

**Kalanchoe pinnata Attenuates Cyclophosphamide-Induced Testicular and Sperm Alterations in Wistar Rats: Histomorphological and Endocrine Evidence**Bolanle Patience OLADIPO<sup>1,2</sup>, Michael Olusegun OLADIPO<sup>3</sup>, Adejoke Y ONAOLAPO<sup>4</sup>, Olakunle J ONAOLAPO<sup>5</sup><sup>1</sup>Department of Pharmacology and Therapeutics, Osun State University, Osogbo, Osun State, Nigeria<sup>2</sup>Department of Pharmacology and Therapeutics, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria.<sup>3</sup>Orthopaedic Surgery Unit, Department of Surgery, Osun State University Teaching Hospital, Osogbo, Osun State, Nigeria<sup>4</sup>Behavioural Neuroscience and Neuropharmacology Unit, Department of Pharmacology and Therapeutics, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria, [ojonaolapo@lautech.edu.ng](mailto:ojonaolapo@lautech.edu.ng), 0000-0003-2142-6046<sup>5</sup>Behavioural Neuroscience and Neurobiology Unit, Department of Anatomy, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria, [ayonaolapo@lautech.edu.ng](mailto:ayonaolapo@lautech.edu.ng), 0000-0001-7126-7050**Correspondence:**

Bolanle Patience Oladipo

Department of Pharmacology and Therapeutics,

Ladoke Akintola University of Technology, Ogbomoso, Oyo State.

**Email:** [morekstop@gmail.com](mailto:morekstop@gmail.com). **Tel:** +2348038056219**Abstract**

This study investigated the protective potential of ethanolic *Kalanchoe pinnata* (KP) leaf extract against cyclophosphamide (CYP)-induced reproductive toxicity in male Wistar rats. Eighty male Wistar rats (120–160 g) were randomly assigned to eight groups (n = 10/group): control; KP alone (200, 400, and 600 mg/kg/day, orally, for 14 days); CYP alone (50 mg/kg, intraperitoneally, on Day 1); and CYP + KP groups receiving the Day 1 CYP injection followed by daily oral KP administration for 14 days. Sperm characteristics, serum FSH, LH, testosterone and oestradiol, MDA, TAC, TNF- $\alpha$  and IL-1 $\beta$ , behavioural performance, and quantitative testicular histomorphometry were assessed. CYP significantly reduced total sperm count, rapid progressive motility, and normal sperm morphology while increasing dead sperm cells. CYP exposure was also associated with reduced testosterone, increased FSH and LH, elevated MDA, reduced TAC, and impaired working-memory performance. Co-treatment with KP attenuated several CYP-associated reproductive alterations. The 400 mg/kg dose produced the greatest improvement in testosterone and TAC, with values of  $20.02 \pm 7.30$  ng/mL and  $13.7 \pm 1.6$ , respectively, whereas 600 mg/kg produced the greatest reduction in MDA ( $0.94 \pm 0.04$   $\mu$ mol/L). Quantitative histomorphometry further indicated preservation of seminiferous tubular architecture and germinal epithelial thickness following KP co-treatment. Ethanolic *K. pinnata* leaf extract attenuated several CYP-associated alterations in sperm characteristics, reproductive endocrine measures, oxidative status, behaviour, and testicular histomorphology. These findings support further investigation of KP as a potential protective intervention against cyclophosphamide-associated reproductive injury.

**KEYWORDS:** Cyclophosphamide, Spermatotoxicity, Testosterone, Reproductive toxicity, Oxidative stress**1. Introduction**

Cyclophosphamide (CYP) is a synthetic alkylating chemotherapeutic and immunosuppressive agent widely used in the management of leukaemias, lymphomas, solid tumours, and severe autoimmune disorders [1-4]. Despite its clinical utility, CYP is associated with substantial gonadotoxicity, reflecting its effects on rapidly proliferating germinal cells [1, 3, 4]. In males, CYP exposure can impair spermatogenesis, disrupt testicular

endocrine function, and reduce sperm production, potentially resulting in oligospermia or azoospermia [5, 6].

Oxidative stress is considered an important contributor to CYP-induced testicular injury. CYP metabolism generates reactive oxygen species (ROS) and toxic metabolites, including acrolein, which can promote lipid peroxidation, cellular apoptosis, and disruption of the blood–testis barrier [1,4]. These processes may compromise the structural and functional integrity of the seminiferous epithelium, resulting in impaired

spermatogenesis and alterations in reproductive endocrine function, including reduced circulating testosterone concentrations [7].

Given the reproductive consequences associated with cyclophosphamide exposure, increasing attention has been directed towards naturally derived compounds with antioxidant and anti-inflammatory properties as potential protective adjuncts. *Kalanchoe pinnata* (Lam.) Pers. is a perennial succulent traditionally used for its wound-healing, antimicrobial, and anti-inflammatory properties. Phytochemical investigations have identified several bioactive constituents in *K. pinnata*, including flavonoids such as quercetin and kaempferol derivatives, bufadienolides, phytosterols, and organic fatty acids [8-10]. Preliminary evidence suggests that *K. pinnata* possesses antioxidant and tissue-protective properties; however, its potential to attenuate chemotherapy-associated reproductive injury remains insufficiently characterised [8].

Accordingly, this study evaluated the effects of an ethanolic leaf extract of *K. pinnata* on sperm characteristics, reproductive endocrine measures, systemic oxidative and inflammatory markers, behavioural performance, and testicular histomorphology in Wistar rats exposed to cyclophosphamide.

## 2. Materials and Methods

### 2.1 Plant Material and Extraction

Fresh leaves of *Kalanchoe pinnata* were harvested before sunrise from Goshen Farms, Osogbo, Osun State, Nigeria. The plant material was authenticated by a taxonomist in the Department of Pure and Applied Biology, Ladoké Akintola University of Technology (LAUTECH), Ogbomoso, Oyo State, Nigeria, and a voucher specimen (LHO 985) was deposited in the departmental herbarium.

The collected leaves were cleaned, air-dried at room temperature (25 °C), and pulverised into a coarse powder. Five hundred grams of the powdered leaves were subjected to Soxhlet extraction using 2.5 L of absolute ethanol. The powdered material was placed in a porous thimble and introduced into the extraction chamber of the Soxhlet apparatus, while the ethanol was placed in the round-bottom flask connected to the extractor and reflux condenser. Extraction proceeded through repeated cycles of solvent vaporisation, condensation, percolation through the plant material, siphoning, and recycling until extraction was considered complete. The resulting extract was separated and concentrated using a rotary evaporator. The concentrated extract was subsequently freeze-dried to obtain a stable preparation and stored in a dark, airtight vacuum vial until required for administration.

### 2.2 Experimental Animals

Eighty adult, male Wistar rats weighing 120–160 g at the beginning of the experiment were obtained from Empire

Breeders, Osogbo, Osun State, Nigeria. The animals were housed in standard wooden cages under a 12-h light/12-h dark cycle at an ambient temperature of  $25 \pm 2.5$  °C. They were allowed to acclimatise for seven days before commencement of treatment and had unrestricted access to drinking water and standard rodent chow containing 29% protein, 11% fat, and 58% carbohydrate. All experimental procedures were conducted following approval by the Institutional Animal Research Ethics Committee of LAUTECH and in accordance with the European Council Directive 2010/63/EU on the protection of animals used for scientific purposes.

### 2.3 Experimental Design and Treatment

Following acclimatisation, the animals were randomly allocated by block randomisation based on baseline body weight into eight experimental groups of 10 rats each. The control group received normal saline vehicle at 10 mL/kg by oral gavage once daily for 14 consecutive days. Three groups received ethanolic *K. pinnata* leaf extract at doses of 200, 400, or 600 mg/kg body weight (based on the results of the acute oral toxicity test), respectively, administered orally once daily for 14 days. A cyclophosphamide-only group received a single intraperitoneal injection of cyclophosphamide at 50 mg/kg on Day 1, followed by oral administration of normal saline vehicle at 10 mL/kg daily until Day 14. The remaining three groups received a single intraperitoneal injection of cyclophosphamide at 50 mg/kg on Day 1, followed by daily oral administration of *K. pinnata* extract at 200, 400, or 600 mg/kg body weight, respectively, from Day 1 through Day 14. Thus, the experimental groups comprised a control group, three *K. pinnata*-only groups, a cyclophosphamide-only group, and three cyclophosphamide and *K. pinnata* treatment groups.

The extract preparations were prepared in saline vehicle and adjusted according to body weight to maintain the intended dose. On Day 15, approximately 24 h after completion of the 14-day treatment period, the rats were euthanised by cervical dislocation, blood was taken for biochemical assays and the epididymis was excised for assessment of sperm parameters while the testis was processed for general histology.

### 2.4 Sperm Analysis and Reproductive Parameters

Epididymal sperm parameters were assessed using the right caudal epididymis as previously described [12, 13]. Following dissection, the epididymis was placed in a pre-warmed dish containing 2 mL phosphate-buffered saline (PBS; pH 7.4) and gently macerated to facilitate the release of spermatozoa into the medium. For sperm counting, aliquots of the resulting suspension were diluted 1:40 with PBS and loaded onto an improved Neubauer haemocytometer. Spermatozoa were counted across eight 1-mm<sup>2</sup> squares using light microscopy, and the sperm concentration was calculated from the haemocytometer count.

Sperm motility was assessed at 37 °C within five minutes of sample preparation. Spermatozoa were classified according to their movement characteristics as rapidly progressive, non-progressive, or immotile, based on observations from eight randomly selected microscopic fields. For assessment of sperm morphology and viability, smears were prepared on clean glass slides and stained with eosin–nigrosin. The stained preparations were examined at 640× magnification to determine the proportions of morphologically normal and abnormal spermatozoa and the proportion of dead spermatozoa.

## 2.5 Assessment of Oxidative Stress and Inflammatory Markers

Blood samples were collected by intracardiac puncture at the end of the experimental period. Samples were immediately centrifuged at 3000 rpm for 15 min at 4 °C to separate the serum, which was subsequently used for biochemical analyses as previously described [14-16]. Serum malondialdehyde (MDA) concentration was determined using a thiobarbituric acid reactive substances (TBARS) spectrophotometric assay kit. In this assay, MDA reacts with thiobarbituric acid under high-temperature conditions to form a coloured chromogen, which was measured spectrophotometrically at 532 nm. MDA concentrations were expressed in  $\mu\text{mol/L}$ . Total antioxidant capacity (TAC) was assessed using the Trolox equivalent antioxidant capacity (TEAC) decolourisation method, with absorbance kinetics recorded at 734 nm [15].

Serum concentrations of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1  $\beta$  (IL-1 $\beta$ ) were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits obtained from BioVision Inc., California, USA, according to previously described protocols [12].

## 2.6 Endocrine Assessment

Serum concentrations of total testosterone, oestradiol, follicle-stimulating hormone (FSH), and luteinising hormone (LH) were determined using commercially available radioimmunoassay (RIA) kits obtained from Tianjin Medical & Bioengineering Co., China. The assays had a reported detection limit of 0.04 ng/mL, with intra-assay coefficients of variation below 5%. All measurements were performed according to previously described protocols [17, 18].

## 2.7 Testicular and Epididymal Histomorphology

Following euthanasia, the left testes and epididymal tissue segments were excised and immediately fixed in Bouin's solution for 24 h. The tissues were subsequently dehydrated through graded concentrations of ethanol, embedded in paraffin wax, and sectioned at 5  $\mu\text{m}$  using a rotary microtome. Tissue sections were stained with haematoxylin and eosin (H&E) for microscopic evaluation. Digital bright-field images were acquired using an Olympus trinocular optical microscope equipped with a digital image-capture system.

## 2.10 Statistical Analysis

Data were expressed as mean  $\pm$  standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism version 7.0. Differences among experimental groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post-hoc test for multiple comparisons. Statistical significance was set at  $p < 0.05$ .

## 3 Result

### 3.1 Effects of *Kalanchoe pinnata* on Body Weight and Feed Intake

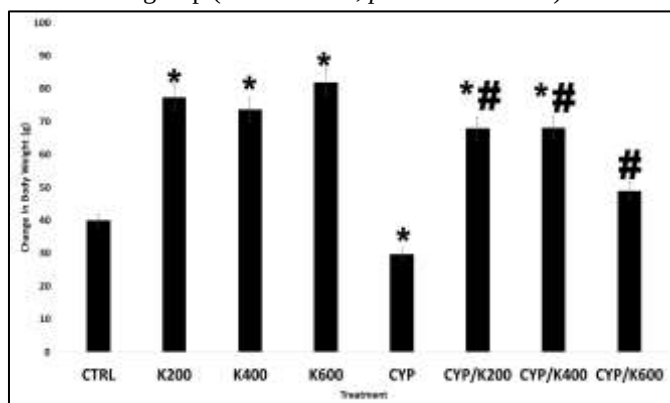
Figures 1 and 2 show *Kalanchoe pinnata* (KP) on body weight and feed intake. There was a significant reduction in total body weight gain and relative feed intake with cyclophosphamide compared with the control group ( $p < 0.05$ ). The CYP-only group gained  $29.7 \pm 3.08$  g, compared with  $40.04 \pm 3.45$  g in the control group. In contrast, rats administered *Kalanchoe pinnata* (KP) extract alone at 200, 400, and 600 mg/kg exhibited greater body weight gains of 77.4, 73.7, and 82.0 g, respectively, which were significantly higher than the control value ( $p < 0.05$ ). Co-treatment with KP was associated with significant improvement in body weight gain relative to the CYP-only group. Body weight gains of  $68.0 \pm 1.94$  g and  $68.2 \pm 4.06$  g were recorded in the CYP/K200 and CYP/K400 groups, respectively ( $p < 0.05$  vs. CYP). Relative feed intake was also improved following KP co-treatment, with the CYP/K400 and CYP/K600 groups showing values that approached or exceeded those of the control group ( $p < 0.05$ ).

### 3.2 Effects on Epididymal Sperm Characteristics

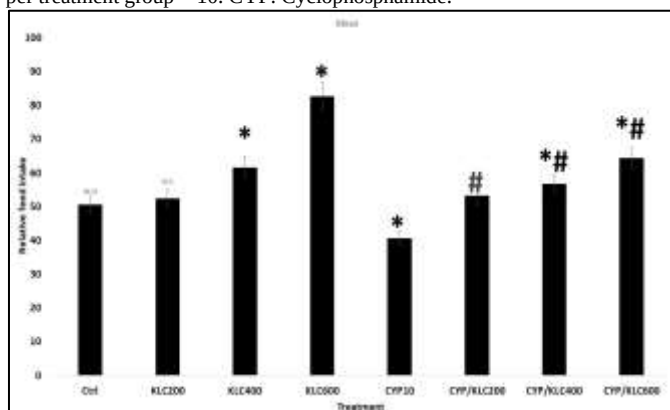
Figures 3, 4, 5, 6 and 7 shows the effect of KP on epididymal sperm parameters following CYP administration in rats. There were significant alterations in epididymal sperm parameters with CYP compared with control. Total sperm count decreased to  $67.2 \pm 10.6\%$ , while normal sperm morphology declined to  $10.0 \pm 0.0\%$ . Rapid progressive motility was reduced to  $40.0 \pm 2.0\%$ , whereas the proportion of dead sperm cells increased to  $25.0 \pm 1.7\%$  ( $p < 0.05$  vs. control). Co-treatment with KP was associated with significant improvement in several of these parameters compared with the CYP-only group. Total sperm count increased to  $110.0 \pm 1.9\%$ ,  $128.0 \pm 4.1\%$ , and  $181.0 \pm 2.9\%$  in the CYP/K200, CYP/K400, and CYP/K600 groups, respectively ( $p < 0.05$  vs. CYP). Normal sperm morphology increased to  $35.0 \pm 0.0\%$  and  $40.0 \pm 0.0\%$  in the CYP/K200 and CYP/K400 groups, respectively. Progressive motility increased to  $60.0 \pm 3.1\%$  in the CYP/K200 group and  $75.0 \pm 3.5\%$  in the CYP/K400 group, while the proportion of dead sperm cells decreased to  $15.0 \pm 0.7\%$ ,  $12.0 \pm 0.6\%$ , and  $10.0 \pm 0.5\%$  in the CYP/K200, CYP/K400, and CYP/K600 groups, respectively.

### 3.3 Effects on Serum Oxidative Stress Markers

The effects of KP on lipid peroxidation measured as malondialdehyde levels (MDA) and Antioxidant status measured as Total antioxidant Capacity (TAC) is shown on Table 1. There was a significant increased serum MDA and a decrease in TAC compared to the control ( $p < 0.05$ ). Serum MDA increased from  $1.21 \pm 0.03 \mu\text{mol/L}$  in controls to  $4.80 \pm 0.03 \mu\text{mol/L}$  in the CYP-only group, whereas TAC decreased from  $10.56 \pm 0.75$  in controls to  $8.14 \pm 0.58$  following CYP administration. Administration of KP alone resulted in a decrease in MDA concentrations at 200 and 400 mg/kg, with the K400 group recording  $1.00 \pm 0.03 \mu\text{mol/L}$  ( $p < 0.05$  vs. control). In the CYP groups, co-treatment with KP significantly reduced serum MDA when compared with CYP. Concentrations of MDA were  $1.08 \pm 0.05$ ,  $1.12 \pm 0.02$ , and  $0.94 \pm 0.04 \mu\text{mol/L}$  in the CYP/K200, CYP/K400, and CYP/K600 groups, respectively ( $p < 0.05$  vs. CYP). Total antioxidant capacity also increased in all CYP/KP groups compared with CYP, with the highest value recorded in the CYP/K400 group ( $13.70 \pm 1.60$ ;  $p < 0.05$  vs. CYP).



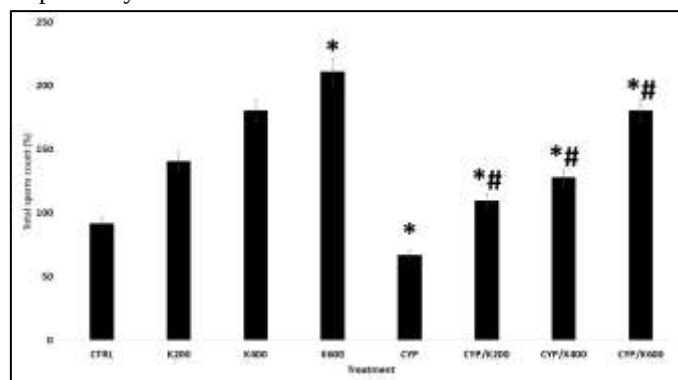
**Figure 1.** Effect of *Kalanchoe pinnata* extract on change in body weight. Each bar represents Mean  $\pm$  S.E.M. \* $p < 0.05$  indicates a significant difference from control. # $p < 0.05$  indicates a significant difference from CYP. Number of rats per treatment group = 10. CYP: Cyclophosphamide.



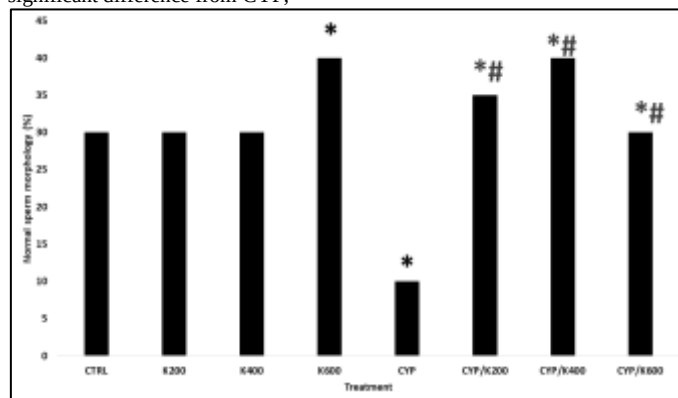
**Figure 2.** Effect of *Kalanchoe pinnata* extract on change in feed intake. Each bar represents Mean  $\pm$  S.E.M. \* $p < 0.05$  indicates a significant difference from control. # $p < 0.05$  indicates a significant difference from CYP. Number of rats per treatment group = 10. CYP: Cyclophosphamide.

### 3.4 Effects on Serum Inflammatory Cytokines

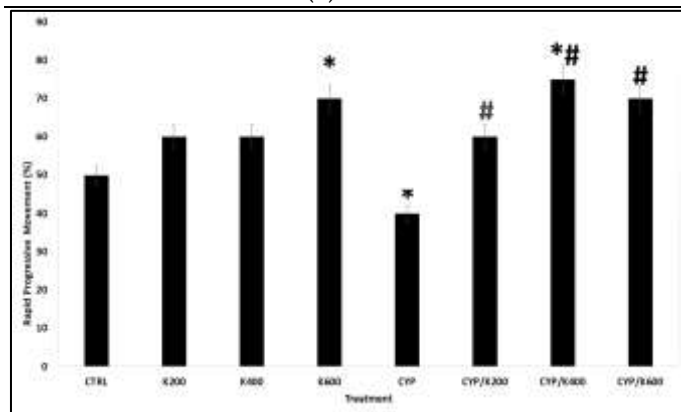
The effects of KP on serum inflammatory cytokine concentrations are shown in Table 2. Serum TNF- $\alpha$  increased significantly with CYP ( $23.00 \pm 3.27 \text{ pg/ml}$ ) compared to control ( $10.65 \pm 1.21 \text{ pg/mL}$ ), whereas IL-1 $\beta$  decreased significantly ( $p < 0.05$ ) with CYP ( $24.64 \pm 7.38 \text{ pg/ml}$ ) compared to control ( $48.32 \pm 0.12 \text{ pg/ml}$ ). Administration of KP alone increased TNF- $\alpha$  concentrations with KP at 400 ( $23.91 \pm 6.97$ ) and 600 ( $16.24 \pm 2.79 \text{ pg/mL}$ ) compared to control. Concentration of IL-1 $\beta$  also increased significantly with KP at 200 ( $64.66 \pm 12.91$ ), 400 ( $139.76 \pm 60.01$ ), and 600 ( $58.92 \pm 17.63 \text{ pg/ml}$ ) compared to control. Co-treatment with KP significantly reduced the TNF- $\alpha$  with CYP/KP200 ( $16.36 \pm 0.57$ ), CYP/KP400 ( $19.29 \pm 2.90$ ), and CYP/KP600 ( $20.47 \pm 2.10 \text{ pg/ml}$ ) compared to CYP control ( $p < 0.05$ ). Levels of IL-1 $\beta$  were also significantly higher in all CYP/KP groups compared to the CYP-only group. Values were  $48.71 \pm 0.67$ ,  $81.36 \pm 16.86$ , and  $53.96 \pm 8.41 \text{ pg/mL}$  in the CYP/K200, CYP/K400, and CYP/K600 groups, respectively.



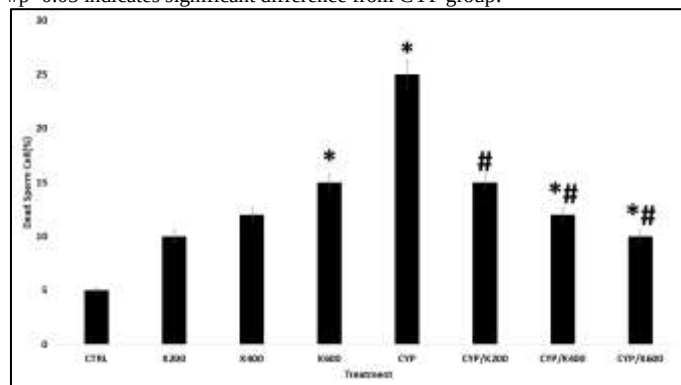
**Figure 3.** Effect of *Kalanchoe pinnata* and cyclophosphamide on total sperm count. Each bar represents Mean  $\pm$  S.E.M, number of rats per treatment group = 10. \* $p < 0.05$  indicates significant difference from control; # $p < 0.05$  indicates significant difference from CYP.



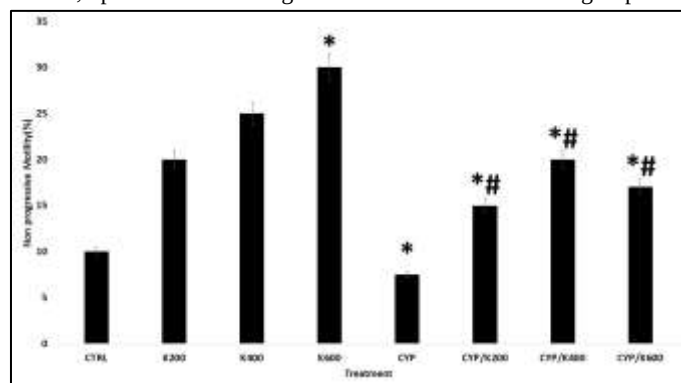
**Figure 4.** Effect of *Kalanchoe pinnata* and cyclophosphamide on a normal sperm morphology. Each bar represents Mean  $\pm$  S.E.M, number of rats per treatment group = 10. \* $p < 0.05$  indicates significant difference from control; # $p < 0.05$  indicates significant difference from CYP group.



**Figure 5.** Effect of *Kalanchoe pinnata* and cyclophosphamide on a rapid progressive movement. Each bar represents Mean  $\pm$  S.E.M, number of rats per treatment group = 10. \* $p < 0.05$  indicates significant difference from control; # $p < 0.05$  indicates significant difference from CYP group.



**Figure 6.** Effect of *Kalanchoe pinnata* and cyclophosphamide on % dead sperm cells. Each bar represents Mean  $\pm$  S.E.M, number of rats per treatment group = 10. \* $p < 0.05$  indicates significant difference from control; # $p < 0.05$  indicates significant difference from CYP group.



**Figure 7.** Effect of *Kalanchoe pinnata* and cyclophosphamide on a non-progressive movement. Each bar represents Mean  $\pm$  S.E.M, number of rats per treatment group = 10. \* $p < 0.05$  indicates significant difference from control; # $p < 0.05$  indicates significant difference from CYP group.

### 3.5 Effects of *Kalanchoe pinnata* on Reproductive Hormones

Table 3 shows the effect of KP on reproductive hormones. There was a significant decrease in serum testosterone levels with CYP ( $6.60 \pm 2.05$  ng/ml) compared to control ( $11.94 \pm 0.03$  ng/ml). *Kalanchoe pinnata* administration alone was associated

with a significant increase in testosterone levels at 400 ( $21.62 \pm 8.21$  ng/ml) and 600 ( $42.97 \pm 1.47$  ng/ml) mg/kg doses compared to control. Testosterone levels increased significantly at all doses compared to the CYP-only group. Testosterone concentrations were  $14.40 \pm 0.67$ ,  $20.02 \pm 7.30$ , and  $12.48 \pm 5.55$  ng/mL in the CYP/K200, CYP/K400, and CYP/K600 groups respectively ( $p < 0.05$  vs. CYP).

There was a significant increase in serum levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) with CYP compared to control ( $p < 0.05$ ). Serum LH was also increased with K200 ( $7.65 \pm 3.80$ ) compared to control. In the CYP/KP groups, FSH concentrations were also altered following co-treatment, with values of  $2.30 \pm 0.21$ ,  $2.80 \pm 0.06$ , and  $2.89 \pm 0.17$  in the CYP/K200, CYP/K400, and CYP/K600 groups, respectively. LH decreased to  $1.28 \pm 0.33$ ,  $0.19 \pm 0.10$ , and  $0.11 \pm 0.02$  in the respective co-treatment groups ( $p < 0.05$  vs. CYP). Serum oestradiol levels decreased significantly with CYP ( $10.81 \pm 0.35$  ng/ml) compared to control ( $12.04 \pm 0.07$ ). Oestradiol levels did not differ significantly in the CYP/KP groups compared to control but compared to CYP alone there was a significant increase in oestradiol levels with were CYP/KP 400 ( $13.60 \pm 0.42$ ) and CYP/KP 600 ( $12.59 \pm 0.67$  ng/ml).

**Table 1.** Effect of *Kalanchoe pinnata* on Oxidative Stress and Antioxidant Markers

| Treatment Group | Malondialdehyde (MDA, $\mu\text{mol/L}$ ) | Total Antioxidant Capacity (TAC) /Trolox Equivalent |
|-----------------|-------------------------------------------|-----------------------------------------------------|
| CTRL            | $1.21 \pm 0.03$                           | $10.56 \pm 0.75$                                    |
| K200            | $1.13 \pm 0.08^*$                         | $9.95 \pm 1.40$                                     |
| K400            | $1.00 \pm 0.03^*$                         | $10.95 \pm 3.64$                                    |
| K600            | $1.39 \pm 0.07$                           | $9.07 \pm 1.04$                                     |
| CYP             | $4.80 \pm 0.03^*$                         | $8.14 \pm 0.58^*$                                   |
| CYP/K200        | $1.08 \pm 0.05^{*}\#$                     | $12.83 \pm 1.52\#$                                  |
| CYP/K400        | $1.12 \pm 0.02^{*}\#$                     | $13.70 \pm 1.60\#$                                  |
| CYP/K600        | $0.94 \pm 0.04^{*}\#$                     | $11.82 \pm 0.98\#$                                  |

Data presented as mean  $\pm$  SEM (n = 10 rats per group). \* $p < 0.05$  versus the control group. # $p < 0.05$  versus the CYP group.

**Table 2.** Effect of *Kalanchoe pinnata* on Inflammatory Cytokines

| Group    | Tumor Necrosis Factor- $\alpha$ pg/mL) | Interleukin-1 beta pg/mL) |
|----------|----------------------------------------|---------------------------|
| CTRL     | $10.65 \pm 1.21$                       | $48.32 \pm 0.12$          |
| K200     | $11.47 \pm 1.71^*$                     | $64.66 \pm 12.91^*$       |
| K400     | $23.91 \pm 6.97^*$                     | $139.76 \pm 60.01^*$      |
| K600     | $16.24 \pm 2.79^*$                     | $58.92 \pm 17.63^*$       |
| CYP      | $23.00 \pm 3.27^*$                     | $24.64 \pm 7.38^*$        |
| CYP/K200 | $16.36 \pm 0.57^{*}\#$                 | $48.71 \pm 0.67^{*}\#$    |
| CYP/K400 | $19.29 \pm 2.90^{*}\#$                 | $81.36 \pm 16.86^{*}\#$   |
| CYP/K600 | $20.47 \pm 2.10^{*}\#$                 | $53.96 \pm 8.41^{*}\#$    |

Data presented as mean  $\pm$  SEM (n = 10 rats per group). \* $p < 0.05$  versus the control group. # $p < 0.05$  versus the CYP group.

**Table 3. Effect of *Kalanchoe pinnata* on reproductive hormones**

| Group    | T (ng/mL)      | FSH (IU)     | LH (IU)       | O(ng/mL)      |
|----------|----------------|--------------|---------------|---------------|
| CTRL     | 11.94 ± 0.03   | 2.25 ± 0.03  | 0.56 ± 0.05   | 12.04 ± 0.07  |
| K200     | 12.25 ± 2.91   | 1.75 ± 0.18  | 7.65 ± 3.80*  | 11.49 ± 0.01  |
| K400     | 21.62 ± 8.21*  | 2.16 ± 0.23  | 0.44 ± 0.11   | 13.45 ± 0.14  |
| K600     | 42.97 ± 1.47*  | 2.32 ± 0.17  | 0.10 ± 0.01   | 12.93 ± 0.73  |
| CYP      | 6.60 ± 2.05*   | 4.47 ± 0.94* | 2.55 ± 1.32*  | 10.81 ± 0.35* |
| CYP/K200 | 14.40 ± 0.67#  | 2.30 ± 0.21# | 1.28 ± 0.33*# | 11.50 ± 0.06# |
| CYP/K400 | 20.02 ± 7.30*# | 2.80 ± 0.06# | 0.19 ± 0.10#  | 13.60 ± 0.42# |
| CYP/K600 | 12.48 ± 5.55#  | 2.89 ± 0.17# | 0.11 ± 0.02#  | 12.59 ± 0.67# |

Data are presented as mean ± SEM (n = 10 rats per group). \*p < 0.05 versus the control group, #p < 0.05 versus the control group. T: Testosterone, FSH: Follicle stimulating hormone, LH: Luteinizing hormone.

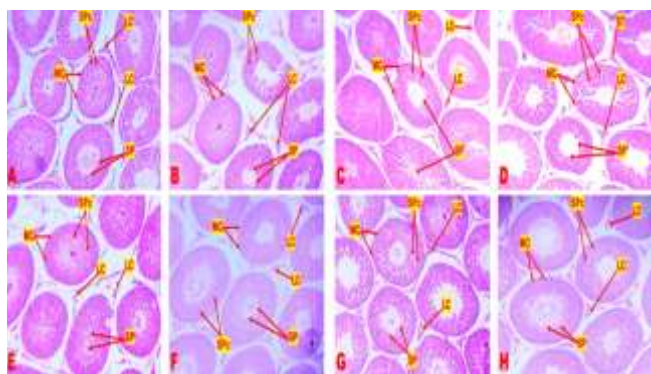
### 3.6 Effect of *Kalanchoe pinnata* on testicular Histomorphology

Histological examination of testicular sections revealed differences in seminiferous tubular architecture among the experimental groups (Figure 8A-H). The control group (8A) revealed preserved testicular histoarchitecture characterized by regularly arranged seminiferous tubules with an organized germinal epithelium and recognizable spermatogenic cells at different stages of development. Spermatocytes and spermatozoa were evident within the seminiferous epithelium and luminal compartments, respectively, while the interstitial compartment containing Leydig cells was preserved. Testicular sections from rats administered *Kalanchoe pinnata* (KP) alone at 200, 400 and 600 mg/kg (Figure 8 B–D) also demonstrated generally well preserved seminiferous tubular organization. The germinal epithelium remained relatively well organized, with recognizable spermatogenic cells and luminal spermatozoa. No obvious widespread degenerative changes were apparent in these groups compared with the control. In the CYP group (8E) there was disruption of the normal seminiferous tubular architecture. The seminiferous epithelium was less uniformly organized, with reduced compactness of the germinal cell layers and altered tubular morphology compared with the control group. These changes were followed by less distinct organization of spermatogenic elements within affected tubules, consistent with CYP-associated testicular injury. The CYP/KP groups were associated with improved preservation of testicular architecture relative to CYP treatment alone. In the CYP/KP200 group (8F), seminiferous tubules and spermatogenic cells remained identifiable, although some alterations in epithelial organization persisted. Sections from the CYP/KP400 group (8G) demonstrated comparatively better preservation of seminiferous tubular organization, with a more clearly defined

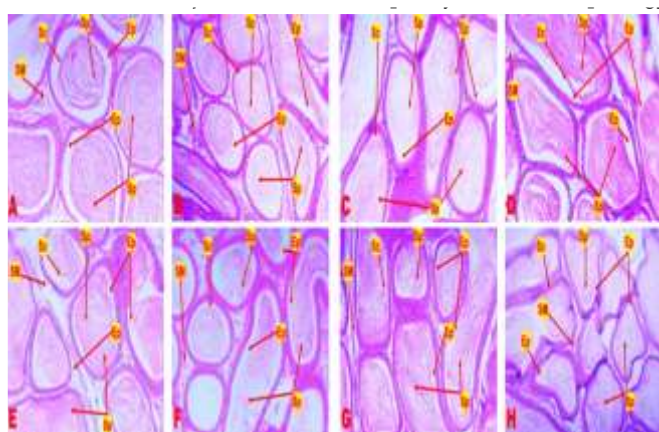
germinal epithelium and recognizable spermatogenic cells and luminal spermatozoa. The CYP/KP600 group (8H) similarly showed preservation of seminiferous tubular profiles and spermatogenic cellular organization relative to the CYP-only group.

### 3.7 Effect of *Kalanchoe pinnata* on Epididymal Histomorphology

Histological examination of the epididymal sections revealed differences in ductal organization and epithelial morphology among the experimental groups (Figure 9 A-H). The control group (9A) showed relatively well-preserved epididymal architecture, characterized by regularly arranged epididymal ducts lined by pseudostratified columnar epithelium with distinct stereocilia. The lumina contained recognizable spermatozoa, while the surrounding smooth-muscle layer appeared continuous. The epididymal sections from rats administered *Kalanchoe pinnata* extract alone at 200, 400 and 600 mg/kg (9B, 9C and 9D) demonstrated generally preserved ductal architecture. The epithelial lining remained recognizable, with stereocilia extending toward the luminal surfaces and spermatozoa identifiable within the ductal lumina. The surrounding smooth-muscle component was also maintained. No widespread epithelial disruption or pronounced loss of luminal spermatozoa was apparent in these groups. The CYP group (9E) exhibited alterations in the normal epididymal architecture. The epithelial lining appeared less uniformly organized, with changes in the configuration of the epididymal ducts and reduced definition of the epithelial–luminal interface. The luminal sperm population also appeared less prominent in some ductal profiles compared with the control and KP-treated groups. These findings are consistent with cyclophosphamide-associated epididymal tissue injury. In the CYP/KP groups there was associated with preservation of epididymal architecture. In the CYP/KP200 group (9F), the epididymal ducts remained identifiable and the epithelial lining was relatively maintained, although some architectural alterations persisted. The CYP/KP400 group (9G) showed comparatively well-preserved ductal profiles, with recognizable epithelial lining, stereocilia and luminal spermatozoa. The smooth-muscle component surrounding the ducts was also maintained. The CYP/KP600 group (9H) demonstrated preservation of several features of epididymal organization, including recognizable epithelial lining and luminal spermatozoa. However, some irregularity in ductal configuration remained evident. Overall, the CYP/KP400 and CYP/KP600 groups showed greater preservation of epididymal histoarchitecture than the CYP-only group.



**Figure 8. Representative photomicrographs demonstrating the effect of ethanolic leaf extract of *Kalanchoe pinnata* on testicular histoarchitecture in cyclophosphamide-treated male Wistar rats.** Sections A–H represent the control, KP200, KP400, KP600, CYP, CYP+KP200, CYP+KP400 and CYP+KP600 groups, respectively. Control and KP-treated sections (A–D) show relatively preserved seminiferous-tubule organization with recognizable spermatogenic cells and spermatozoa. CYP exposure (E) is associated with disruption of seminiferous-tubule architecture and reduced organization of the germinal epithelium. Co-treatment with *K. pinnata* (F–H) is associated with progressive preservation of tubular organization and spermatogenic cellular architecture, particularly at 400 and 600 mg/kg. SPc, spermatocytes; SP, spermatozoa; LC, Leydig cells; MC, myoid cells; ST, seminiferous tubule. Haematoxylin and eosin stain; magnification: [X160].



**Figure 9. Representative photomicrographs showing the effect of ethanolic leaf extract of *Kalanchoe pinnata* on epididymal histoarchitecture in male Wistar rats exposed to cyclophosphamide.** A: control; B: KP 200 mg/kg; C: KP 400 mg/kg; D: KP 600 mg/kg; E: CYP 50 mg/kg; F: CYP + KP 200 mg/kg; G: CYP + KP 400 mg/kg; H: CYP + KP 600 mg/kg. Control and KP-only groups demonstrate relatively preserved epididymal ductal organization, epithelial lining and luminal spermatozoa. Cyclophosphamide exposure is associated with disruption of epididymal architecture and reduced apparent luminal sperm density, whereas co-treatment with *K. pinnata* is associated with preservation of epithelial and ductal organization. Sc, stereocilia; Sp, spermatozoa; Ep, epithelium; SM, smooth muscle. Haematoxylin & Eosin stain, X160

## Discussion

This study demonstrates that ethanolic leaf extract of *Kalanchoe pinnata* was associated with attenuation of several cyclophosphamide (CYP)-induced reproductive, biochemical and neurobehavioural alterations in male Wistar rats. The effects were observed across sperm characteristics, oxidative and inflammatory biomarkers, reproductive endocrine indices, and the histological organization of the testes and epididymis. The findings therefore suggest a broad protective profile of *K. pinnata* against CYP-associated toxicity, although the magnitude of response varied among endpoints and doses. Cyclophosphamide is metabolized to reactive intermediates, including acrolein, which can promote oxidative injury and

lipid peroxidation [6, 12]. The male reproductive system is particularly vulnerable to oxidative damage because of the high content of polyunsaturated fatty acids in testicular and sperm membranes and the continuous proliferative activity of germ cells. Such oxidative injury has been implicated in disruption of spermatogenesis, deterioration of seminiferous tubular architecture and impairment of sperm quality [1, 3]. In the present study, CYP administration was accompanied by substantial deterioration in sperm characteristics, including reduced sperm count and progressive motility, reduced normal sperm morphology and increased dead sperm cells. These functional alterations were accompanied by conspicuous histological changes in both the testis and epididymis [12, 13]. The testicular photomicrographs provide structural evidence supporting the functional reproductive findings. The control and *K. pinnata*-only groups showed relatively preserved seminiferous tubular organization, with recognizable spermatogenic cells and spermatozoa. In contrast, the CYP-treated group demonstrated disruption of the normal organization of the seminiferous tubules and germinal epithelium. These findings are consistent with previous reports describing testicular architectural disruption following cyclophosphamide exposure and the potential of antioxidant interventions to reduce such injury [1, 3, 12, 13].

Importantly, the epididymal photomicrographs extend the structural findings beyond the testis. The control and *K. pinnata*-treated animals exhibited relatively preserved epididymal ducts, recognizable epithelial lining, stereocilia and luminal spermatozoa. In contrast, the CYP-treated section showed altered ductal and epithelial organization and reduced apparent luminal sperm density. Following co-administration of *K. pinnata*, the epithelial and ductal architecture appeared comparatively preserved, particularly in the CYP/KP400 and CYP/KP600 groups. These observations are consistent with the improved sperm characteristics observed in the corresponding treatment groups and suggest that the protective effect of KP extended to both the testicular and post-testicular reproductive compartments.

The biochemical findings provide a possible context for the observed structural preservation. Administration of CYP was associated with a marked increase in MDA together with a decrease in TAC levels, indicating an imbalance between oxidative processes and endogenous antioxidant defence. In the present study, testicular MDA increased from  $1.21 \pm 0.03$  nmol/g in the control group to  $4.80 \pm 0.03$  nmol/g in the CYP group, whereas co-treatment with KP markedly reduced MDA, with the CYP/KP600 group showing the lowest reported value ( $0.94 \pm 0.04$  nmol/g). This finding is compatible with the established contribution of oxidative stress to cyclophosphamide-associated tissue injury [1, 3, 12, 13]. The reduction in lipid peroxidation following KP administration

may therefore have contributed to preservation of cellular and tissue architecture. The antioxidant response, however, was not uniformly dose-dependent. Although the greatest reduction in MDA occurred in the CYP/KP600 group, the highest TAC response was reported in the co-treatment groups at different doses, with the source dataset identifying the intermediate dose as particularly responsive. This differential response suggests that individual components of the crude extract may influence different biological endpoints at different concentrations. Such a non-linear response is important because it indicates that increasing the dose of a complex botanical preparation does not necessarily produce proportional improvement across all outcome measures.

The inflammatory findings further suggest that KP exerted a modulatory rather than uniformly suppressive effect. CYP exposure increased TNF- $\alpha$  but was associated with a reduction in IL-1 $\beta$  in the present dataset. Following KP co-treatment, TNF- $\alpha$  concentrations were reduced relative to the CYP group, whereas IL-1 $\beta$  values shifted toward or above the control range depending on the dose. The data therefore indicate differential modulation of inflammatory biomarkers rather than generalized suppression of inflammatory signalling. This interpretation is consistent with the broader immunomodulatory activities reported for *K. pinnata* preparations [8]. However, the precise mechanisms underlying the divergent cytokine responses cannot be established because tissue-specific cytokine expression and upstream inflammatory signalling pathways were not examined.

The endocrine findings provide additional evidence of CYP-associated reproductive dysfunction. CYP exposure reduced circulating testosterone from  $11.94 \pm 0.03$  ng/mL in the control group to  $6.60 \pm 2.05$  ng/mL and was accompanied by increases in FSH and LH. Such a pattern is compatible with disruption of testicular endocrine function and altered negative feedback within the hypothalamic-pituitary-gonadal axis. Cyclophosphamide-associated gonadal injury and disruption of male reproductive endocrine function have previously been reported [1, 3]. Co-administration of KP increased testosterone and reduced the CYP-associated elevations in FSH and LH. The CYP/KP400 group showed a particularly notable endocrine response, with testosterone increasing to  $20.02 \pm 7.30$  ng/mL compared with  $6.60 \pm 2.05$  ng/mL in the CYP group. However, the endocrine response was not linearly dose-dependent. Although KP600 produced the highest testosterone concentration among the KP-only groups, the CYP/KP600 group did not produce the highest testosterone concentration among the CYP co-treatment groups. This parameter-specific response is consistent with the complex biological activity expected from a crude botanical extract [19, 20].

The endocrine findings should nevertheless be interpreted cautiously in relation to the histological observations. Although

Leydig cells were recognizable within the interstitial compartments of the testicular sections, routine H&E staining does not permit definitive assessment of Leydig-cell functional activity or steroidogenic capacity. Consequently, the present histological findings cannot independently establish that CYP specifically destroyed Leydig cells or that KP directly restored Leydig-cell steroidogenesis. Rather, the combined increase in circulating testosterone and preservation of testicular architecture provide evidence consistent with attenuation of CYP-associated reproductive endocrine disturbance. Direct evaluation of steroidogenic proteins and enzymes would be required to establish the underlying mechanism. The epididymal findings are particularly relevant to the interpretation of the sperm data. The epididymis provides an essential microenvironment for sperm maturation and storage, and disruption of its epithelial architecture may adversely influence post-testicular sperm quality.

In the present study, the CYP group exhibited altered epididymal epithelial organization and reduced apparent luminal sperm density, whereas KP co-treatment was associated with preservation of the ductal epithelium and recognizable luminal spermatozoa. These morphological observations correspond with the improved sperm count, motility and morphology observed following KP administration. Nevertheless, the present study measured epididymal morphology rather than specific molecular markers of epididymal maturation or sperm transport; therefore, the findings should be described as preservation of epididymal architecture rather than direct restoration of epididymal function.

The concordance between the biochemical, endocrine, sperm and histomorphological findings strengthens the overall interpretation of the study. CYP exposure was associated with oxidative imbalance, altered reproductive hormones, impaired sperm characteristics and disruption of testicular and epididymal organization. Conversely, KP co-treatment was generally associated with improvement across these domains. Previous studies have similarly emphasized the potential of antioxidant and phytochemical interventions in mitigating chemically induced reproductive injury [8, 21]. The present findings extend this concept by demonstrating structural preservation of both the seminiferous and epididymal compartments alongside biochemical and functional reproductive measures.

Overall, the present findings suggest that ethanolic *K. pinnata* leaf extract attenuated several manifestations of CYP-associated reproductive toxicity in male Wistar rats. The evidence is strengthened by the agreement between functional sperm measures, biochemical indices, endocrine parameters and the newly evaluated testicular and epididymal histomorphology. The photomicrographs demonstrate

preservation of seminiferous tubular and epididymal ductal organization in KP co-treated animals relative to CYP alone, while the quantitative and biochemical findings provide complementary evidence of improved reproductive status. The response was, however, parameter- and dose-specific rather than uniformly dose-dependent. Further studies incorporating longer recovery periods, functional fertility assessment, sperm DNA integrity, apoptosis markers, steroidogenic pathway measurements and molecular assessment of oxidative and inflammatory signalling will be necessary to clarify the mechanisms and determine the durability of the observed protective effects.

### Conclusion

Ethanollic leaf extract of *Kalanchoe pinnata* attenuated several cyclophosphamide-induced reproductive alterations in male Wistar rats. The protective profile was characterized by improvement in sperm characteristics, attenuation of oxidative stress and inflammatory disturbances, modulation of reproductive endocrine parameters, and preservation of testicular and epididymal histoarchitecture. The histological findings were consistent with the functional and biochemical observations, particularly the preservation of seminiferous tubular and epididymal ductal organization in animals receiving *K. pinnata* alongside cyclophosphamide. The observed effects were not uniformly dose-dependent, with some endpoints showing greater responses at the intermediate or higher extract doses. Collectively, these findings indicate that *K. pinnata* has reproductive-protective potential in this experimental model of cyclophosphamide exposure. However, the findings should be regarded as preclinical evidence, and further studies are required to establish the durability, mechanisms and functional reproductive significance of this protective effect before any consideration of translational application as an adjuvant to cyclophosphamide therapy.

### Limitations of the Study

Several limitations should be considered when interpreting the findings. First, the study employed male Wistar rats as an experimental model, and the physiological and pharmacological responses observed in rodents may not fully reproduce the complex reproductive, endocrine and metabolic responses to cyclophosphamide or *K. pinnata* in humans. Second, although sperm characteristics and testicular and epididymal histomorphology provided important indicators of reproductive status, functional fertility was not directly assessed through mating studies, pregnancy outcomes, litter size or related reproductive endpoints. Consequently, the extent to which the observed improvements in sperm parameters and tissue architecture translate into preservation of actual fertility remains uncertain. Third, the study did not investigate specific upstream molecular pathways that could explain the observed antioxidant, inflammatory and endocrine effects. In particular,

markers of apoptosis such as caspase-3, antioxidant signalling involving Nrf2 and its downstream pathways, and key steroidogenic enzymes were not assessed. Therefore, the present findings establish associations between *K. pinnata* administration and improvement in biochemical, endocrine and structural outcomes but do not definitively establish the molecular mechanisms responsible for these effects. Further investigations incorporating molecular pathway analysis, longer follow-up periods and functional fertility assessments are warranted to address these limitations.

### Funding.

None

### Availability of data and materials

Data generated during and analysed during the course of this study are available from the corresponding author on request.

### Competing interests

All authors of this paper declare that there is no conflict of interest related to the content of this manuscript.

Received: 11th July, 2026, Revised 24th August, 2026, Accepted: 6th September, 2026, **Published online: 25/09/26**

### References

1. Abdi M, Fadaee M, Jourabchi A, Karimzadeh H, Kazemi T. Cyclophosphamide-Induced Infertility and the Impact of Antioxidants. *Am J Reprod Immunol*. 2024 Dec;92(6):e70014. Doi: 10.1111/aji.70014.
2. Onaolapo AY, Adebayo AA, Onaolapo OJ. Oral phenytoin protects against experimental cyclophosphamide-chemotherapy induced hair loss. *Pathophysiology*. 2018 Mar;25(1):31-39. doi: 10.1016/j.pathophys.2017.12.001.
3. Olowe TG, Oyovwi MO, Nwangwa KE, Ohwin EP, Oghenetega OB. Cytotoxic Properties of Cyclophosphamide: A Focus on Its Mechanistic Impacts on Male Gonadal Functions. *J Explor Res Pharmacol*. 2024;9(2):106-115. doi: 10.14218/JERP.2023.00025S.
4. Olofinnade AT, Ajifolawe OB, Onaolapo OJ, Onaolapo AY. Dry-feed Added Quercetin Mitigates Cyclophosphamide-induced Oxidative Stress, Inflammation and Gonadal Fibrosis in Adult Male Rats. *Antiinflamm Antiallergy Agents Med Chem*. 2025;24(1):43-56. doi: 10.2174/0118715230316410240821105658.
5. Medrano JV, Hervás D, Vilanova-Pérez T, Navarro-Gomezlechon A, Goossens E, Pellicer A, Andrés MM, Novella-Maestre E. Histologic Analysis of Testes from Prepubertal Patients Treated with Chemotherapy Associates Impaired Germ Cell Counts with Cumulative Doses of Cyclophosphamide, Ifosfamide, Cytarabine, and Asparaginase. *Reprod Sci*. 2021 Feb;28(2):603-613. doi: 10.1007/s43032-020-00357-6.
6. Saleh DO, Abo El Nasr NME, Hussien YA, El-Baset MA, Ahmed KA. Cyclophosphamide-induced testicular injury: the role of chrysin in mitigating iron overload and ferroptosis. *Naunyn Schmiedeberg Arch Pharmacol*. 2025 May;398(5):5475-5489. doi: 10.1007/s00210-024-03519-4.
7. Ahmed HA, Gatea FK, Hussein ZA. Azilsartan as a preventive agent against cyclophosphamide-induced testicular injury in male rats. *Naunyn Schmiedeberg Arch Pharmacol*. 2025 Jan;398(1):979-990. doi: 10.1007/s00210-024-03339-6
8. Omoruyi F, Tatina L, Rios L, Stennett D, Sparks J. Insights into the Therapeutic Use of *Kalanchoe pinnata* Supplement in Diabetes

- Mellitus. Pharmaceuticals (Basel). 2025 Oct 10;18(10):1518. doi: 10.3390/ph18101518.
9. Kendeson CA, Kagoro ML, Adelaku EA. Phytochemical And Pharmacological Evaluation Of Nigerian Kalanchoe Pinnata (Lam.) Stem-Bark *Journal of Chemical Society of Nigeria*, 2021; 46(4). <https://doi.org/10.46602/jcsn.v46i4.654>
  10. Assis de Andrade E, Machinski I, Terso Ventura AC, Barr SA, Pereira AV, Beltrame FL, Strangman WK, Williamson RT. A Review of the Popular Uses, Anatomical, Chemical, and Biological Aspects of Kalanchoe (Crassulaceae): A Genus of Plants Known as "Miracle Leaf". *Molecules*. 2023 Jul 21;28(14):5574. doi: 10.3390/molecules28145574.
  11. Nascimento LBDS, Casanova LM, Costa SS. Bioactive Compounds from *Kalanchoe* Genus Potentially Useful for the Development of New Drugs. *Life* (Basel). 2023 Feb 26;13(3):646. doi: 10.3390/life13030646.
  12. Onaolapo AY, Ojo FO, Onaolapo OJ. Biflavonoid quercetin protects against cyclophosphamide-induced organ toxicities via modulation of inflammatory cytokines, brain neurotransmitters, and astrocyte immunoreactivity. *Food Chem Toxicol*. 2023 Aug;178:113879. doi: 10.1016/j.fct.2023.113879.
  13. Onaolapo AY, Oladipo BP, Onaolapo OJ. Cyclophosphamide-induced male subfertility in mice: An assessment of the potential benefits of Maca supplement. *Andrologia*. 2018 Apr;50(3). doi: 10.1111/and.12911. Epub 2017 Oct 18. PMID: 29047156.
  14. Onaolapo OJ, Onaolapo AY, Omololu TA, Oludimu AT, Segun-Busari T, Omoleke T. Exogenous Testosterone, Aging, and Changes in Behavioral Response of Gonadally Intact Male Mice. *J Exp Neurosci*. 2016 May 2;10:59-70. doi: 10.4137/JEN.S39042.
  15. Olofinnade AT, Onaolapo AY, Onaolapo OJ, Olowe OA. The potential toxicity of food-added sodium benzoate in mice is concentration-dependent. *Toxicol Res (Camb)*. 2021 May 17;10(3):561-569. doi: 10.1093/toxres/tfab024.
  16. Olofinnade AT, Onaolapo OJ, Onaolapo AY Clomiphene/letrozole administration can modulate neurobehavioural and neurochemical indices in hyperandrogenic female rats. *Acta Bioscientia* 2025;1(3);111-122. <https://doi.org/10.71181/actabioscientia12260>
  17. Adesope KA, Onaolapo AY, Onaolapo OJ. Assessment of the Effects of Muira Puama Root Extract on Biochemical Indices, and Testicular Histomorphology in Cyclophosphamide-treated Rats *Acta Bioscientia* 2025;1(4);182-189 <https://doi.org/10.71181/actabioscientia12330>.
  18. Aworinde OO, Adebayo A, Olufemi-Aworinde KJ, Adeyemi A, Onaolapo AY, Onaolapo OJ. Synergistic Modulation of Hormonal Balance and Ovarian Architecture by Clomiphene–Letrozole Co-Administration in a Rodent Model of Hyperandrogenism. *Acta Bioscientia* 2026;2(3);377-390. <https://doi.org/10.71181/actabioscientia12550>
  19. Hernández-Caballero ME, Sierra-Ramírez JA, Villalobos-Valencia R, Seseña-Méndez E. Potential of *Kalanchoe pinnata* as a Cancer Treatment Adjuvant and an Epigenetic Regulator. *Molecules*. 2022 Sep 29;27(19):6425. doi: 10.3390/molecules27196425.
  20. Menu WMS. In vitro, Anti-oxidant, and Anti-inflammatory Activity of *Kalanchoe pinnata*. *Arch Pharm Pharma Sci*. 2025; 9(1): 001-008. 10.29328/journal.apps.1001064
  21. Bhusal, D., Poudel, M., Tamang, A., Dhital, S., Parajuli, N., Shrestha, T., Bharati, S., et al (2025). Exploring the Therapeutic Potential of *Kalanchoe Pinnata*: A Comprehensive Analysis of Bioactive Compounds and Pharmacological Activities. *Biology, Medicine, & Natural Product Chemistry*, 13(2), 617-625. doi:<https://doi.org/10.14421/biomedich.2024.132.617-625>

Cite as: Oladipo B.P, Oladipo MO, Onaolapo AY, Onaolapo OJ *Kalanchoe pinnata* Attenuates Cyclophosphamide-Induced Testicular and Sperm Alterations in Wistar Rats: Histomorphological and Endocrine Evidence *Acta Bioscientia* 2026:2(5);657-666. <https://doi.org/10.71181/actabioscientia12705>