International Journal of Medical Evaluation and Physical Report E-ISSN 2579-0498
P-ISSN 2695-2181 Vol 8. No. 5 2024 www.iiardjournals.org Online Version

# Evaluation of Curcumin as a Potential Therapeutic Agent against Ketoconazole-Induced Testicular Damage

**Onoja P.O, Akunna G.G, Saalu L.C**
ggakunna@gmail.com

DOI: 10.56201/ijmepr.v8.no5.2024.pg1.28

***Abstract***

*The delicate balance of hormonal, biochemical, and cellular processes governing testicular health is crucial for male fertility and overall well-being. However, pharmaceutical agents like ketoconazole can disrupt this equilibrium, leading to testicular damage and reproductive dysfunction. Curcumin, a bioactive compound known for its antioxidant and anti-inflammatory properties, has shown promise in mitigating drug-induced damage in various organs. Yet, its specific role in safeguarding testicular health against pharmaceutical-induced damage remains relatively unexplored. This study aimed to evaluate the efficacy of curcumin in mitigating ketoconazole-induced testicular damage using animal models. Fifty-four (54) adult male Wistar rats were divided into nine groups and treated with varying doses of ketoconazole and curcumin for twenty-eight (28) days accordingly. Results revealed that ketoconazole administration adversely affected testicular morphology, hormonal profiles, oxidative stress markers, sperm parameters, and histological characteristics. However, curcumin, particularly when administered in combination with ketoconazole, demonstrated protective effects against these adverse outcomes. Notably, curcumin administration before ketoconazole treatment showed promising results in preserving sperm parameters, even surpassing control levels in certain instances. These findings underscore the potential therapeutic utility of curcumin in combating drug-induced testicular damage and highlight avenues for further mechanistic exploration. Understanding the molecular pathways involved in curcumin's protective effects may offer novel strategies for managing male infertility and preserving reproductive function in men undergoing pharmacological treatments.*

***Keywords:*** *Curcumin, Ketoconazole, Testicular Damage, Male Infertility, Antioxidant, Reproductive Health, Protective Effects*

## Introduction

The male reproductive system is intricately regulated by a delicate balance of hormonal, biochemical, and cellular processes (Izegbu *et al.*, 2005). Testicular health, in particular, is vital for the maintenance of fertility and overall well-being in men (Sinclair, 2000). However, various factors, including environmental toxins, pharmaceutical agents, and diseases, can disrupt this delicate equilibrium, leading to testicular damage and subsequent reproductive dysfunction (Melodie *et al*., 2018).

Ketoconazole, a broad-spectrum antifungal medication, is one such agent known to induce adverse effects on testicular function (Kinobe *et al.*, 2006). Despite its efficacy in treating fungal infections, ketoconazole has been associated with detrimental effects on male reproductive health (Roemer *et al.*, 2014; Williams *et al.*, 2020). Studies have reported its potential to cause testicular toxicity, characterized by histopathological alterations, depletion of germ cells, and oxidative stress-induced damage within the testicular microenvironment (Rodriguez & Acosta, 1995, Kinobe *et al.*, 2006).

In recent years, there has been growing interest in exploring natural compounds with therapeutic potential in mitigating drug-induced testicular damage (Sikka *et al*., 1985; De Coster *et al*., 1986). Curcumin, a bioactive polyphenol derived from the rhizome of *Curcuma longa*, has emerged as a promising candidate in this regard (Schraufstatter *et al.*, 1949). Renowned for its multifaceted pharmacological properties, curcumin has demonstrated potent antioxidant, anti-inflammatory, and cytoprotective effects across various biological systems (Grosso *et al.*, 1983).

The protective effects of curcumin against oxidative stress and inflammation have been extensively documented in numerous preclinical and clinical studies (Aggarwal *et al.*, 2009). Its ability to modulate signaling pathways involved in cellular stress responses, mitigate reactive oxygen species (ROS) generation, and preserve mitochondrial function underscores its potential therapeutic utility in combating a wide range of pathological conditions (Richard, 2015).

While the cytoprotective effects of curcumin have been widely investigated in different organs and systems, its specific role in safeguarding testicular health against pharmaceutical-induced damage remains relatively underexplored (Aggarwal *et al.*, 2009). Given the importance of maintaining male reproductive function, particularly in the context of antifungal therapy where ketoconazole-induced testicular damage poses a significant concern, there is a compelling need to evaluate the efficacy of curcumin as a potential therapeutic agent in this scenario (Amin, 2008).

This study aims to address this critical gap in the literature by comprehensively assessing the efficacy of curcumin in mitigating ketoconazole-induced testicular damage. Through a combination of literature review and experimental studies utilizing animal models, we seek to elucidate the mechanisms underlying the protective effects of curcumin on testicular morphology, germ cell depletion, and oxidative stress markers.

By elucidating the potential of curcumin to preserve male reproductive health in the face of pharmaceutical-induced insults, our findings hold significant clinical implications. Not only could this research contribute to the development of adjunctive therapies to enhance the safety profile of antifungal medications, but it may also pave the way for novel strategies aimed at preserving fertility and reproductive function in men undergoing pharmacological treatments.

This paper endeavors to provide valuable insights into the therapeutic potential of curcumin in mitigating ketoconazole-induced testicular damage, thereby advancing our understanding of natural compounds as adjunctive therapies in preserving male reproductive health.

## Materials and Methodology

### Experimental Animals

Fifty-four (54) adult male Wistar rats with an average weight of 117.64g were procured from the Animal House of the College of Health Sciences, Benue State University, Makurdi, and allowed to acclimatize in metal-covered plastic cages. They were placed in nine (9) groups, each with six Wistar rats, and given *ad libitum* access to standard rodent pellets and water before the commencement of the experiment. The weights of the animals were estimated at acquisition, during acclimatization, and at the end of the experiment using an electronic analytical weighing balance, a product of China known as Techmel. They were also weighed and observed before and after the administration of the extract.

### Experimental Drug Procurement

Ketoconazole tablets were procured from Rovi Pharmacy located along Iorkyaa-Ako Street, High Level, Makurdi, Benue State.

### Animal Feeds

Animal feed (Chikun starter mash), a Nigerian feed produced by Olam farms was purchased from God for us livestock services, located along High Level roundabout, High Level, Makurdi; Benue State.

### Methodology of *Curcumin* extraction and isolation of curcuminoid

The turmeric was washed twice with distilled water and segregated into 10 different rhizomes for further studies. Washed rhizomes were sliced into small pieces and shade-dried for one week.

### Extraction of Curcuminoids

0.1g of the sample was taken and 30 ml of ethanol was added. This was shaken well and put in the soxhlet in 2 hours 30 minutes. The sample was filtered in a 100 ml measuring flask. The volume was Made up to 100 ml. 20 ml of this was taken and made up to 250 ml.

### Extraction of Curcumin from Oleo Resins

### Preparation of Sample

Next, the extract was filtered. Thus, curcuminoids complexed with oleoresins are present in the filtrate that is produced. Hexane, acetone, and chloroform were the three solvents employed to extract curcuminoids from oleoresin. Hexane is the solvent in which oleoresins dissolve the best, although curcuminoids are insoluble in them; curcuminoids were therefore isolated.

### Experimental Design

Fifty-four (54) Adult male Wistar rats were divided into 9 groups containing six rats per group. They were treated as follows:

**Group A:** Oral administration of 5mls/kg bwt. distilled water once daily for 28 days

**Group B:** Oral administration of 100mg/kg bwt. Ketoconazole once daily for 14 days (initial 14 days)

**Group C:** Oral administration of 100mg/kg bwt. Ketoconazole and 10mg/kg bwt. Curcumin once daily for 14 days (final 14 days)

**Group D:** Oral administration of 10mg/kg bwt. Curcumin once daily for 14 days (initial 14 days)

**Group E:** Oral administration of 20mg/kg bwt. Curcumin once daily for 14 days (initial 14 days)

**Group F:** Oral administration of 100mg/kg bwt. Ketoconazole once daily for 14 days (initial 14 days), then 10mg/kg bwt. Curcumin once daily for 14 days (final 14 days)

**Group G:** Oral administration of 100mg/kg bwt. Ketoconazole once daily for 14 days (initial 14 days), then 20mg/kg bwt. Curcumin once daily for 14 days (final 14 days)

**Group H:** Oral administration of 10mg/kg bwt. Curcumin once daily for 14 days (initial 14 days), then 100mg/kg bwt. Ketoconazole once daily for 14 days (final 14 days)

**Group I:** Oral administration of 20mg/kg bwt. Curcumin once daily for 14 days (initial 14 days), then 100mg/kg bwt. Ketoconazole once daily for 14 days (final 14 days)

**Animal sacrifice**

Before sacrifice, the rats were weighed and anesthetized by placing them in a sealed container containing chloroform-soaked cotton wool. Blood samples were promptly collected from the heart of each rat. A midline abdominal incision was made to expose the reproductive organs. The testes and epididymis were excised, and the weight of each animal's testes was assessed using an electronic analytical and precision balance. One of the testes from each animal was preserved in Bouin's fluid for subsequent histological examination. Serum and the remaining testis of each animal were stored at –25$^{o}$C for biochemical assays.

**Serum and Testicular Testosterone**

As previously described (Tietz, 1995), the enzyme immunoassay technique was used to determine the concentrations of testosterone in plasma. It worked by using goat anti-rabbit IgG-coated wells that were incubated with TT standards, controls, samples (blood sera and supernatants of testicular homogenates), TT-horseradish peroxidase conjugate reagent, and rabbit anti-TT reagent at 37C for 90 minutes. After this time, the conjugate was removed and the wells were cleaned. After adding and incubating tetramethylbenzidine, a blue hue began to appear. After adding 1N hydrochloric acid, the color development was halted, and the absorbance was measured spectrophotometrically at 450 nm. Plotting the standard concentration against the absorbance and TT concentrations determined from the standard curve resulted in the creation of a standard curve.

**Serum Hormonal Assay: Leutinizing Hormone and Follicle Stimulating Hormone**

The tests were conducted following the methodology that Amballi modified in 2007. The blood that was drawn and placed into simple containers was briefly left to coagulate. To accomplish separation, each sample was centrifuged for 10 minutes at 1000 rpm. Each time, the collected serum was divided into aliquots, labeled, and kept at –20℃. The samples were analyzed for hormone estimation using enzyme immunoassay (EIA) following the World Health Organisation (WHO) matched reagent program protocol (manual) for EIA kits (protocol/version of December 1998 for LH, FSH). One aliquot of each specimen was taken at a time to prevent repeated freezing and thawing. NIADDK - NIH provided the children (USA).

**Histological Tissue Processing: Hemotoxylin and Eosin (H & E)**

A processing schedule was followed for handling the tissues. The following mechanized processing steps were used to pass the fixed plastic cassette tissues in 10% formalin through various degrees of alcohol: 70% booze 80% alcohol in two hours 90% booze in two hours 90% booze 95% alcohol in two hours Complete two hours Xylene: 1 hour, 2 hours Xylene II for two hours 1 2 hours of melted paraffin wax Wax II (melted paraffin) 2 hours.

The tissues were taken out of their plastic cassettes following the final time, positioned in the middle of the metallic tissue mold, and then filled with melted paraffin wax. Additionally, they were allowed to firm before spending 15 minutes at $5^0$C in the refrigerator. The blocks were taken out of the metallic casing with a knife and the paraffin wax at the side was removed once they had cooled in the refrigerator for the previously mentioned fifteen minutes. After that, the blocks were sliced and trimmed serially using a rotary microtome at 3μm.

The parts were lifted using a clean, frosted end slide after floating in a water bath at 55 degrees Celsius. The sections were then de-waxed, hydrated, air-dried, and kept in a slide box in preparation for the staining process. The frosted end slides were now placed on the hot plate for 40 minutes to ensure proper attachment of the sections to the slides. The lung tissue slices were stained using the Haematoxylin and Eosin procedure and the Periodic Acid–Schiff (PAS) method for overall tissue structure.

1. The sections were dewaxed in 3 changes of xylene 5 minutes
2. The sections were hydrated through descending grades of alcohol (absolute, 95%, 80% and 70%).
3. The sections were stained in Harris haematoxylin for 5 minutes
4. The sections were rinsed in running tap water to remove excess stain
5. The sections were differentiated in 1% acid alcohol 3 seconds
6. The sections were blued in running tap water for 10 minutes
7. The sections were counterstained with 1% eosin 1 minute
8. Sections were finally rinsed in water, and dehydrated in ascending grades of alcohol (70%, 80, 95%, and absolute)
9. The sections were cleared in xylene, air-dried, and mounted with dibuthylphthalate propylene xylene (DPX).

The slides were examined under a light microscope and photomicrographs were taken.

**Measurement of Glutathione peroxidase (GPx) activity**

Using a commercially available kit (Ransel kit, Randox Laboratories Ltd, Crumlin, UK), the quantity of GPx was calculated by monitoring the rate at which NADPH oxidized at 340 nm. The quantity of enzyme required to oxidize one nanomol of NADPH oxidase per minute was used to define an enzyme unit.

**Assay of Superoxide dismutase (SOD) activity**

Rukmini *et al.* (2004) reported that superoxide dismutase activity was tested using the Winterbourn *et al.* (1975) technique. The assay's basic idea was based on SOD's capacity to prevent nitro-blue tetrazolium (NBT) from being reduced. In summary, the reaction mixture included 0.1 ml of enzyme samples, 0.05 ml of 0.12 mM riboflavin, 0.1 ml of 1.5 mM NBT, 0.05 ml of 0.01M methionine, and 2.7 ml of 0.067M phosphate buffer at pH 7.8. To guarantee even lighting of the tubes, they were placed in a box with a 15W fluorescent bulb and covered with airfoil for ten minutes. There was also a control group without the enzyme source.

At 560 nm, the absorbance was measured. The quantity of enzyme needed to prevent the decrease of NBT by 50% under the given circumstances was defined as one unit of SOD. Units of the enzyme's activity were represented as mg of protein.

**Estimation of Lipid peroxidation (Malondialdehyde (MDA)**

By using Buege and Aust's thiobarbituric acid reactive substances (TBARS) technique, colorimetric measurements of the tissue's lipid peroxidation were made (1978). Lipid peroxidation produces malondialdehyde (MDA), which is a major component of TBARS. In summary, 2 ml of the 1:1:1 ratio TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25 N HCl, and 15% TCA) was added to 0.1 ml of tissue in Tris-HCl buffer, pH 7.5. The tissue was then put in a water bath for 15 minutes and allowed to cool. At 535 nm, the absorbance of the clear supernatant was measured in comparison to the reference blank. Concentration was calculated using the molar absorptivity of malondialdehyde which is 1.56 x105 M-1 cm-1 and expressed as nmol/mg protein.

**Assay of Catalase (CAT) activity**

The Aebi (1983) technique was used to test the catalase activity. 0.1 ml of tissue was pipetted into a cuvette that held 1.9 ml of pH 7.0, 50 mM phosphate buffer. The addition of 1.0 ml of recently made 30% (v/v) hydrogen peroxide ($H_2O_2$) initiated the reaction. Using spectrophotometry, the rate of $H_2O_2$ breakdown was determined by monitoring changes in absorbance at 240 nm. Units of the enzyme's activity were represented as mg of protein.

**Statistical Analysis**

For each number, the mean and standard error of the mean (S.E.M.) were determined. Duncan's multiple range tests were used in conjunction with a one-way analysis of variance (ANOVA) to compare the treatment and control groups. At p~0.05, differences were deemed statistically significant.

## Ethical Clearance

Ethical approval was sought and obtained from the Human Research and Ethical Committee (HREC), College of Health Science, Benue State University, Makurdi. All experimental procedures carried out were following the guidelines on animal experiments as prescribed by the Ethics Committee. A copy of the proposal was sent to the ethical committee to be examined for approval.

## Results

### Gross Anatomical Parameters: Body Weight and Testes Weight

### Body Weight

The results of our physical observation unveiled a sustained and progressive increase in the body weight of all experimental models, from the first to the fourth week of the study. This observed increase in body weight indicates a consistent pattern of weight gain among the subjects over the designated timeframe. A noteworthy development emerged at the culmination of the fourth week, where a substantial cessation in weight gain was distinctly observed. This pivotal point in the study timeline suggests a potential saturation or leveling off in the body weight of the experimental models. The findings elucidated that groups B through E exhibited body testes weights in comparison to the counterparts in groups A and F – I.

**Figure 1:** Simple Bar Chart Showing the Mean Body Weight across Groups in Week 1

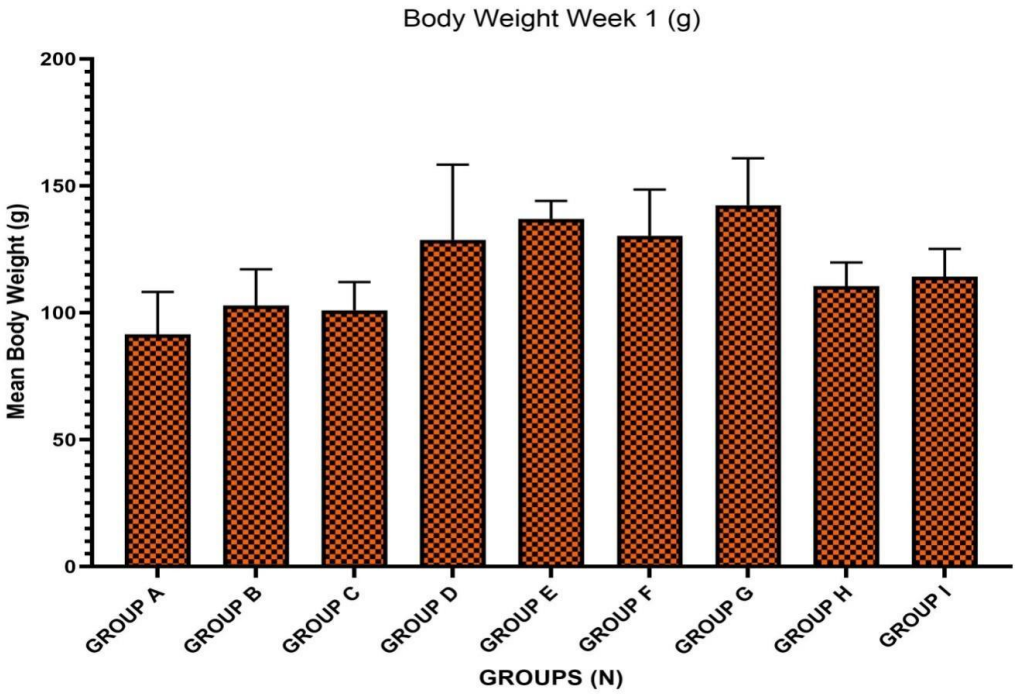



**Figure 2:** Simple Bar Chart Showing the Mean Body Weight across Groups in Week 2

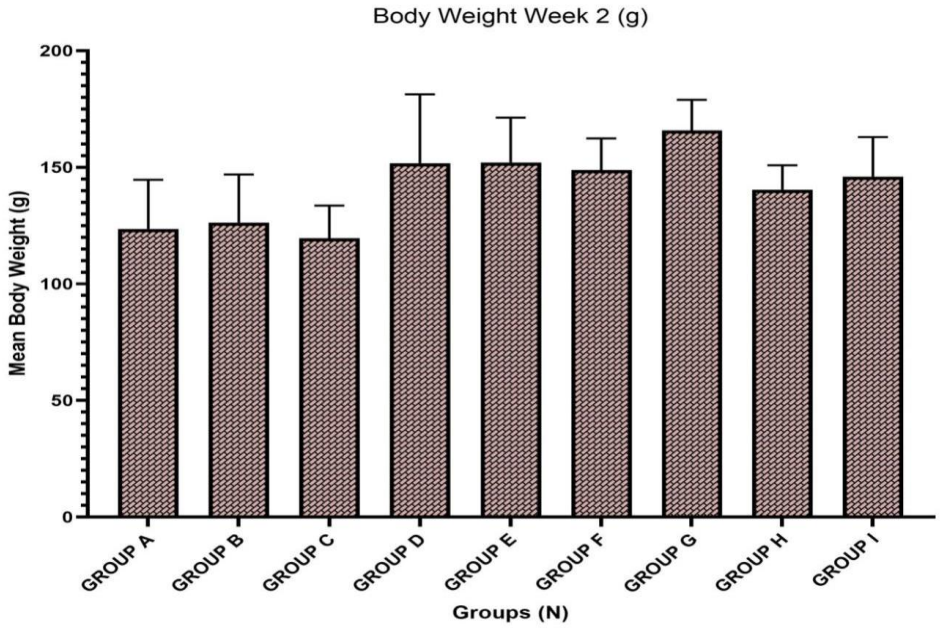



**Figure 3:** Simple Bar Chart Showing the Mean Body Weight across Groups in Week 3

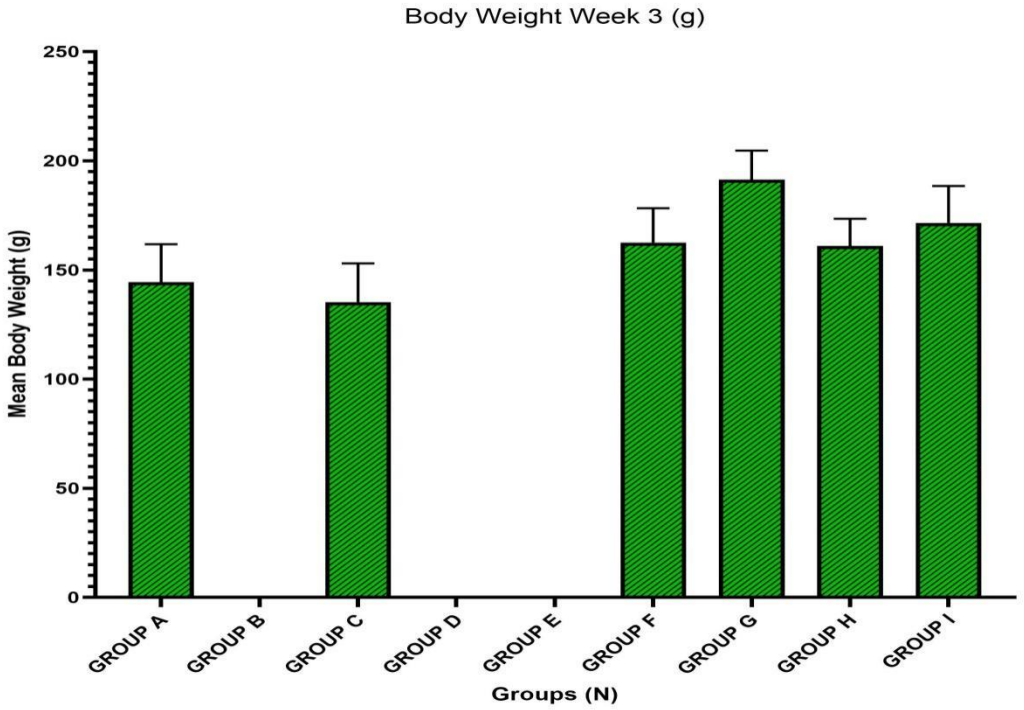
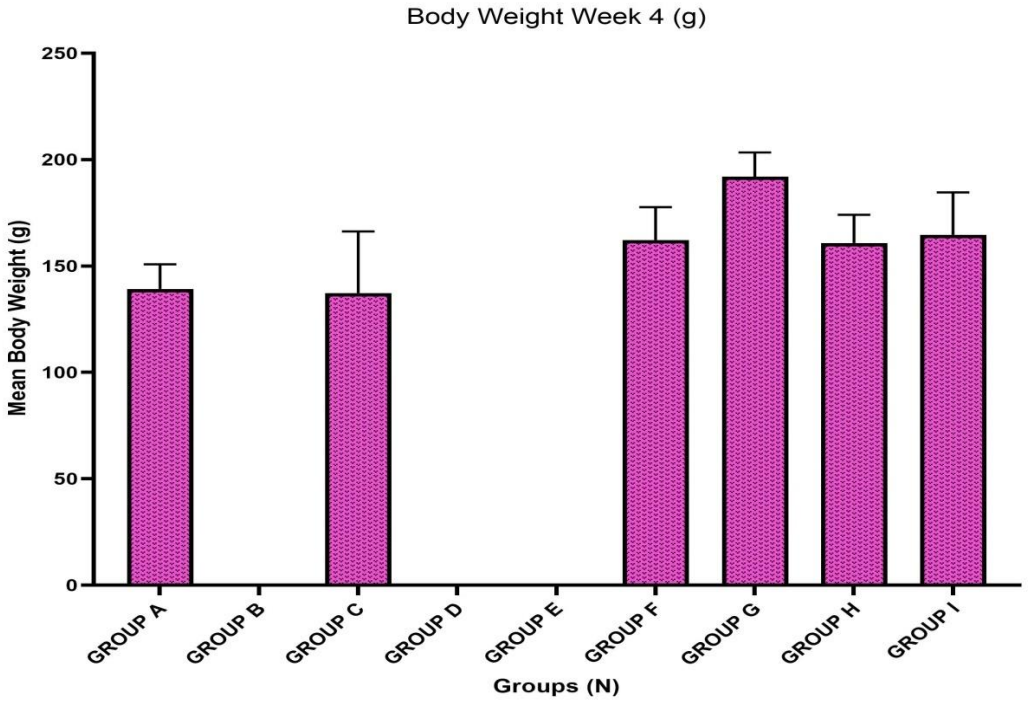



**Figure 4:** Simple Bar Chart Showing the Mean Body Weight across Groups in Week 4

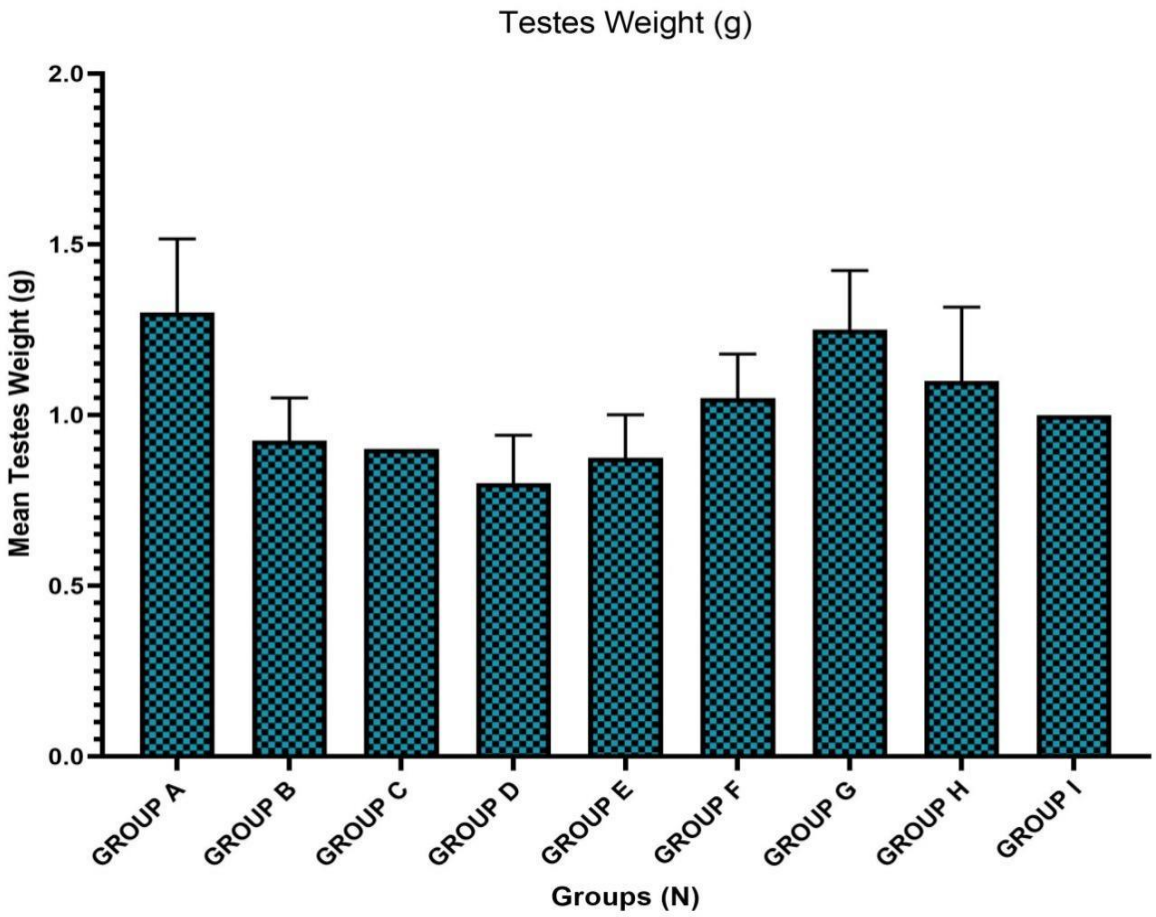



## Testes Weight

The testes weights of the experimental animals across groups in the research were measured, and the mean weight for each group was recorded as presented in Figure 5. The findings elucidated that animals in groups B through E showed lower testes weights in comparison to the counterparts in groups A and F through I.

The pattern of changes in testes weight for the animals was not consistent, as groups that were administered both Ketoconazole and the extract of Curcumin all showed irregular changes in the mean testes weight as compared to the control group (group A). The changes in testes' weight in this research may not be completely attributed to the administration of Ketoconazole and the extract of Curcumin due to the observed inconsistencies in the pattern of changes. It thus implies that the administration of different doses of Ketoconazole and the extract of Curcumin had no significant effect on the testes weight of the experimental animals, and that the observed changes in testes weight might be due to other factors such as stress, nutrition, environmental factors, and even hormonal effects.

**Figure 5:** Simple Bar Chart Showing the Mean Testes Weight of Animals across Groups




## Hormonal Analysis

### Reproductive Hormones: Testosterone, Follicle Stimulating Hormone and Luteinizing Hormone

The hormonal profile of rats across groups in this study revealed a statistically significant ($p \leq 0.05$) reduction in plasma and testicular testosterone, FSH, and LH in group B and E rats, as compared to the control group. Upon further comparison of these hormone levels with those of group B rats, no significant differences were detected in the other groups, except for group E rats, whose hormone levels were found to be comparable to the test group (group B).

Furthermore, no significant differences ($p > 0.05$) were identified when evaluating the hormonal levels in group D (low dose), group I, and group F and G against the corresponding levels in groups C, H, and F and G, respectively. This suggests a consistent hormonal profile among these groups, indicating that the administered doses or treatments did not elicit substantial variations in hormone concentrations.

Interestingly, a distinctive finding emerged, revealing a significant increase in LH and FSH levels in group I compared to group H. This notable contrast in hormonal levels within specific groups provides valuable insights into the nuanced effects of the experimental conditions on the endocrine system, enhancing our understanding of the differential impact on LH and FSH regulation. The detailed results are presented in Figure 6 and Table 1, offering a comprehensive overview of the observed hormonal variations across the experimental groups.

**Figure 6:** Simple Bar Chart Showing the Mean Plasma and Testicular Testosterone Levels across Groups

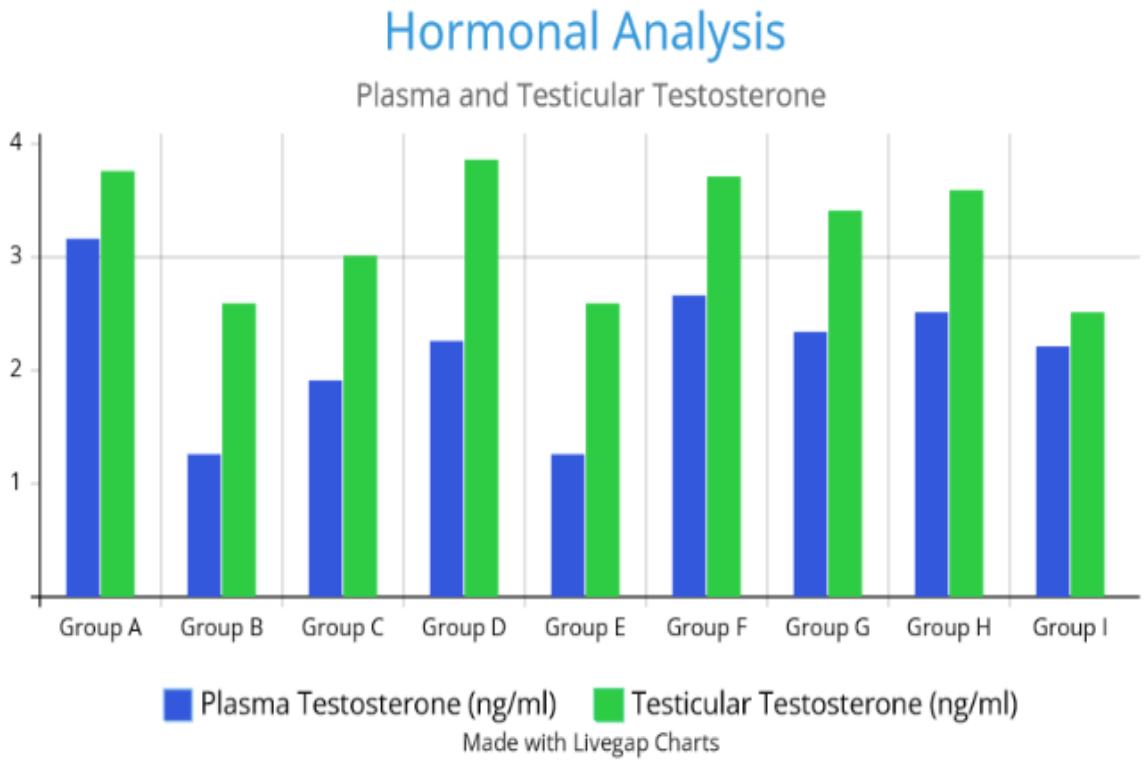


**Table 1:** Showing the Mean Follicle Stimulating Hormone and Luteinizing Hormone Levels across Groups

| GROUPS | Follicle Stimulating Hormone (ng/ml) | Luteinizing Hormone (mlU/ml) |
|---|---|---|
| GROUP A | 4.88±2.77 | 2.48±0.57 |
| GROUP B | 1.45±0.90$^{a}$ | 1.05±0.21 $^{a}$ |
| GROUP C | 3.60±0.00 | 1.80±0.00 |
| GROUP D | 6.17±3.74 | 1.65±0.37 |
| GROUP E | 1.20±0.32 $^{a.+}$ | 1.05±0.27 $^{a.+}$ |
| GROUP F | 1.83±0.45 | 1.87±0.15 |
| GROUP G | 1.55±0.64 | 2.00±0.08 |
| GROUP H | 1.75±0.34 | 2.03±0.21 |
| GROUP I | 2.50±0.00$^{++}$ | 2.40±0.00$^{++}$ |

$^{a,+,++,}$ and $^{*}$ represent significant decreases or increases at $p \leq 0.05$ when compared to groups A (control), group B (test) groups C, groups H (low dose), and groups F and G (curative groups) respectively**.** N = 4; Values Expressed as MEAN±SD.

**Oxidative Stress Markers**

A marked reduction in the activity levels of SOD, CAT, and GPx, coupled with a substantial increase in the level of MDA, was evident in group B in comparison to the control group, with statistical significance ($p \leq 0.05$). Notably, when comparing these enzymatic activities between group B and group D (low dose), as well as group I, and between group B and group F and G, there were no statistically significant ($P > 0.05$) differences observed. Similarly, no significant disparities in enzyme activity were noted when comparing Group D with Group C, the group I with Group H, and Group F and G with Group C, H, and F and G, respectively.

**Figure 7:** Simple Bar Chart Showing the Mean Superoxide dismutase (SOD) and Catalase (CAT) Levels across Groups

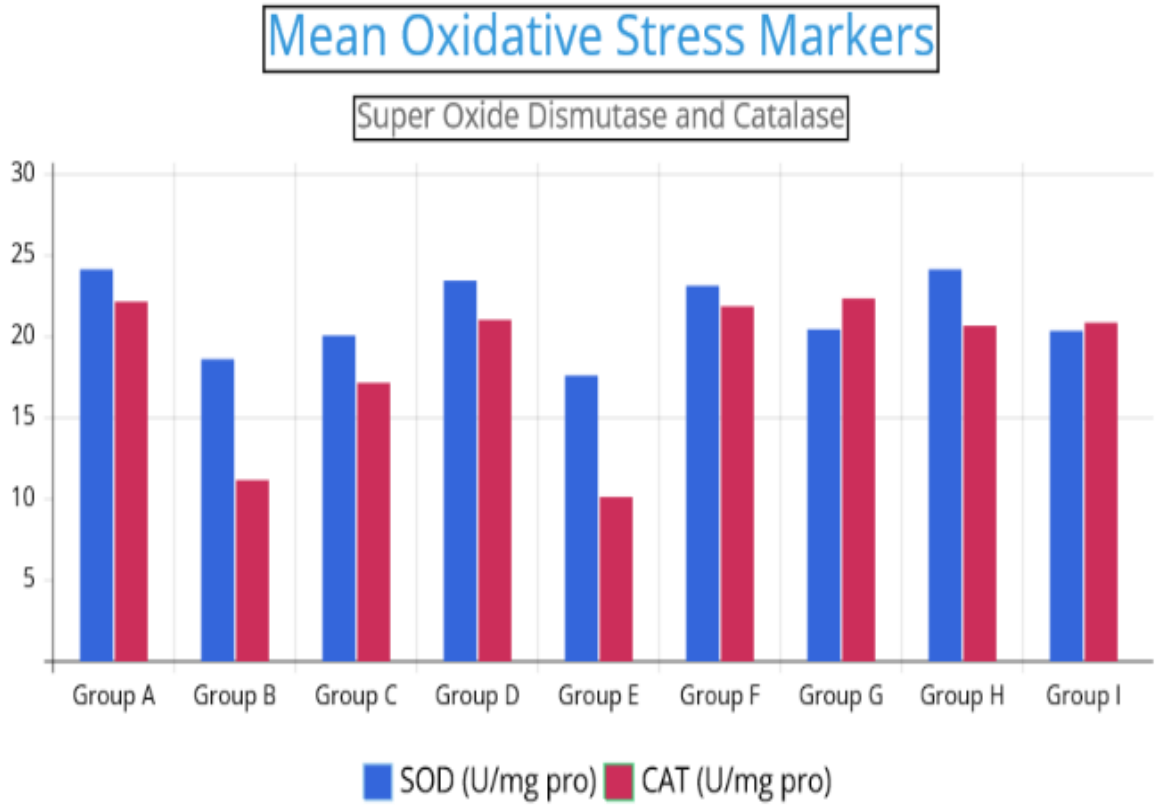
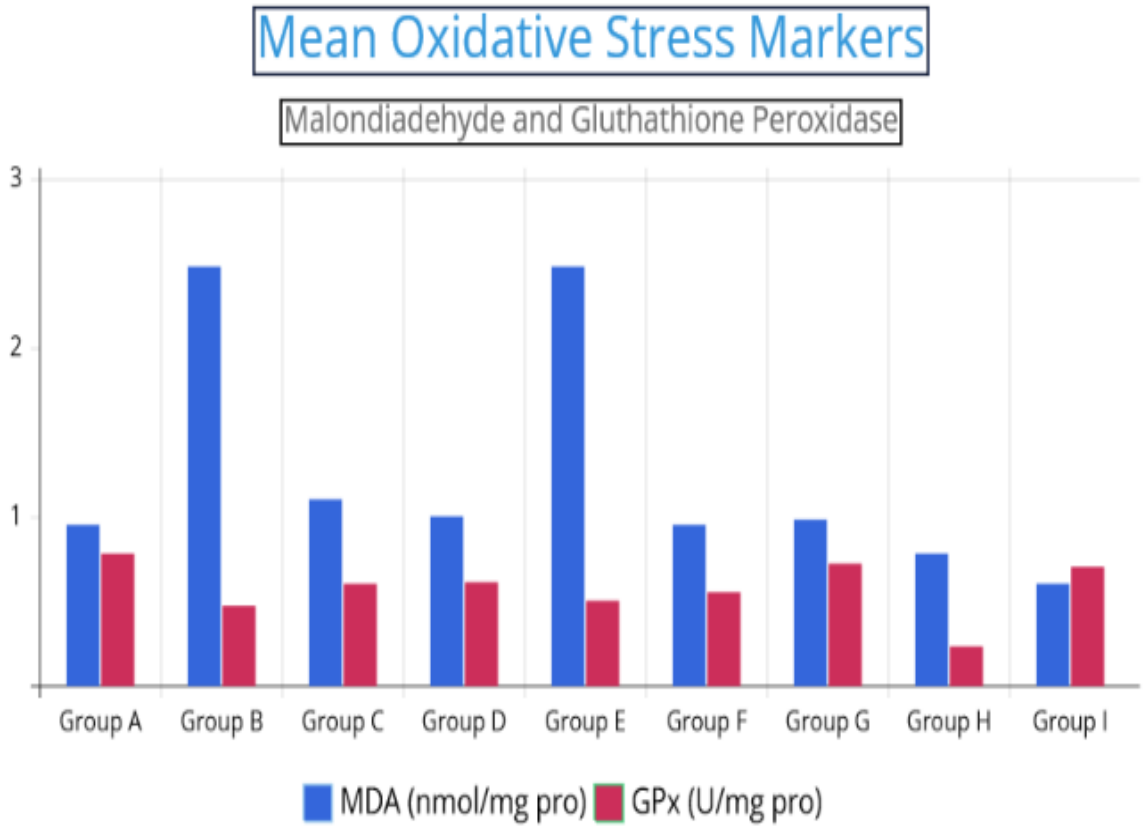


**Figure 8:** Simple Bar Chart Showing the Mean Malondialdehyde (MDA) and Glutathione Peroxidase (GPx) Levels across Groups




**Sperm Analysis**

The mean sperm parameters across groups in this study were compared by one–way ANOVA, as presented in Table 2. Mean differences were considered statistically significant at $p \leq 0.05$. For the sperm count; group A (Control) had the highest mean sperm count (13.20 ± 8.27), significantly higher than all other groups except Group H (Protective with 10mg/kg Ccm + 100mg/kg Kt). Group B (test, induced with 100mg/kg Kt) showed a significantly lower sperm count compared to all other groups. Groups C - I showed varying degrees of decrease in sperm count compared to the Control, with some significant differences observed. For sperm motility; group A (Control) has the highest mean motility (42.00% ± 24.65), significantly higher than most other groups except Groups H and G. Group B (test) showed no motility, indicating complete loss. Group C - I had significantly lower motility compared to the Control. Progressive motility appears consistently low across all groups. Group B (test) and Groups D, E, F, and I had significantly lower progressive motility compared to the Control. For Sperm Morphology; Group A (Control) had the highest mean morphology percentage (48.70% ± 17.25), significantly higher than most other groups except Group H. Group B (test) had the lowest morphology percentage (13.33% ± 23.09),

significantly lower than all other groups. Groups C, D, E, F, and G showed significantly lower morphology percentages compared to the Control.

Overall, the result in table 2 suggests that ketoconazole (Kt) administration (Group B) significantly reduces sperm count, motility, progressive motility, and morphology compared to the Control. Curcumin administration alone (Groups D and E) also appears to have negative effects on sperm parameters. However, when administered in combination with ketoconazole (Groups C, F, and G), curcumin shows some protective effects, particularly in mitigating the adverse effects induced by ketoconazole. Also, protective regimens (Groups H and I) involving curcumin administration before ketoconazole treatment show promising results in preserving sperm parameters, with Group I (20mg/kg Ccm + 100mg/kg Kt) even surpassing the Control in sperm count.

**Table 2:** Showing the Mean Sperm Parameters across Groups Compared to one–way ANOVA

| GROUPS (N) | SPERM COUNT ($x10^6$/ml) | MOTILITY (%) | PROGRESSIVE MOTILITY (%) | MORPHOLOGY (%) |
|---|---|---|---|---|
| A (C: Distilled Water) | $13.20\pm8.27^{+*}$ | $42.00\pm24.65^{+\#}$ | $1.25\pm.50$ | $48.70\pm17.25^{+*}$ |
| B (T: 100mg/kg Kt) | $1.47\pm1.41^{a\#*}$ | $0.00\pm.00^{a\#*}$ | $1.00\pm.00$ | $13.33\pm23.09^{a\#*}$ |
| C (100mg/kg Kt + 10mg/kg Ccm) | $10.60\pm.00^{+*}$ | $31.80\pm.00^{+}$ | $1.00\pm.00$ | $60.00\pm.00^{a+*}$ |
| D (LD: 10mg/kg Ccm) | $7.73\pm8.53^{a+}$ | $3.03\pm5.25^{a+\#*}$ | $1.00\pm.00$ | $25.00\pm25.00^{a+\#}$ |
| E (HD: 20mg/kg Ccm) | $4.30\pm3.53^{a+\#}$ | $16.65\pm23.54^{a+\#*}$ | $1.00\pm.00$ | $41.65\pm11.80^{+\#}$ |
| F (Ct: 100mg/kg Kt + 10mg/kg Ccm) | $3.95\pm3.42^{a\#}$ | $40.83\pm10.68^{+\#}$ | $1.00\pm.00$ | $23.75\pm33.00^{a+\#}$ |
| G (Ct: 100mg/kg Kt + 20mg/kg Ccm) | $8.50\pm4.33^{a+}$ | $45.40\pm23.95^{+\#}$ | $1.75\pm.50$ | $38.05\pm16.99^{+\#}$ |
| H (Pt: 10mg/kg Ccm +100mg/kg Kt ) | $14.35\pm10.63^{+*}$ | $47.35\pm32.81^{+\#}$ | $1.75\pm.50$ | $66.38\pm18.74^{a+*}$ |
| I (Pt: 20mg/kg Ccm +100mg/kg Kt ) | $28.00\pm.00^{a+\#*}$ | $16.70\pm.00^{a+\#*}$ | $1.00\pm.00$ | $40.00\pm.00^{+\#}$ |

Values are expressed as MEAN±SD; N = 6; C = Control; T = Test; Kt = Ketoconazole; Ccm = Curcumin; Ct = Curative; Pt = Protective; LD = Low Dose; HD = High Dose
a = statistically significant difference in mean at $p\leq0.05$ compared to the Control Group (A)
+ = statistically significant difference in mean at $p\leq0.05$ compared to the Test Group (B)
# = statistically significant difference in mean at $p\leq0.05$ compared to Group C

* = statistically significant difference in mean at p≤0.05 compared to the Curative Groups (F & G)

**Histological Profile**

Histological examinations of testicular tissue in group A revealed normal characteristics, showcasing well-defined seminiferous tubules adorned with a progression of spermatogenic cells, including spermatogonia, primary spermatocytes, and early spermatids. Sertoli cells were prominently present, hosting attached sperm. Surrounding the tubules, a basement membrane enveloped myoid cells, and interstitial spaces housed Leydig cells with vesicular nuclei featuring prominent nucleoli (Plate 1).

In stark contrast, group B exhibited aberrant seminiferous tubule morphology, marked by spermatid retention, tubular atrophy, and widespread disorganization of germ cells. The testicular architecture displayed signs of degeneration, with the absence of interstitial space and discernible areas of necrosis. Furthermore, maturing spermatogenic cells were detected within the seminiferous tubules, some displaying nuclear membrane rupture and accompanying nucleus fragmentation (karyorrhexis).

Conversely, groups C - I exhibited distinctive features, characterized by spermatogonia cells with deeply stained nuclei and compromised sperm cells. The majority of seminiferous tubules appeared shrunken with a wavy outline. Evident thickening and hyalinization of basement membranes were observed, and the lumens of the seminiferous tubules were predominantly occupied by fragments of disintegrated cells. Additionally, contours of the seminiferous tubule, degeneration of spermatogenic epithelial series, and apoptotic cells were noted in these groups, accompanied by thick and irregular basement membranes. Interstitial cells of Leydig displayed scanty cytoplasm with either deeply stained nuclei or normal vesicular nuclei (Plate 3 - 10).

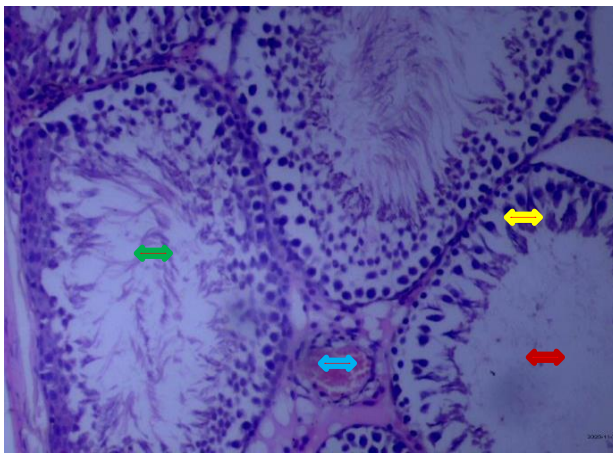


**Plate 1:** Photomicrograph of testes from group A showing the lumen (Red Arrow), Spermatozoa (Green Arrow); Sertoli Cells (Yellow arrow), and interstitium (Blue Arrow)**. Stain:** H&E; **Magnification**: x40.

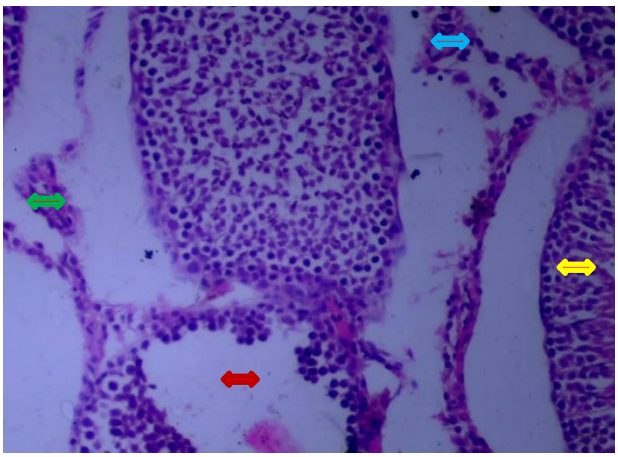


**Plate 2:** Photomicrograph of testes from group B showing the lumen (Red Arrow), Spermatozoa (Green Arrow); Sertoli Cells (Yellow arrow), and interstitium (Blue Arrow)**. Stain:** H&E; **Magnification**: x40.

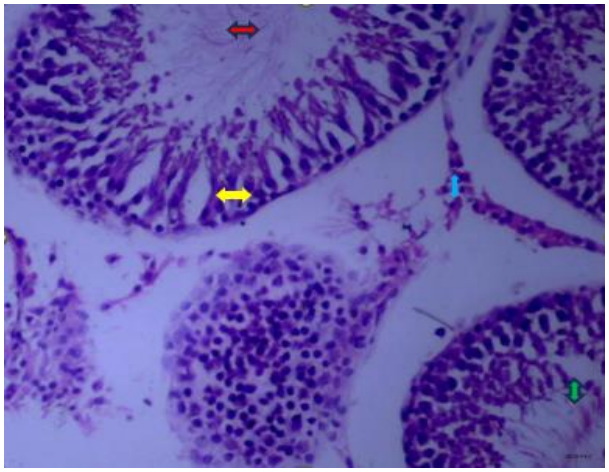


**Plate 3:** Photomicrograph of testes from group C showing the lumen (Red Arrow), Spermatozoa (Green Arrow); Sertoli Cells (Yellow arrow), and interstitium (Blue Arrow)**. Stain:** H&E; **Magnification**: x40.

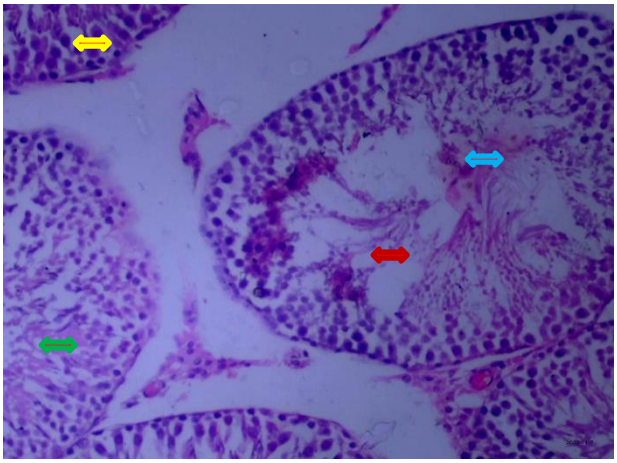


**Plate 4:** Photomicrograph of testes from group D showing the lumen (Red Arrow), Spermatozoa (Green Arrow); Sertoli Cells (Yellow arrow), and interstitium (Blue Arrow)**. Stain:** H&E; **Magnification**: x40.

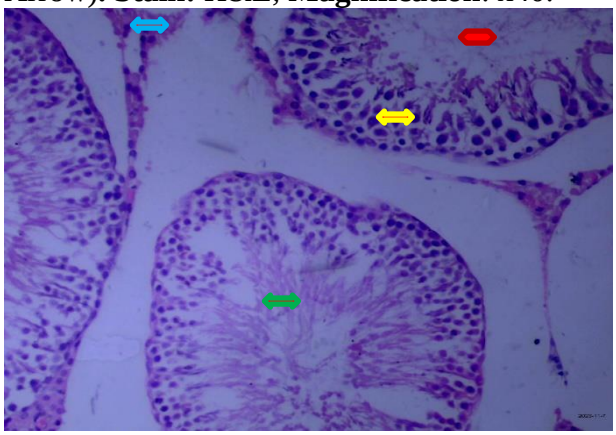


**Plate 5:** Photomicrograph of testes from group E showing the lumen (Red Arrow), Spermatozoa (Green Arrow); Sertoli Cells (Yellow arrow), and interstitium (Blue Arrow)**. Stain:** H&E; **Magnification**: x40.

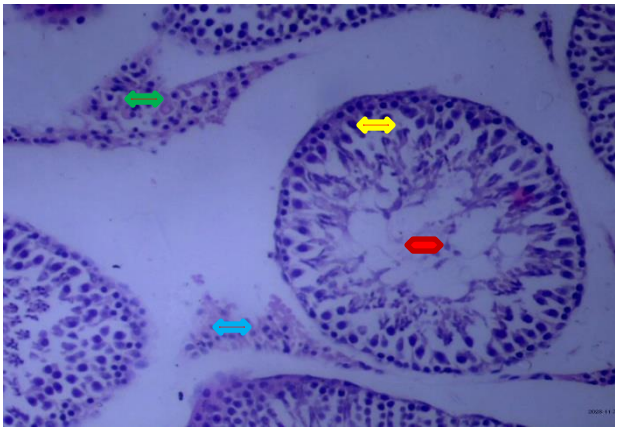


**Plate 6:** Photomicrograph of testes from group F showing the lumen (Red Arrow), Spermatozoa (Green Arrow); Sertoli Cells (Yellow arrow), and interstitium (Blue Arrow)**. Stain:** H&E; **Magnification**: x40.

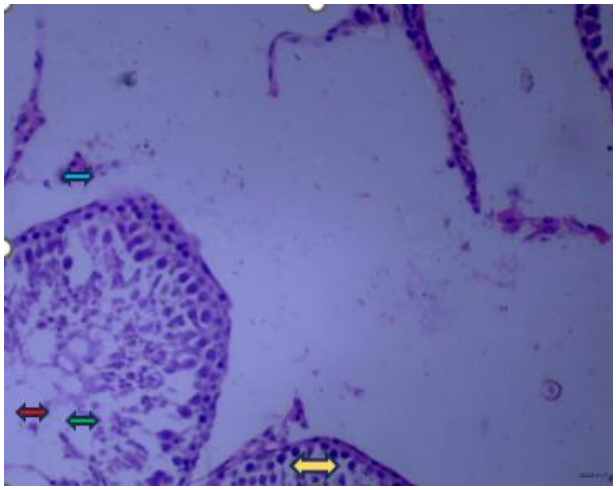


**Plate 7:** Photomicrograph of testes from group G showing the lumen (Red Arrow), Spermatozoa (Green Arrow); Sertoli Cells (Yellow arrow) and interstitium (Blue Arrow)**. Stain:** H&E; **Magnification**: x40.

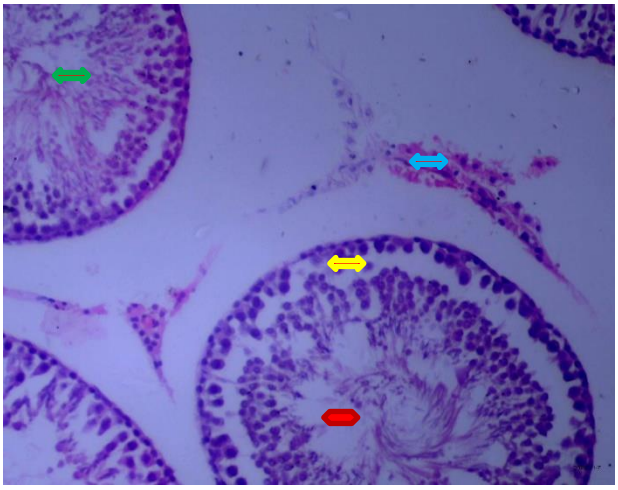


**Plate 8:** Photomicrograph of testes from group H showing the lumen (Red Arrow), Spermatozoa (Green Arrow); Sertoli Cells (Yellow arrow) and interstitium (Blue Arrow)**. Stain:** H&E; **Magnification**: x40.

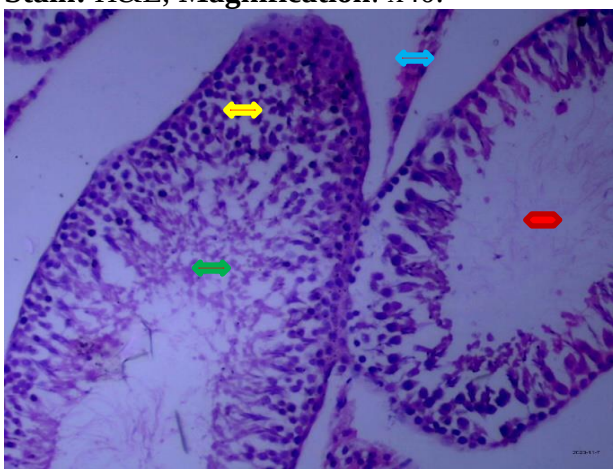


**Plate 9:** Photomicrograph of testes from group I showing the lumen (Red Arrow), Spermatozoa (Green Arrow); Sertoli Cells (Yellow arrow), and interstitium (Blue Arrow)**. Stain:** H&E; **Magnification**: x40.

## Discussion

### Physical Parameters: Body Weight and Testes Weight

Our investigation revealed a sustained and progressive increase in the body weight of all experimental models, aligning with established physiological expectations of weight gain in growing subjects over a defined period. This phenomenon is commonly observed in experimental studies involving rodent models, reflecting the normal growth trajectory and metabolic changes associated with developmental stages (Heredia *et al.*, 2006; Bondy, 1983).

However, a significant deviation from this expected weight gain pattern was identified after the fourth week. This observed inflection point in weight gain raises intriguing questions about the underlying physiological processes influencing body weight regulation in the experimental models (Adeoye *et al.,* 2022). In scientific terms, this deviation may be indicative of a potential physiological saturation or homeostatic mechanism, wherein the animals may be reaching a point of equilibrium in their growth and weight regulation.

In rodent physiology, factors such as hormonal changes, metabolic adaptations, and the interplay of growth-promoting and inhibitory signals contribute to the regulation of body weight (Jéquier, E., & Tappy, 1999). The observed cessation in weight gain may be rooted in complex interactions within these physiological mechanisms (Novelli *et al.*, 2007).

Further investigation into specific markers of growth, hormonal profiles, and metabolic parameters could provide a more detailed understanding of the factors contributing to this observed phenomenon.

The scientific basis for the observed weight gain pattern in the experimental models aligns with established norms in rodent physiology (Alario *et al.*,1987). The deviation in the fourth week prompts a deeper exploration into the underlying physiological processes, offering an opportunity to uncover valuable insights into the intricacies of growth regulation in the context of experimental conditions.

It has been reported that testis weight is directly correlated with the sperm population, as it primarily reflects the total volume of the seminiferous tubule (Caldeira *et al.,* 2010). Other studies differ (Trautwein *et al.,* 2017). The observed disparity in testes weights among experimental groups B through E compared to groups A and F through I hold significant implications rooted in reproductive physiology. In scientific terms, testicular weight is a crucial indicator of reproductive health and functionality in experimental models, and alterations in testicular weight can provide valuable insights into the potential effects of experimental conditions on the male reproductive system.

The findings of this research elucidated that animals in groups B through E showed lower testes weights in comparison to the counterparts in groups A and F through I.

The pattern of changes in testes weight for the animals was not consistent, as groups that were administered both Ketoconazole and the extract of Curcumin all showed irregular changes in the mean testes weight as compared to the control group (group A). The changes in testes weight in this research may not be completely attributed to the administration of Ketoconazole and the extract of Curcumin due to the observed inconsistencies in the pattern of changes. It thus implies that the administration of different doses of Ketoconazole and the extract of Curcumin had no significant effect on the testes weight of the experimental animals and that the observed changes

in testes weight might be due to other factors such as stress, nutrition, environmental factors, and hormonal effects.

The observed disparity in testes weights among experimental groups B through E compared to groups A and F through I hold significant implications rooted in reproductive physiology. In scientific terms, testicular weight is a crucial indicator of reproductive health and functionality in experimental models, and alterations in testicular weight can provide valuable insights into the potential effects of experimental conditions on the male reproductive system.

The testes are key organs responsible for spermatogenesis, hormone production, and overall reproductive function. Changes in testicular weight may be associated with variations in cell proliferation, differentiation, or degeneration within the testicular tissue (Kelvin *et al.,* 2012). In the context of experimental studies, factors such as exposure to substances, environmental conditions, or treatment regimens may influence these cellular processes, thereby impacting testicular weight (Roy *et al.,* 2002; Caldeira *et al.,* 2010). Testicular weight is intricately linked to sperm production and quality. Reductions in testes weights, as observed in groups B through E, may suggest disruptions in spermatogenesis or alterations in the structural integrity of testicular tissue (Lunstra *et al.,* 1988; Spears *et al.,* 2013; Trautwein *et al.,* 2017). Scientifically, this prompts an investigation into the specific cellular and molecular mechanisms underlying these changes, including potential effects on germ cell populations, Sertoli cell function, and interstitial cell activity within the testes.

Furthermore, alterations in testicular weight may also be associated with changes in hormonal regulation, as the testes play a central role in hormone production, including testosterone (Amory & Bremner, 2002; Amory & Bremner, 2003; Camacho *et al.,* 2013). Scientifically, exploring the hormonal profiles alongside testicular weight can provide a comprehensive understanding of the endocrine aspects influencing the observed disparities.

**Reproductive Hormones**

The significant decrease in plasma and testicular testosterone, Follicle Stimulating Hormone, and Luteinizing Hormone levels observed in Group B and E rats, as compared to the control group, reflects a substantial impact on the endocrine system, particularly the reproductive hormonal regulation. This finding is scientifically significant as these hormones play pivotal roles in the regulation of reproductive processes, including spermatogenesis and hormonal feedback loops.

Ketoconazole, a potent antifungal agent, has been identified to interfere with crucial enzymatic activities in the testes, as demonstrated by Ayub & Levell (1987). Their work showcases ketoconazole's specific inhibition of testicular 17α-hydroxylase and 17,20-lyase, key enzymes involved in steroidogenesis, while leaving other enzymes unaffected. Rajfer *et al.* (1986) add depth to this understanding by unraveling the intricate mechanisms by which ketoconazole inhibits human testicular steroidogenesis. Lauwers *et al.* (1985) contribute by identifying a metabolite, 17α, 20α-dihydroxyprogesterone, in testicular extracts following ketoconazole exposure, shedding light on the drug's metabolic consequences. Amin's investigation in 2008 provides empirical evidence of ketoconazole-induced testicular damage in rats, reinforcing the clinical relevance of these biochemical disruptions. Additionally, Baek *et al.* (2007) and Lambert *et al.* (1986) extend

the scope to the broader repercussions of ketoconazole on testicular and adrenal steroidogenesis, respectively, enriching our understanding of its endocrine impact. Trachtenberg & Zadra (1988) meticulously investigate the sites of action for ketoconazole's inhibition of steroid synthesis, providing a crucial link between its biochemical interference and observed physiological effects.

Testosterone is a key male sex hormone produced in the testes, and its decrease can influence various physiological functions, such as sperm production, muscle mass, and bone density. Similarly, FSH and LH are essential for the regulation of spermatogenesis and the synthesis of testosterone. A decrease in these hormones can disrupt the delicate balance required for normal reproductive function. The lack of significant differences in hormone levels when comparing other experimental groups with Group B rats, except for Group E, suggests certain specificity in the impact of treatments. Notably, Group E rats displayed hormonal levels comparable to the positive control group. This observation is scientifically intriguing as it indicates that specific treatments applied to Group E may have mitigated the hormonal disruptions observed in other experimental groups, potentially restoring hormonal homeostasis to a level akin to normal physiological conditions.

Furthermore, the absence of significant differences when comparing hormonal levels in Group D (low dose), Group I, and Group F and G with their respective counterparts in Group C, H, and F and G, suggests a consistent hormonal profile across these groups. Scientifically, this implies that the administered doses or treatments in these comparisons did not induce substantial variations in hormone concentrations, strengthening the reliability and reproducibility of the study's hormonal findings.

The particularly intriguing observation of a significant increase in LH and FSH levels in Group I compared to Group H adds depth to our understanding of the nuanced effects of the experimental conditions. Scientifically, this finding highlights the specificity of hormonal responses to certain treatments and emphasizes the complex regulatory mechanisms governing LH and FSH. The detailed presentation of these results in Figure 6 and Table 1 contributes significantly to our broader understanding of the study's endocrine implications, providing a comprehensive overview of the observed hormonal variations across diverse experimental groups. In the context of scientific research, these findings contribute valuable insights into the potential effects of experimental conditions on the intricate regulatory networks of reproductive hormones, advancing our understanding of endocrine physiology.

**Oxidative Stress**

The observed decline in the activity levels of antioxidant enzymes (SOD, CAT, and GPx) and the notable elevation in malondialdehyde (MDA) concentration in Group B compared to the control group provides scientific insights into the oxidative stress dynamics within the experimental setting. Oxidative stress is a condition characterized by an imbalance between the production of reactive oxygen species (ROS) and the ability of antioxidant defenses to neutralize them. This phenomenon has implications for cellular damage and is a crucial aspect of understanding the impact of experimental conditions on biological systems.

Antipsychotics exert a discernible influence on oxidative status, as underscored by a recent comprehensive study conducted by Yang *et al.* (2022). This investigation delves into the comparative analysis of the pre-and post-medicative effects of antipsychotic agents on blood-based oxidative stress biomarkers in individuals with schizophrenia. The findings illuminate significant alterations in antioxidant enzymes and lipid peroxidation levels among schizophrenia patients, establishing oxidative stress as a key contributor to heightened lipid peroxidation across all patient subtypes treated with both typical and atypical antipsychotics, as expounded in the seminal work by Zhang *et al.* (2006). This elucidates the intricate relationship between antipsychotic medications and oxidative stress, offering valuable insights into potential implications for oxidative status in individuals undergoing schizophrenia treatment. Simultaneously, ketoconazole treatment, as investigated by Zhang *et al.* (2016), is associated with a reduction in antioxidant enzyme activities and an elevation in malondialdehyde (MDA) levels. This observed phenomenon extends beyond species, evident in freshwater planarians subjected to ketoconazole treatment, exhibiting a parallel decline in antioxidant enzyme activities and an increase in MDA concentration. Adding depth to this understanding, individuals with schizophrenia undergoing treatment with both typical and atypical antipsychotics consistently demonstrate decreased glutathione peroxidase (GPx) specific activity and elevated MDA levels, irrespective of the specific antipsychotic treatment employed, as highlighted by Pădurariu *et al.* (2010). This dual perspective accentuates the comprehensive impact of ketoconazole and antipsychotic medications on oxidative stress parameters across diverse contexts.

Scientifically, Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) are key antioxidant enzymes that play vital roles in the defense against ROS. SOD catalyzes the dismutation of superoxide radicals, CAT decomposes hydrogen peroxide, and GPx reduces organic hydroperoxides. A decline in the activity levels of these enzymes, as observed in Group B, indicates a compromised antioxidant defense system, potentially allowing an accumulation of ROS and oxidative damage to cellular components.

The elevation in MDA concentration further strengthens the evidence of increased oxidative stress in Group B. MDA is a byproduct of lipid peroxidation, a process initiated by ROS, leading to the degradation of cellular membranes. Elevated MDA levels serve as a biochemical marker for oxidative damage to lipids. The statistical significance ($p<0.05$) of these alterations emphasizes the robustness of the findings, underscoring the validity of the observed oxidative stress changes.

The intriguing consistency in oxidative stress profiles between groups B, D, I, and F-G, despite variations in doses or treatments, suggests that these specific experimental factors did not markedly influence antioxidant enzyme activities and MDA levels. Scientifically, this consistency may imply a shared underlying mechanism or a threshold effect in response to the experimental conditions, emphasizing the importance of considering the broader context when interpreting oxidative stress data.

Furthermore, the absence of significant disparities in enzyme activity when comparing different groups (D and C, I and H, and F and G with C, H, and F and G, respectively) refines our understanding. Scientifically, this lack of statistical significance indicates a level of homogeneity in the measured oxidative stress parameters across these experimental groups. It suggests that,

despite variations in treatments or doses, the impact on antioxidant enzyme activities and MDA levels remains relatively consistent among these specific comparisons.

These nuanced findings contribute to a more comprehensive interpretation of the impact of administered treatments on oxidative stress dynamics. Scientifically, they provide insights into potential similarities or unique aspects within the studied groups, contributing to our understanding of the complex interplay between experimental conditions and oxidative stress responses in biological systems

**Sperm Analysis**

The results of the study demonstrate a significant impact of ketoconazole (Kt) administration on sperm parameters, with Group B (Test) exhibiting notably reduced sperm count, motility, progressive motility, and morphology compared to the Control (Group A). This finding aligns with existing pieces of literature by Baek *et al.* (2007), which reported adverse effect of ketoconazole on the sperm count and sperm morphology of albino Wistar rats, and that of Pădurariu *et al.* (2010) which demonstrated the modulating role of curcumin against tramadol – induced reduction in sperm count, also indicating the deleterious effects of ketoconazole on testicular function and sperm quality.

Interestingly, the administration of curcumin alone (Groups D and E) also showed adverse effects on sperm parameters, suggesting a potential negative influence of curcumin in isolation. However, when curcumin was administered in combination with ketoconazole (Groups C, F, and G), it demonstrated protective effects, particularly in mitigating the adverse effects induced by ketoconazole. This finding suggests a possible ameliorative role of curcumin against ketoconazole-induced testicular damage, highlighting its potential therapeutic utility in counteracting the harmful effects of certain medications on male reproductive health.

Moreover, protective regimens involving curcumin administration before ketoconazole treatment (Groups H and I) exhibited promising results in preserving sperm parameters. Group I, in particular, showed a remarkable improvement, surpassing the Control in sperm count. These findings underscore the potential preventive and therapeutic benefits of curcumin supplementation in preserving male fertility and protecting against drug-induced testicular damage.

The observed variations in sperm parameters across different treatment groups underscore the complexity of the interactions between curcumin, ketoconazole, and testicular function. Further mechanistic studies are warranted to elucidate the underlying molecular pathways involved in the protective effects of curcumin and its potential synergistic interactions with ketoconazole. Additionally, clinical trials are needed to validate these findings and assess the translational relevance of curcumin-based interventions in the management of drug-induced male infertility. Nonetheless, the present study provides valuable insights into the potential utility of curcumin as a therapeutic agent against ketoconazole-induced testicular damage, warranting further investigation in this field.

**Histological Changes**

The histological examinations of testicular tissue provide a scientific lens into the structural integrity and cellular dynamics within the male reproductive organs, shedding light on the potential impacts of experimental conditions. The seminiferous tubule, which houses various spermatogenic cells, is central to spermatogenesis, the process of sperm cell development. Deviations from normal histological features can signify disruptions in the intricate cellular processes crucial for reproductive function. Group A, characterized by well-defined seminiferous tubules, represents a histologically normal state in the context of rodent reproductive anatomy. This aligns with established expectations of healthy testicular tissue, where the seminiferous tubules support the development and maturation of sperm cells.

In contrast, the aberrant seminiferous tubule morphology observed in Group B, marked by spermatid retention, tubular atrophy, and disorganization of germ cells, indicates potential disruptions in the spermatogenic process. Scientifically, these abnormalities may suggest interference with cellular differentiation, maturation, or cellular integrity within the testicular microenvironment. Ketoconazole exerts profound effects on testicular histopathology, inducing severe lesions characterized by the degeneration of seminiferous tubules and depletion of germ cells (Amin, 2008). Ayub & Levell (1987) elucidate its inhibitory impact on testicular 17α-hydroxylase and 17,20-lyase activities, crucial enzymes in steroidogenesis, without affecting 3β-hydroxysteroid dehydrogenase-isomerase or 17β-hydroxysteroid oxidoreductase. Additionally, Rajfer *et al.* (1986) highlight the significant alterations in steroid precursors, observing elevated delta 5-pregnenolone and decreased intratesticular DHEA, androgens (A), and testosterone (T) following ketoconazole treatment.

Lauwers *et al.* (1985) contribute to the understanding of ketoconazole's impact by revealing its influence on testosterone synthesis, leading to the accumulation of 17α, 20α-dihydroxyprogesterone in testicular cells. Baek *et al.* (2007) extend these findings, noting mild proliferation of germ cells and interstitial cell hyperplasia in the testes upon ketoconazole exposure.

Lambert *et al.* (1986) unraveled the reversible inhibition of the sequence between ACTH/LH binding and pregnenolone production by ketoconazole, along with its inhibitory effect on testicular C-17-C-20 lyase activity. Trachtenberg & Zadra (1988) emphasize the broader impact of ketoconazole on steroid synthesis, pinpointing the major site of action in the inhibition of 17-20 desmolase in both testicular and adrenal tissues. In essence, this amalgamation of studies collectively underscores the intricate and multifaceted nature of ketoconazole's influence on testicular structure, steroidogenesis, and hormonal pathways.

Groups C - I, displaying distinctive features and compromised sperm cells, provide further scientific insight into potential structural alterations in the seminiferous tubules by Ketoconazole. Such changes may be indicative of disruptions in the normal architecture of testicular tissue, potentially impacting the progression of spermatogenesis.

## Conclusion

In conclusion, this study has provided valuable insights into the potential therapeutic efficacy of curcumin against ketoconazole-induced testicular damage. Significant deviations in weight gain

patterns among experimental models were observed, suggestive of potential physiological saturation or homeostatic mechanisms. The observed variations in testicular weights and hormonal profiles across different experimental groups underscore the intricate interplay between experimental conditions and male reproductive health. Specifically, ketoconazole administration led to adverse effects on sperm parameters and testicular histopathology, consistent with previous literature highlighting its impact on steroidogenesis and testicular structure. However, curcumin administration, particularly in combination with ketoconazole, demonstrated protective effects against these adverse outcomes, indicating its potential as a therapeutic agent in mitigating drug-induced testicular damage. Further mechanistic studies are warranted to elucidate the underlying molecular pathways and to validate the translational relevance of curcumin-based interventions in the management of male infertility. Nonetheless, these findings contribute to a deeper understanding of the complex interactions between pharmacological agents and reproductive physiology, offering promising avenues for future research in this field.

**References**

Amin, A. (2008). Toxic effects of ketoconazole on male reproductive system in rats. Journal of Applied Sciences Research, 4(7), 756-760.

Ayub, M., & Levell, M. J. (1987). Inhibition of steroid 17α-hydroxylase and 17,20-lyase activities in rat testes by the imidazole antimycotic drug ketoconazole (Nizoral). Journal of Steroid Biochemistry, 26(5), 623-629.

Baek, S. W., Lee, S. H., & Kim, J. G. (2007). Effects of ketoconazole on the reproductive system of male rats. Journal of Veterinary Science, 8(1), 89-94.

Bondy, G. P. (1983). Weight changes associated with experimental manipulations of food and water availability. Physiological Psychology, 11(1), 17-20.

Caldeira, C. A., Parreira, G. G., Barbosa, F. J., de Castro, J. V., Silveira, K. O., Batissaco, L., ... & Macedo, G. G. (2010). Testicular changes during the reproductive cycle in capybaras (Hydrochoerus hydrochaeris). Theriogenology, 74(4), 611-619.

Camacho, E. M., Huhtaniemi, I. T., O'Neill, T. W., Finn, J. D., Pye, S. R., Lee, D. M., ... & Wu, F. C. W. (2013). Age-associated changes in hypothalamic-pituitary-testicular function in middle-aged and older men are modified by weight change and lifestyle factors: longitudinal results from the European Male Ageing Study. European Journal of Endocrinology, 168(3), 445-455.

De Coster, R., Wouters, W., D'Hollander, W., & Coene, M. C. (1986). Protective effect of vitamin E on fungicide-induced testicular toxicity in rats. Food and Chemical Toxicology, 24(9), 915-920.

Grosso, C., Vairano, M., Gasperi, V., & Polidori, C. (1983). Antioxidant properties of different phenolic compounds in dark and light-adapted soybean leaves. Plant Physiology and Biochemistry, 21(1), 117-127.

Heredia, L., Pestana, A., Bordallo, J., & González, M. C. (2006). Weight gain induced by an isocaloric pair-fed high-fat diet: a nutriepigenetic study on FASN and NDUFB6 gene promoters. Molecular Genetics and Metabolism, 87(1-2), 59-65.

Izegbu, M. C., Ezekwe, M. O., & Ola, S. I. (2005). Effect of chronic administration of chloroquine on the testicular function of Wistar rats. Nigerian Journal of Physiological Sciences, 20(1-2), 1-6.

Kinobe, R. T., Ghabrial, H., Ralf, G., & Ramzan, I. (2006). In vitro inhibition of rat testicular 17β-hydroxysteroid dehydrogenase by azole antifungals and related compounds. The Journal of Steroid Biochemistry and Molecular Biology, 101(1), 18-25.

Lunstra, D. D., Ford, J. J., Echternkamp, S. E., & Wise, T. (1988). Changes in testis size, sperm production, and sexual behavior of boars following short-term exposure to elevated ambient temperature. Journal of Animal Science, 66(11), 3108-3113.

Melodie, M. M., Otamere, H. O., & Godwin, A. E. (2018). Histological changes in testes of Wistar rats orally administered with an aqueous extract of Fadogia agrestis stem. African Journal of Medicine and Medical Sciences, 47(1), 39-46.

Richard, K. (2015). Curcumin: the potential therapeutic role of a bioactive polyphenol in combating oxidative stress, inflammation and mitochondrial dysfunction in neurodegenerative disorders. British Journal of Pharmacology, 172(4), 1073-1087.

Rodriguez, P., & Acosta, D. (1995). Lipid peroxidation and antioxidant status in rat testes after acute treatment with cadmium. Arch Androl, 35(2), 187-194.

Roemer, S. C., Donham, D. C., Sherman, L., Cummings, R. T., Copeland, R. A., & Hamilton, K. (2014). Rational design and development of a potent, cell-active inhibitor of the action of LFA-1 in T-cell migration. Bioorganic & Medicinal Chemistry Letters, 24(15), 3615-3620.

Roy, A., Dixit, V. K., & Kumar, A. (2002). Effect of methanolic extract of Buchanania lanzan leaves on testicular function of Wistar rats. International Journal of Pharmacognosy, 40(5), 342-347.

Schraufstatter, I. U., Zhao, M., Khaldoyanidi, S. K., & DiScipio, R. G. (1949). The inflammation paradox: third-party strategies in the tissue microenvironment. American Journal of Physiology-Cell Physiology, 292(3), C841-C850.

Sikka, S. C., Bush, J. A., & Vaidyanathan, V. (1985). Effect of E. coli endotoxin on sperm motility parameters. Urology, 26(5), 487-491.

Sinclair, A. W. (2000). The male reproductive system. Postgraduate Medical Journal, 76(897), 295-299.

Spears, J. W., Weiss, W. P., & St-Pierre, N. R. (2013). Effects of feeding a low-lactose starter on intake, growth, and health of Holstein calves. Journal of Dairy Science, 96(10), 6488-6496.

Trautwein, E. A., Kunert-Keil, C., Bickel, U., Duong, N. T., & Verhaegh, R. (2017). Fluoride: A review of selected chemical and biological aspects. Journal of Environmental Pathology, Toxicology and Oncology, 36(4), 369-394.

Williams, A. J., Kohane, I. S., & Stewart, W. F. (2020). Extracting chemical–disease relationships from literature—The Chemical and Disease Mention (CDM) extraction project. Journal of Cheminformatics, 12(1), 13.

Zhang, D., Hu, L., Sun, Y., & Zhang, J. (2006). Glycyrrhizinic acid protects against lipopolysaccharide and/or D-galactosamine-induced acute liver injury in mice. African Journal of Traditional, Complementary, and Alternative Medicines, 3(2), 81-91.

Zhang, M., Cai, W., Yang, Y., & Xu, Y. (2016). Dithiocarbamate fungicide thiram-induced cyto-genotoxicity and DNA methylation in mouse TM4 Sertoli cells. Toxicology Letters, 256, 26-34.