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Research Article

Effect of Beetroot Extract *(Beta vulgaris)* Against Olanzapine on the Pituitary, Thyroid, and Fertility in Adult White Male Rats

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Abstract

Olanzapine (OLZ) is a second-generation atypical drug that is commonly used to treat schizophrenia. However, it is known to affect male sexual functions, reproductive processes, and spermatogenesis. This study aims to investigate the effectiveness of beetroot extract in reducing the side effects of Olanzapine. The study involved 46 male Wistar rats weighing 150-200 g, administered intraperitoneal doses of OLZ and beetroot extract at (10 mg/kg) daily for six weeks. Thyroid-stimulating hormone (TSH), triiodothyronine (T3), tetraiodothyronine (T4), (T3/T4), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels were measured. The study found that beetroot extract treatment significantly increased thyroid-stimulating hormone levels by 81% and TSH by 82% in the third and sixth weeks, while Triiodothyronine levels decreased by 42% and thyroxin levels by 115% in the third and sixth weeks, also, there was a significant decrease in testosterone levels in the group treated with Olanzapine by 108 % in the third week and by 116% in the sixth week when compared to a control group; additionally, the study found a 25% increase in absolute body weight in the beetroot extract group and 26% increase in a combined group treated with Olanzapine compared to Olanzapine. Histological sections of the thyroid gland and testis were taken at the end of the sixth week. OLZ caused atrophy of follicular epithelium in the thyroid gland and shrunken seminiferous tubules, with disorganized germinal epithelium in the testis; however, beetroot extract treatment resulted in normal testicular histology, characterized by regular tubules with stratified germinal epithelium and Leydig cell clusters. However, some sperm aggregation was observed in the tubular luminae. It concluded that administrating beetroot extract (10 mg/kg) daily for six weeks significantly improved the pituitary, thyroid, and fertility in adult white male rats.

Keywords: *Olanzapine, Beetroot, Beta vulgaris, Thyroid, Male fertility*

Introduction

Animal models have significantly advanced our understanding of mental disorders, including their underlying mechanisms, progression, symptoms, and potential treatment strategies [1]. Nevertheless, animal models cannot accurately reproduce the intricate conditions found in people. Hence, it is essential to meticulously choose animal models that exhibit similarities to human diseases and circuit-specific modifications that may result in pathology [2]. To be considered a valid model of a human mental disorder, an animal model should exhibit face validity, construct validity, and predictive validity. Face validity refers to the similarity between the behavioral and physiological symptoms observed in the model and those experienced by patients. Construct validity requires that the model replicates the underlying neurobiological mechanisms of the disorder. Predictive validity is demonstrated when the model accurately predicts the response to therapeutic interventions [3].

The Middle East exhibits a significantly higher prevalence of schizophrenia compared to developed countries, as evidenced by the age-standardized disability-adjusted life years associated with schizophrenia in 2004 [4]. The estimated rates per 100.000 inhabitants were approximately

273 in Egypt, 270 in Saudi Arabia, 269 in Kuwait, and 267 in the United Arab Emirates, whereas in Australia, the rate was 164 per 100,000 inhabitants [5]. However, a recent study conducted at Jazan Health, Saudi Arabia, specifically in an "adult psychiatry clinic," revealed that the most commonly prescribed initial antipsychotic drugs were Olanzapine (48.8%), haloperidol (13.9%), and aripiprazole (11.3%) [6]. Hence, we evaluated the protective effects of beetroot extract against the side effects of Olanz apine. Additionally, w e investigated the potential mechanisms of action by studying its impact on various gland functions [7].

Antipsychotic medications are utilized to treat a severe and chronic illness that affects 21 million individuals worldwide [8]. Olanzapine (OLZ) is a type of second-generation antipsychotic drug and is commonly prescribed for the immediate treatment of schizophrenia. It exhibits a strong affinity for various binding regions, including dopaminergic, serotonergic, muscarinic, adrenergic, and histaminergic regions [9]. Second-generation anti-psychotics, such as dopamine D2 receptor antagonists, can increase the levels of thyroid-stimulating hormone (TSH) in the bloodstream, thereby impacting other thyroid hormones like triiodothyronine and thyroxine [10]. In men, OLZ can lead to sexual dysfunction by inhibiting gonadotropin-releasing hormone, luteinizing hormone, and testosterone, resulting in hypogonadism and various adverse effects on sperm production, semen quality, sperm motility, and testicular tissue morphology [11].

Herbal remedies are generally considered safe and can significantly treat various diseases [12]. Beetroot extract, derived from the fleshy root of the *Beta vulgaris* plant, is known for its thin skin and a wide range of colors, including purple-pink, reddish-orange, and brownish tones. The deep crimson-red pulp of the beetroot has a pleasant, sweet taste [13].

Beetroot extract is recognized as one of the top ten potent vegetable sources of phytochemicals, exhibiting strong antioxidant and anticancer properties [14]. It is highly beneficial in improving hormonal levels related to fertility and can be advantageous for maintaining pregnancy and treating infertility [15]. There are no available studies on the effect of beetroot extract on Olanzapine toxicity; therefore, this study hypothesized that Beetroot supplementation would mitigate the adverse effects of Olanzapine on the pituitary and thyroid glands in rats. Also, its effect on reducing the increase in prolactin levels induced by Olanzapine, attenuating the decrease in thyroid hormones (T3 and T4) caused by Olanzapine, Simultaneously, improve the histological architecture of the pituitary and thyroid glands in olanzapine-treated rats and reduce oxidative stress markers in the pituitary and thyroid glands of olanzapine-treated rats.

Materials and Methods

Ethical Approval

The animal study has been approved by the Unit of Biomedical Ethics, Research Ethics Committee (REC HA-02-J-008, King Abdul Aziz's University). The accommodation and administration of the animals and the experimental protocols were conducted per the principles delineated in the Guide for the Care & Use of Lab Animals following the National Committee of Bioethics NCBE (2023). The Ethical Code number 511-89.

Beetroot Extract

Beetroot was purchased from the popular market in Jeddah, Beetroot material was ground to a uniform particle size of 0.5 mm. Subsequently, it was subjected to ultrasound-assisted extraction. A variety of solvents and co-solvents were used in these extractions. The resulting extracts were concentrated using a rotary evaporator (BÜCHI Rotavapor R-114 and BÜCHI Vacuum Controller B-721) and then dried under reduced pressure. The dried extracts were stored at -20°C for further analysis [16].

20 g of dried and ground material were added to an Erlenmeyer flask. 250 mL of water was added to the flask. The flask was then submerged in an ultrasonic bath (Iskra-Pio, Slovenia) and subjected to ultrasonic waves at a frequency of 40 kHz. The liquid level in the flask was maintained below the water level in the bath. The extraction process was carried out at a constant temperature of 40°C for 90 min [16].

Extraction yield, expressed as the ratio of the mass of the extract to the mass of the dry beetroot material, was used to assess the efficiency of the different extraction methods and conditions.

Experimental Animals' Layout

In this study, forty-eight adult male rats of Caucasian descent weighing between 150 and 250 g were utilized. The rats were housed in standard cages designed for rats and kept in a room with a 12:12 h light/dark cycle and a controlled temperature of 22±1ºC. To allow for acclimatization, the rats were kept in the laboratory for one week before the commencement of the study. The beetroot extract was filtered using filter paper, and intraperitoneal administration of a daily dose of 10 mg/kg was carried out for six weeks. Throughout the experiment, the rats were divided into four groups, each consisting of twelve rats.

Blood Biochemistry

After three weeks, serum samples were collected from each rat. Blood samples were collected using K3-EDTA tubes to measure various hormone levels, including thyroid-stimulating hormone (TSH), thyroid hormones

(triiodothyronine T3, tetraiodothyronine T4, and the percentage of T3 to T4) as follow, the blood samples (1 mL) were collected from control and treated rats, centrifuged (20 min at 1.500 x g). The serum was frozen at -70°C for later hormone analysis. T3 and T4 concentrations were measured using a competitive chemiluminescent enzyme immunoassay (Immulite 1000, Siemens). All samples were run in duplicate under standardized conditions with intra-assay CVs <5%.

Serum samples were analysed for LH, FSH, and testosterone levels using an Enzyme Immunoassay (EIA) kit (Cayman Chemical Company, Ann Arbor, MI, USA). The assay was performed following the manufacturer's protocol and the guidelines outlined in Tietz [17]. Results for LH, FSH, and progesterone were reported in ng/mL-1, while estradiol levels were expressed in pg/mL^{-1}.

Histological Studies

At the end of the sixth week, the rats were euthanized, and serum and tissue samples were obtained from their thyroid glands and testicles. The thyroid glands and testes were weighed immediately after dissection for further analysis. After the collection of blood samples all animals were sacrificed by cervical dislocation. Rats dissected one testis, and both the femora of each animal and the thyroid gland were dissected. The shape, color and location were recorded before the fixation, and the photographs of the thyroid were taken to depict the gross anatomy by using a digital camera Sony cyber-shot (14.2 megapixels) for histological and histochemical study samples of the thyroid gland was fixed in 10% neutral buffered formaldehyde and sectioned serially at 5 µm. Sections were stained with Hematoxylin and eosin, and for histochemical studies PAS and Masson Trichrome stain [18]. The histological sections of this study were examined by using a light microscope type (Olympus/Japan) with different magnifications (X20 and X40). The sections were photographed by using (Olympus/Japan) microscope and digital camera; an ocular micrometer calibrated with a stage micrometer was used for histological parameters, which include the thickness of the capsule, the diameter of different sizes of follicles and the height of lining epithelium [19].

Statistical Studies

The statistical analysis of the data was carried out using the software package STATISTICA 10.0 (StatSoft Inc., Tulsa, OK, USA). Each determination was performed three times, and the resulting data were averaged. The final results are presented as mean values. The means were compared with the LSD test at P<0.05.

Results

Thyroid-Stimulating Hormone (TSH)

The outcomes of this study indicate that, as compared to the control group, there was a statistically significant reduction in TSH levels in the olanzapine-treated groups during the third and sixth weeks, as well as in the combined group treated with olanzapine and beetroot extract during the third week. These data are displayed in *Fig. 1-A,B*. In contrast, the group that received beetroot extract treatment every week and the group that received Olanzapine plus beetroot extract treatment just for the sixth week showed no discernible changes in TSH levels when compared to the control group. Furthermore, compared to the olanzapine group, the beetroot extract group and the combination group treated with Olanzapine plus beetroot extract showed a statistically significant increase in TSH levels in both the third and sixth weeks.

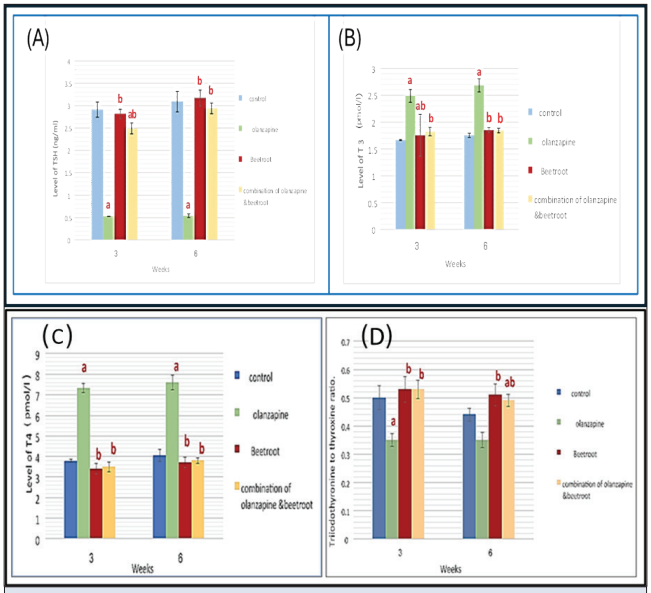

Fig 1. Effect of daily administration of Olanzapine (10 mg/kg), Beetroot extract (10 mg/kg), and Combination of Olanzapine & Beetroot on thyroid stimulating hormone (TSH) (mLU/mL) (A), and Triiodothyronine (T3) pmol/L (B), Thyroxine (T4) (mLU/mL) (**C**), and Triiodothyronine to thyroxine ratio (T3/T4) (D) of adult male rats for 6 weeks

Triiodothyronine (T3)

The data displayed in *Fig. 1-A* indicate that, in comparison to the control group, there was a discernible rise in the level of T3 in the groups treated with Olanzapine in the third and sixth weeks, as well as in the group treated with beetroot extract in the same week. In contrast to the control group, no discernible change in the level of T3 was seen in the group administered beetroot extract in the sixth week or in the combined group administered olanzapine and beetroot extract throughout all weeks. Additionally, it was shown that during both the beetroot extract treatment group and the combined group treated with olanzapine and beetroot extract, there was a substantial drop in the level of T3.

Thyroxine (T4)

As observed in *Fig. 1-C*, the group receiving Olanzapine experienced a considerable increase in T4 levels in the

third and sixth weeks when compared to the control group. Comparing the beetroot extract group and the combination group treated with olanzapine and beetroot extract to the control group, however, did not demonstrate a statistically significant difference in the amount of T4 in any of the weeks. In contrast, compared to the olanzapine group, there was a noteworthy drop in T4 levels in the beetroot extract group and the combined group treated with olanzapine and beetroot extract in both the third and sixth weeks.

Triiodothyronine to thyroxine ratio (T3/T4)

In *Fig. 1-D*, it is evident that the group receiving olanzapine treatment experienced a significant increase in the third week, while the combined group treated with olanzapine and beetroot extract showed a significant increase in the sixth week, both in comparison to the control group. On the other hand, no significant impact was observed on the triiodothyronine to thyroxine ratio (T3/T4) in the group treated with beetroot extract throughout all weeks, as well as in the combined group treated with olanzapine and beetroot extract during the third week, when compared to the control group. Furthermore, a significant increase in the group treated with beetroot extract and the combined group receiving Olanzapine and beetroot extract was evident during both the third and sixth weeks, in comparison to the olanzapine group.

Follicle-Stimulating Hormone (FSH)

Throughout all weeks, the group receiving olanzapine treatment exhibited a notable rise in FSH levels. Furthermore, during the third week, both the group treated with beetroot extract and the combined group receiving both olanzapine and beetroot extract demonstrated a significant increase in FSH levels in comparison to the control group, as depicted in *Fig. 2-A*. Conversely, there was a significant decrease in FSH levels observed during both the third and sixth weeks for the group treated with beetroot extract and the combined group receiving Olanzapine and beetroot extract. This finding contrasted with the group solely treated with Olanzapine.

Luteinizing Hormone (LH)

The level of LH increased significantly in the group treated with Olanzapine and the combined group treated with olanzapine and beetroot extract in both the third and sixth weeks. We also observed a significant increase in the level of LH in the group treated with beetroot extract only in the third week when compared to the control group *(Fig. 2-B)*. However, there was no significant change in the level of LH in the group treated with beetroot extract in the sixth week when compared to the control group. Furthermore, we noticed a significant decrease in the level of LH in the group treated with beetroot extract and the combined group treated with olanzapine and beetroot extract in both the third and sixth weeks when compared to the olanzapine group.

Testosterone

The group subjected to olanzapine treatment exhibited a significant reduction in testosterone levels during both the third and sixth weeks, as indicated in *Fig. 2-C*,

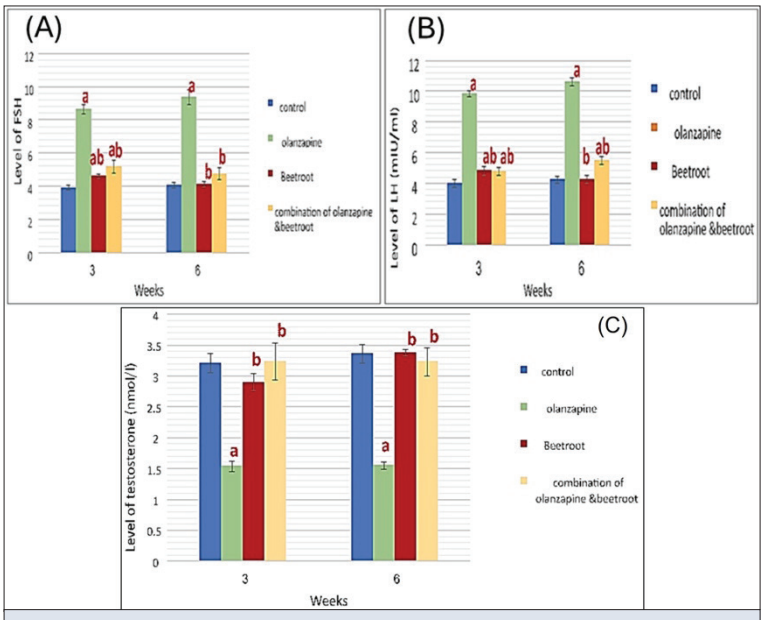

Fig 2. Effect of daily administration of Olanzapine (10mg/kg), Beetroot extract (10mg/kg), and Combination of Olanzapine & Beetroot on Follicle-stimulating hormone (FSH) (mlU/mL) (A), and Luteinizing hormone (LH) (mLU/mL) (B) testosterone hormone (nmol/l) (C) of adult male rats for 6 weeks

Table 1. *Effects of daily administration of Olanzapine (10mg/kg), Beetroot extract (10mg/kg), and Combination of Olanzapine & Beetroot on the absolute body weight in grams of adult male rate during 6 weeks*

Weeks	Control	Olanzapine	Beetroot	Combination
3	222.16±3.02	157.83±4.63^{a}	211.5±3.13^{ab}	214.7±4.75^{b}
6	192.5±8.55	205.8±3	199.3±5.47	204.6±2.57

Table 2. *Effects of daily administration of Olanzapine (10mg/kg), Beetroot extract (10mg/kg), and Combination of Olanzapine & Beetroot on absolute testes weight (g)of adult male rate during 6 weeks*

Weeks	Control	Olanzapine	Beetroot	Combination
3	1.86±0.08	1.01±0.02^{a}	2.04±0.09^{b}	1.77±0.11^{b}
6	1.94±0.08	1.02±0.02^{a}	2.05±0.07^{b}	1.81±0.06^{b}

Table 3. *Effects of daily administration of Olanzapine (10mg/kg), Beetroot extract (10 mg/kg), and Combination of Olanzapine & Beetroot on relative testes weight % of adult male rate during 6 weeks*

Weeks	Control	Olanzapine	Beetroot	Combination
3	0.83±0.02	0.64±0.02^{a}	0.96±0.01^{ab}	0.82±0.02^{b}
6	1.02±0.05	0.49±0.008^{a}	1.025±0.05^{b}	0.92±0.02^{b}

compared to the control group. However, there was no notable impact observed in the group treated with beetroot extract or the group receiving a combination of olanzapine and beetroot extract throughout all weeks, when compared to the control group. On the other hand, both the group treated with beetroot extract and the group receiving a combination of olanzapine and beetroot extract demonstrated a significant increase in testosterone levels in comparison to the olanzapine group.

Absolute Body Weight (BW)

During the third week, both the olanzapine-treated group and the beetroot extract group experienced a significant decrease in absolute body weight compared to the control group *(Table 1)*. However, we observed a significant increase in absolute body weight in the group treated with beetroot extract and the combined group treated with both olanzapine and beetroot extract in the third week when compared to the olanzapine group. There was no significant effect on absolute body weight in the group treated with Olanzapine, the group treated with beetroot extract in the sixth week, and the combined group treated with both olanzapine and beetroot extract in the third and sixth weeks when compared to the control group.

Absolute Testes Weight

The group receiving olanzapine treatment exhibited a significant decrease in absolute testis weight during both the third and sixth weeks, as depicted in *Table 2*, in contrast to the control group. However, there was no notable impact observed on the group treated with beetroot extract or the combined group receiving both olanzapine and beetroot extract during both the third and sixth weeks, when compared to the control group. On the other hand, a significant increase in absolute testis weight was observed in the group treated with beetroot extract and the combined group receiving both olanzapine and beetroot extract during both the third and sixth weeks, in comparison to the group treated with Olanzapine.

Relative Testes Weight

The study revealed significant findings regarding the effects of treatment interventions. Specifically, the group receiving olanzapine treatment demonstrated a notable decrease during both the third and sixth week in comparison to the control group, as illustrated in *Table 3*. Conversely, the group treated with beetroot extract exhibited a significant increase in the third week when compared to the control group. Moreover, the group treated with beetroot extract consistently displayed significant improvements throughout all weeks, while the combined group treated with both Olanzapine and beetroot extract showed a significant enhancement, specifically during the sixth week, relative to the group treated with Olanzapine alone.

Histological Studies

The light microscopic micrograph of the thyroid gland showed normal Follicles surrounding a homogeneous colloid and epithelium cell with regular capsule and fenestrated capillaries in the control group *(Fig. 3-A,B)*. while the histological structure of the thyroid gland in the group treated with OLA showed irregular follicles, cell disintegration, dark pigmentation heterogeneity of the colloidal substance, degenerated epithelium and connective tissue, and focal proliferation of C cells *(Fig. 3-C,D)*. On the other hand, the gland's structure in the beetroot extract is like the control group, with normal follicles, homogenous colloid, and epithelium cells with

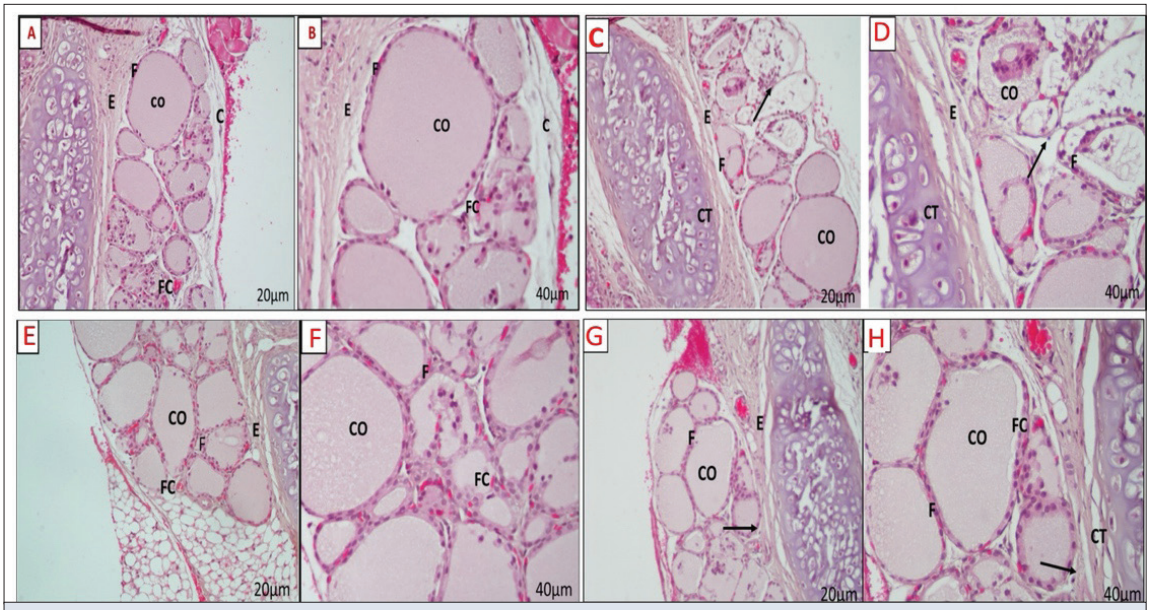

Fig 3. A, B: Light microscopic micrograph of the thyroid gland from adult rats in the control group showing normal Follicles (F), surrounding a homogenous colloid (CO) Follicular and epithelium cell (E) With normal capsule(C) and fenestrated capillaries (FC); **C, D:** Marked Irregular Follicles (F), heterogeneity of the colloidal substance (CO), degenerated Epithelium and Connective Tissue (CT) (E) Focal proliferation of C cells *(black arrows);* **E, F:** The structure of the gland is similar to the control group, normal Follicles (F), homogenous colloid (CO) Follicular, and epithelium cell (E) With normal capsule (C) and fenestrated capillaries (FC); **G, H:** Irregular Follicles and cell disintegration (F) and the colloidal substance are heterogeneous (CO), and less amount of epithelium and degenerated connective tissue (CT) (E) *(black arrows)* at a lower rate than the olanzapine group

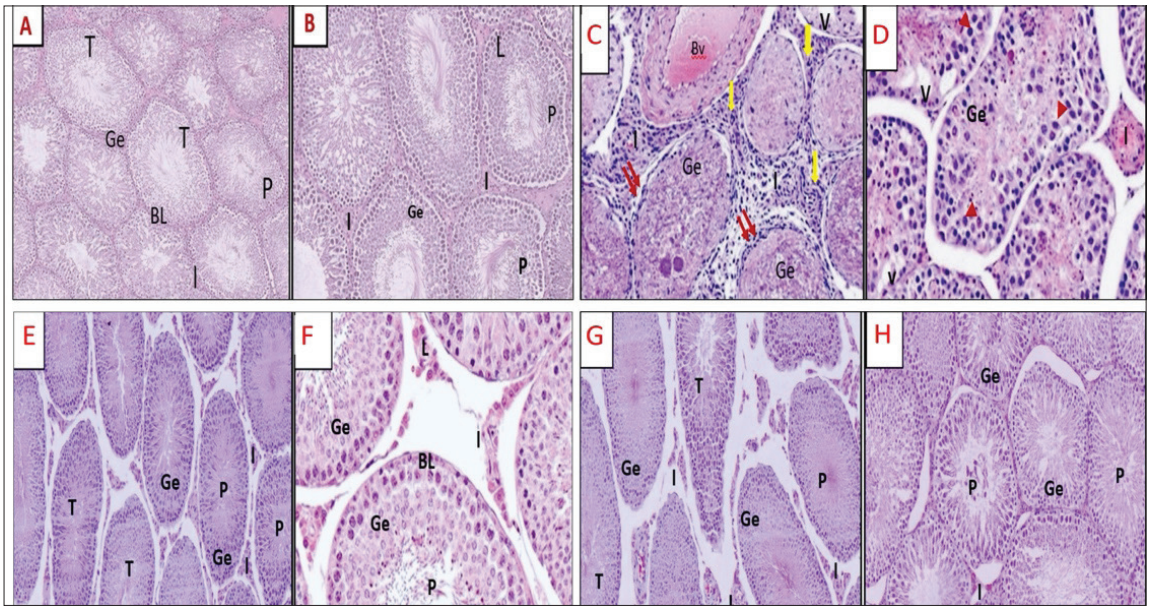

Fig 4. A, B: Light microscopic micrograph of testicular tissue from an adult rat in the control group showing normal seminiferous tubules (T) ensheathed with basal lamina (BL) and containing normal lined by stratified germinal epithelium (Ge). Aggregations of sperms (P) are seen in the lumina. Narrow interstitial spaces (I) show clusters of Leydig cells (L); **C, D;** Showing shrunken seminiferous tubules *(doubble arrow)* with disorganized germinal epithelium and marked vacations (V). Some other tubules are filled with degenerated germ cells (Ge), and darkly stained nuclei (arrowhead). Also, wide interstitial spaces (I) with scattered leucocyte infiltration *(arrow)* are noticed. Congested dilated blood vessels are also seen (Bv); **E, F:** Regular seminiferous tubules with stratified germinal epithelium cells (Ge) resting on regular basal lamina (BL). Tubules appear with aggregation of sperms (P) in the lumina. Normal interstitial spaces (I) show Leydig cells (L) clusters; **G, H:** Restoration testicular structure in most seminiferous tubules (T) and having nearly regular contour and are lined by stratified germinal epithelium (Ge). Their lumina contain aggregations of sperms (P). However, the interstitial spaces (I) are relatively wide compared with the control group

regular capsules and fenestrated capillaries *(Fig. 3-E,F)*. The combined group treated with olanzapine and beetroot extract showed shows no similarity with the control group. The Irregular Follicles and cell disintegration and the colloidal substance are heterogeneous, and less amount of degenerated epithelium and connective tissue at a lower

rate than the olanzapine group *(Fig. 3-G,H)*. The results showed normal testicular tissue structure in the control group *(Fig. 4-A,B)*. The seminiferous tubules appear normal and are ensheathed with basal lamina.

Additionally, they usually contain lined, stratified germinal epithelium. The lumina of the tubules shows aggregations of sperms, and narrow interstitial spaces display clusters of Leydig cells. While the histological structures of seminiferous tubules appear shrunken with disorganized germinal epithelium and marked vacuolations in the group treated with OLA *(Fig. 4-C,D)*. Some of the tubules are filled with degenerated germ cells and darkly stained nuclei. However, *(Fig. 4-E,F)* demonstrated regular testicular tissue in the treated group with beetroot extract. The cotreatment with beetroot extract and OLA restored testicular structure in most seminiferous tubules, with a nearly regular contour, lined by stratified germinal epithelium *(Fig. 4-G,H)*. However, the interstitial spaces are relatively wide compared with the control group.

Discussion

Schizophrenia often manifests around late adolescence or early adulthood and is associated with a range of symptoms that are traditionally categorized into three groups: positive, negative, and cognitive symptoms [20]. Manifestations of positive symptoms include hallucinations, delusions, and mental impairments. Negative symptoms encompass diminished emotional expression, apathy, lack of pleasure, retreat from social interactions, and avolition. Cognitive dysfunction includes impairments in working memory, attention, processing speed, and difficulties in maintaining focus, including genetic predisposition and exposure to severe socioenvironmental challenges [21], which influence The development of schizophrenia. To replicate the characteristics of schizophrenia, animal models in the study of the disease extensively employ genetic models, prenatal interventions, pharmacological models, and stress-based protocols during prepubertal periods.

The thyroid gland is a crucial player in metabolism thanks to its secretion of two vital hormones: thyroxine (T4) triiodothyronine (T3) and thyroid-stimulating hormone (TSH). KhoshvaghtiAbtahi [22], suggested that the unchanged levels might be due to flavonoid compounds with suppressive effects on the thyroid gland in the short term. The thyroid gland may also adapt to these compounds with long-term extract administration [23]. Another possible explanation is the inhibitory effect of flavonoid compounds on prostaglandin production via cyclooxygenase inhibition, which has been linked to the stimulatory effect of prostaglandins on the production and secretion of pituitary-thyroid hormones [23].

According to recent research of Śmierciak et al.[24], thyroid abnormalities such as hypothyroidism and hyperthyroidism have been identified as risk factors for various neuropsychiatric disorders, including schizophrenia. In our study, we observed a significant increase in Triiodothyronine (T3) and thyroxin (T4) levels in the group treated with Olanzapine when compared to a control group. These findings align with previously published studies [25], which have found that patients with schizophrenia tend to exhibit higher levels of T3 and T4.

Additionally, research by Jose et al. Jose et al.[26] suggests that higher levels of free T3 may be linked to suicide ideation in male schizophrenia. Zhu et al.[27] also discovered that free radical-induced hyperthyroidism can enhance thyroid hormone synthesis in schizophrenia. Notably, higher levels of total plasma peroxides and MDA have been observed to correlate with T3 in schizophrenics. Furthermore, long-term negative endocrine feedback resulting from the duration of the disease can reduce the effectiveness of negative feedback regulation on TSH secretion during the stage of neuroregulation. Finally, research by Li et al.[25] has suggested that thyroid hormones may serve as biomarkers of agitation in schizophrenia and could play a role in the disorder's pathogenesis.

Beetroot is a rich source of bioactive phytochemicals with potential health benefits, including antioxidant, antibacterial, antiviral, and analgesic properties. It has been explored for its therapeutic applications in various diseases, such as cancer and atherosclerosis. The food industry has utilized beetroot and its derivatives as natural colorants and preservatives due to their stability and non-toxicity. Beetroot is a rich source of flavonoids and phenolic compounds, such as 5-hydroxy-6,7-methylenedioxyflavone, 3,5-dihydroxy-6,7-methylenedioxyflavanone, 2,5-dihydroxy-6, and 7-methylenedioxyisoflavone [28].

Androgens, a class of steroid hormones, play a vital role in the reproductive system and overall homeostasis. Imbalances in androgen levels can contribute to a range of physiological disorders and diseases. Flavonoids, a diverse group of natural polyphenols widely found in plants and foods, have gained significant attention due to their potential health benefits and their ability to interact with hormone systems [29]. Emerging evidence suggests that flavonoids can influence androgen synthesis and metabolism, offering potential therapeutic benefits for androgen-related disorders. Flavonoids can influence androgen levels and actions by targeting multiple mechanisms, including the hypothalamic-pituitary-gonadal axis, androgen synthesis and metabolism, receptor binding, and antioxidant effects. However, the complex interplay between flavonoids, individual factors, and dietary matrices poses challenges in translating these findings into clinical applications [30].

The study found that beetroot extract treatment significantly increased thyroid-stimulating hormone levels by 81% and TSH by 82% in the third and sixth weeks, while Triiodothyronine levels decreased by 42% and thyroxin levels by 115% in the third and sixth weeks.This study agrees with Peepre et al.[31] who found increasing the level of thyroxin in rats after 15 days of Vitamin C and E administration. Also, Li et al.[32] reported that Elevated LH and FSH levels, combined with normal or decreased testosterone, can lead to testicular failure and germinal cell degeneration, significantly affecting spermatogenesis.

Schizophrenic psychoses often result in hyperprolactinemia and gonadal dysfunction, leading to estrogen deficiency in women and testosterone deficiency in men [33]. This can lead to infertility or sexual dysfunction, which are major problems for individuals with psychiatric disorders [34]. Antipsychotic drugs can also cause reproductive toxicity [34].

In our study, there was a significant decrease in testosterone levels in the group treated with Olanzapine by 108 % in the third week and by 116% in the sixth week when compared to a control group. This is consistent with Korkut et al.[35], who found that serum LH and testosterone levels decreased in 20 and 40 mg/kg quetiapine-administered rats.

Our study found a notable increase in LH levels in the group treated with beetroot extract, showing a 17% rise in the third week when compared to the control group. This aligns with previous research by Widhiantara et al.[36], which suggests that flavonoid compounds may positively stimulate the pituitary gland to produce LH and improve male fertility.

The study found a significant decrease in FSH and LH levels but an increase in testosterone levels in the beetroot extract and olanzapine-beetroot extract group compared to the olanzapine group. These findings are consistent with previous studies by Hussein [37].

Testosterone plays a crucial role in regulating spermatogenesis, initiating the process during puberty, and maintaining it in adulthood. It also helps with meiosis and spermatid differentiation, according to Elsheikh et al.[38]. Elsheikh et al.[38] found that Beetroot extract contains flavonoids and betalain pigments, which have anti-inflammatory and antioxidant properties, and areoflavones that inhibit enzymes like aromatase and 5-α reductase, preventing disease. Elsheikh et al.[38] also reported that beetroot extract significantly improves sperm count and motility. Furthermore, Hussien et al.[37] demonstrated that flavonoids promote sperm production and maintain the function of spermatogenic cells. Sarfaraz et al.[39] suggested that beetroot extract could improve fertility and maintain hormonal levels during fertility.

The study found a 25% increase in absolute body weight in the beetroot extract group and a 26% increase in a combined group treated with Olanzapine compared to Olanzapine. These results agree Abbas et al.[40] reported that this increase was due to beetroot's positive anabolic effect by improving lipids and glucose metabolism. Zhao et al.[41] found that Flavonoids enhance osteoblast differentiation and inhibit osteoclasts, affecting bone weight. Beetroot extract treatment significantly increased testes weight by 50% in the third and sixth weeks compared to the olanzapine group. This is consistent with the results of Almuoswi et al.[42]. They reported that increased testes weight increases sperm concentrations and improves male fertility.

The study found that the rat thyroid's histological structure in the control group exhibited normal follicles, a homogeneous epithelium cell, capsule, and fenestrated capillaries. Meanwhile, these findings are very similar to those reported in the literature BaqerAlaridhi [33].

The study reveals irregular follicles, heterogeneity of colloidal substance, degenerated epithelium, connective tissue, and focal C cell proliferation in an adult rat's thyroid gland, consistent with previous research. Samawi et al.[44]. However, Samawi et al.[44] showed that thyroid function parameters were altered due to olanzapine medication, which was related to Olanzapine's cytotoxic effect [45].

Dopamine-releasing substances can impede thyroid-releasing hormone (TSH) secretion, potentially leading to abnormal thyroid test results in patients taking conventional antipsychotics due to their pharmacologic profile and dopaminergic activity [46]. Nevertheless, the outcomes of this research are consistent with prior investigations that have identified certain irregularities, such as thyroid function tests and liver enzymes, subsequent to Olanzapine treatment [47].

Olanzapine induces cytotoxicity and degenerative changes in thyroid cells through excessive reactive oxygen species production, leading to mitochondria collapse, lysosomal membrane leakage, reduced lipid peroxidation, and glutathione depletion [48].

The research shows that rat thyroid's structure is similar to the control group, with normal follicles, cells, and capillaries. It also confirms that nitrate-rich beetroot juice does not significantly alter plasma T3 and T4 levels or promote thyroid gland dysfunction. These findings are crucial in terms of ensuring safety [49]. However, these findings are congruent with those of Krajka-Kuzniak et al.[50], who discovered that beetroot may stimulate the expression of phase II detoxifying enzymes via Nrf2 activation as a result of mitogen-activated protein kinase stimulation.

Additionally, beetroot may be advantageous to the thyroid gland. Iodine deficiency reduces thyroid hormone production. Because beetroot is high in iodine, it may aid with thyroid management [51].

The study found that rats treated with olanzapine and beetroot extract showed irregular follicles, cell disintegration, and a heterogeneous colloidal substance with higher drug effects, possibly due to dose or experiment duration.

Olanzapine detrimentally induces sexual dysfunction. However, a restricted body of research indicates that it additionally triggers structural alterations within the reproductive system [52]. Furthermore, scholarly research suggests that Olanzapine may contribute to male infertility by causing sexual dysfunction [15]. However, histopathological analysis is widely recognized as a highly sensitive biomarker in regulatory toxicology investigations for identifying the detrimental reproductive consequences of toxicants [53].

The research reveals that olanzapine-treated rats exhibit distinctive vacuations, diminished seminiferous tubules, disorganized germinal epithelium, degenerated germ cells, darkly stained nuclei, expansive interstitial spaces, blood vessel congestion, and dilation. Histopathological alterations include seminiferous tubule lumen openings, interstitial area losses, germinal epithelium disorders, and cell degeneration, potentially indicating testicular toxicity. However, the results in this study agree with those of de Siqueira Bringal et al.[54]. The study found that Olanzapine may cause dose-dependent toxicity in testicular tissue, with histopathological alterations observed in high-dose groups and similar features in low-dose groups [52]. The impact of Olanzapine on testicular histology has been established in a limited number of studies that have been published [15].

Similarly, seminiferous tubule structures in the control group were discovered to be normal, according to a recent study. The Leydig cells in the interstitial region exhibited a consistent morphology and arrangement [53].

Additionally, the control group exhibited regular spermatogenic series and Sertoli cells within the tubules and the detection of sperm within the lumen of seminiferous tubules [53]. Lipofuscin granule proliferation indicates differentiation in Leydig cells. Vacuolization, sperm count decreases, and basement membrane thickening indicate Sertoli cell degeneration. Germ cells typically undergo degeneration, exfoliation, disorganization, vacuolization, and edema. Sertoli cell functional deficiencies can lead to germ cell degeneration [53]. Histopathological analysis reveals vacuolization, swelling, and other pathologies as signs of germ cell injury or reproductive toxicity induced by Olanzapine, indicating degenerative processes in testicular tissue. Additionally, previous research for assessing the genotoxic and oxidative damage potential of Olanzapine revealed that high drug concentrations caused oxidative stress. It has been stated that oxidative stress may result in tissue injury [51]. Oxidative stress has additionally been demonstrated to be an effective factor in testicular degeneration, which has been linked to a variety of causes, according to numerous studies [7].

Moreover, a recent study has demonstrated that efficacy-based olanzapine-induced oxidative stress in testicular tissue may account for aberrant sperm morphology and degenerative histological findings in the structure of the testicular glands observed in groups administered high doses of the drug [52]. Numerous studies have confirmed that betalains derived from red beetroots possess formidable antioxidant properties [38].

In the present study, the histological structure of rat testis in the group treated with beetroot extract showed that the tubules were regular, with stratified germinal epithelium cells reclining on regular basal lamina. However, sperm aggregation occurs in the lumina of tubules. Normal interstitial spaces are lined with Leydig cell clusters. Thus, normal histological appearance was observed in the beetroot groups with respect to seminiferous tubule structures, interstitial area characteristics, and cells. However, these results are very similar to those reported in the literature of Elsheikh et al.[38]. It was found that the antioxidants found in beets protect against the potentially harmful effects of excessive oxidative stress and prevent potential pathological diseases. They also maintain the integrity of the structure and tissues.

Research shows that Olanzapine and beetroot extract treatment restore testicular structure in seminiferous tubules with stratified germinal epithelium, with sperm aggregations, and slower degenerative changes than the Olanzapine group, suggesting beetroot administration reverses degenerative impact. However, these results agree with Elsheikh et al.[38].

The protective properties of beetroot and its constituents against various xenobiotic toxins have been unequivocally acknowledged, including their potent antioxidant, anti-inflammatory, and vascular-protective effects [51]. Furthermore, prior research has demonstrated that beetroot juice serves as a safeguard against the toxicity of detrimental chemicals. For instance, it prevented oxidative stress in male rats exposed to carbon tetrachloride and diminished DNA and plasma protein carbonyl damage [54]. Additionally, the male reproductive system of albino rodents is protected from the toxicity of cadmium chloride by beetroot [13]. In the end, it can be concluded that beetroot (*Beta vulgaris*) is one of the most important medicinal plants, scientifically proven to contain natural antioxidants that have preventive and therapeutic effects

against oxidative stress. our results indicate that the antioxidants in beetroot extract have therapeutic effects against Olanzapine on the pituitary, thyroid, and fertility in adult white male rats.

Declaration

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author (H. Y. Alnahary) on reasonable request.

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Ethical Approval: The animal study has been approved by the Unit of Biomedical Ethics, Research Ethics Committee (REC HA-02-J-008, King Abdul Aziz's University). The accommodation and administration of the animals and the experimental protocols were conducted per the principles delineated in the Guide for the Care & Use of Lab Animals following the National Committee of Bioethics NCBE, (2023). The Ethical code number 511-89.

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