimulating hormone levels were significantly lower in the groups given OLZ (2.5, 5, 10 mg/kg), as well as in the groups given OLZ (5 and 10 mg/kg) in combination with vitamin E, compared to the control group. The OLZ had much reduced FSH levels (10 mg/kg) compared to the OLZ (2.5 mg/kg).

The results are in agreement with Ardç et al., 2021(11), who found that the groups given OLZ had much lower FSH levels than the control group.

The current findings show that compared to the control group, all groups that got OLZ (2.5, 5, 10mg/kg & OLZ 5 and 10mg/kg with Vit E) had significantly lower mean serum luteinizing hormone levels. Additionally, there was a notable difference in LH levels between the OLZ (2.5 mg/kg) and the OLZ (10 mg/kg).

Similarly, Zhang et al., 2020 (24), who administered OLZ for 8 weeks, found that the treatment group's blood FSH and LH levels were significantly lower than the control group's. An essential set of hormones that control the spermatogenesis process include gonadotropin-releasing hormone, luteinizing hormone, and follicle-stimulating hormone. The reduction of GnRH, which in turn decreases FSH, LH, and testosterone, is caused by hyperprolactinemia, which can cause infertility and reproductive dysfunction. (25). The findings showed

changes in reproduction after OLZ treatment, and hyperprolactinemia may have played a role in these changes.

Oxidative stress is the most common contributor to male infertility and germ line DNA damage. (26). In humans, oxidative stress is linked to reduced sperm motility and viability, as well as complications in sperm-oocyte fusion. (27 &28). The findings demonstrated that when OLZ was administered to rats, there was a significant reduction in superoxide dismutase (SOD) and catalase (CAT) levels in testicular tissue across all OLZ-treated groups as compared to the control group. Additionally, when comparing OLZ 2.5 mg/kg to 5 and 10 mg/kg, there is a statistically significant drop in SOD levels, and a similar trend is seen with CAT levels, which also fall significantly as OLZ dosage increases. Akintunde et al. (2018) (29), who found that adult male wistar rats had reduced levels of GSH after receiving an antipsychotic medication, was in agreement with these results. The researchers found that the testes of the rats had depleted antioxidant enzymes such CAT and SOD. The results were corroborated by a study conducted by Hamza et al., 2017(30), which indicated that SOD activity was higher in mice treated with both sodium azide (SA) and vitamin E compared to the SA group. This finding lends credence to the idea that supplementing OLZ with vitamin E improves testicular SOD and CAT levels.

The most abundant replicative process, spermatogenesis, requires a high rate of oxygen consumption and generates significant quantities of reactive oxygen species (ROS) (31). Idiopathic infertility has been linked to a drop in semen antioxidant activity (32).

When compared to the control group, OLZ (2.5, 5, and 10 mg/kg) resulted in a decrease in GSH levels in testicular tissue. High dosages of OLZ (10 mg/kg) significantly reduced tissue GSH levels compared to lower doses (5 mg/kg and 2.5 mg/kg) of OLZ. This was in the line with Abdel Moneim, 2014 (33) and Elghaffar et al., 2016 (34). Administration of Vit E had improved this decline. These results matched those of the study by Zhou et al., 2006. (35).

The current findings revealed that testicular MDA level was significantly increased after administration of OLZ at 3 distinct dosages (2.5, 5 & 10mg/kg), suggesting oxidative stress compared to the control group. These results corroborate those of Abd el-Hameed et al., 2020 (36).

Recent research suggests that vitamin E may have a protective impact against lipid peroxidation and oxidative stress due to its

influence on lowered malondialdehyde (MDA) levels. Findings from this study corroborated those from prior research by Rahangadale et al., 2012 (37).

Testicular histopathology confirmed the biochemical findings in the OLZ-treated group and revealed male reproductive toxicity characterized by mild to moderate vacuolation, degenerative tubular epithelial changes, and degenerated seminiferous tubules with small amounts of sperm in their lumens and thickened basement membranes. Degeneration was so severe in certain instances with OLZ 10 mg/kg that the germ cells and sertoli cells were reduced to a single layer or completely or partially destroyed. Ardç et al., 2021(11) results are supported by these results. Compared to those who received OLZ alone, the histological alterations were reduced to varying degrees following the administration of Vit E with OLZ. These was in accordance with Fahim et al., 2013 (38).

5. Conclusion:

Co-administration of vitamin E with OLZ may mitigate its reproductive toxicity which appeared by increasing CAT, SOD, and GSH levels while reducing MDA levels in tissues. The toxicity of OLZ on the reproductive system and the protective benefits of vitamin E require more research with greater dosages and longer durations.

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