Journal of Experimental Pharmacology Dovepress

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The Potential Protective Role of Ascorbic Acid Against Testicular Toxicity Induced by Fluoxetine in Male Wistar Rats

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Background: Fluoxetine (FLX) is a Selective Serotonin Re-uptake Inhibitor (SSRI) commonly used as a first-line treatment for depression, anxiety, and mood disorders. It can cause infertility in the male reproductive system through the release of Reactive Oxygen Species (ROS). This study aimed to evaluate the testiculo-protective potential of ascorbic acid against fluoxetine-induced spermatotoxicity in male Wistar rats.

Methods: This study assessed Vitamin C's effect on male fertility in fluoxetine-treated Wistar rats. Thirty rats (130 ± 40 g) were divided into six groups (n=5): Control (distilled water), fluoxetine 20 mg/kg, Vitamin C 100 mg/kg, fluoxetine 20 mg/kg + Vitamin C 50 mg/kg, fluoxetine 20 mg/kg + Vitamin C 100 mg/kg, and fluoxetine 20 mg/kg + Vitamin C 150 mg/kg. Treatments were administered daily via oral gavage for 60 days, followed by assessments of testicular weight, semen analysis, oxidative stress biomarkers (CAT and GPx), and histomorphology. The data was analyzed using one-way ANOVA and Turkey's post-hoc multiple comparison test, reporting as mean±SEM using The GraphPad Prism version 6.0 for Windows, with significance set at $p<0.05$.

Results: Vitamin C, administered particularly at higher doses, significantly increased body weight, testicular weight, and antioxidant enzyme levels (glutathione peroxidase and catalase) while improving fertility parameters such as sperm count, motility, and viability in treated rats ($P<0.05$). Fluoxetine alone led to a significant reduction ($P<0.05$) in these parameters, but the combination with Vitamin C mitigated these effects. Histological analysis showed improved testicular structure in Vitamin C-treated groups, highlighting its protective role against fluoxetine-induced testicular damage.

Conclusion: Ascorbic acid has testiculoprotective potential in fluoxetine-induced spermatotoxicity, mainly owing to its antioxidant properties.

Keywords: testiculo-protection, oxidative stress, histomorphology, spermatotoxicity, ascorbic acid, vitamin C, male fertility

Introduction

Depression has become a common mental disorder worldwide, affecting more than 280 million people of all ages,[1] and approximately 5.0% of adults.[1] It persistently causes feelings of sadness and loss of interest.[2] Depression differs from short-lived emotional responses to mood fluctuations.[3] Depression has been noted to be one of the leading causes of disability and suicide, and it is one of the major contributors to the global burden of diseases.[4–6]

One or more antidepressant medications have been used to manage depressive disorders.[7] According to,[8] antidepressants comprise selective 5-hydroxytryptamine (serotonin 5-HT) reuptake inhibitors (SSRIs), serotonin agonists and reuptake inhibitors (SARIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), norepinephrine-dopamine reuptake inhibitors (NDRIs), tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), noradrenergic, and

Received: 5 July 2024
Accepted: 14 November 2024
Published: 23 November 2024

specific serotonergic antidepressants (NaSSAs). Increased serotonin levels in the central nervous system are associated with reduced anxiety and elevated mood.[9]

Fluoxetine (FLX), a Selective Serotonin Re-uptake inhibitor (SSRIs), is considered a first-line antidepressant in this class and has been used for the management of anxiety and mood disorders.[10,11] SSRIs have detrimental effects on male sexual function, such as reduction in male fertility.[12] Studies have shown that approximately 25–73% of people treated with SSRIs experience sexual dysfunction compared to those treated with other classes of antidepressants.[13]

[14] Reported that fluoxetine is associated with physiological sexual disorders. It also hinders follicle-stimulating hormone (FSH) and luteinizing hormone (LH) output from the adenohypophysis,[15] decreases spermatogenesis,[16] decreases sperm count,[17] and decreases sperm motility and viability.[16] Fluoxetine also negatively affects sperm morphology and maturity;[18] decreases reproductive organ weight[19] and decreases testosterone.[20]

Reactive Oxygen Species (ROS) are partly responsible for the initiation and development of physiological disorders that affect reproductive processes in both males and females.[21,22] Excessive ROS production disrupts male reproductive functions through germ cell apoptosis, lipid peroxidation, and sperm Deoxyribonucleic Acid (DNA) fragmentation.[23] This contributes to the initiation and development of reproductive disorders in both males and females.[21–23] Antioxidants have also been shown to protect, reduce, or eliminate excess reactive oxygen species (ROS)[23,24] via enzymatic (eg, superoxide dismutase-SOD, catalase-CAT, peroxidases, and thioredoxin system) and non-enzymatic molecules (eg, Vitamin A, Vitamin B complex, Vitamin C, Vitamin E, Glutathione, and transferrin).[25]

Vitamin C plays a significant role in reducing testiculotoxicity and oxidative stress by acting as a potent antioxidant. It neutralizes reactive oxygen species (ROS), which are implicated in damaging sperm cells and testicular tissues.[26] By reducing ROS levels, Vitamin C helps protect the testes from oxidative damage, thus preserving sperm count, motility, and overall testicular health.[26,27] This protective effect can mitigate the adverse impacts of toxic agents on male reproductive function. This stirred the impetus of this current study. Although researchers have provided insight on depression and the detrimental effects of SSRIs like fluoxetine on male reproductive health, a focused exploration of the mechanisms by which oxidative stress contributes to these adverse effects with a clear view of the testicular histomorphology is lacking. Secondly, there is a paucity of information regarding the effect of ascorbic acid on fluoxetine-induced testicular toxicity. This study seeks to address this gap by investigating the protective role of ascorbic acid (Vitamin C) against fluoxetine-induced oxidative stress, assessing its impact on seminal parameters and testicular histomorphology in male Wistar rats. This could identify Vitamin C as a natural antioxidant approved by FDA capable of ameliorating fluoxetine-induced testiculotoxicity, adding to potential therapeutic interventions.

Hence, the present study investigated the effects of ascorbic acid on oxidative stress, seminal analytical parameters, and testicular histomorphology in male Wistar rats, thereby providing insights into how this natural antioxidant can protect rats from fluoxetine-induced testicular toxicity.

Materials and Methods

Ethical Approval

Approval and permission to conduct the research were provided by the Kampala International University (KIU)-Western Campus Institutional Review and Ethics Committee (IREC) after scrutiny by the Directorate of Higher Degrees and Research (DHDR). The study animals were treated humanely according to the Uganda National Council for Science and Technology's ethical research guidelines.[28]

Preparation of Drugs

Distilled water (10 mL) was added to 3.68 grams of fluoxetine (20 mg, Brown and Burk UK Ltd, LOT FTAHH0233) at a concentration of 30 mg/mL to make up the fluoxetine solution, which was administered to the rats daily[29] while two grams of ascorbic acid (L-ascorbic acid; Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 10 mL of distilled water to make an ascorbic acid solution of concentration 20 mg/mL, which was then administered to the rats daily.[30]

Animals, Treatment and Organ Collection

Thirty adults male Wistar rats (body weight range of 130±40g), minimum of 8 weeks old were obtained from the animal facility at Kampala International University-Western Campus in Ishaka, Bushenyi District, Uganda.

Animals were randomly divided into six groups of five animals each (n=5 per group). Group 1 received distilled water (2 mL/kg), Group 2 received fluoxetine (20 mg/kg), Group 3 received Vitamin C (100 mg/kg), Group 4 received fluoxetine (20 mg/kg/day) + Vitamin C (50 mg/kg), Group 5 received fluoxetine (20 mg/kg/day) + Vitamin C (100 mg/kg), and Group 6 received fluoxetine (20 mg/kg/day) + Vitamin C (150 mg/kg).

Properly dissolved fluoxetine or Vitamin C was administered directly into the stomachs of rats by oral gavage. The procedure uses flexible or rigid gavage needles, with softer, flexible tubing reducing the risk of tissue damage.[31] The duration of drug administration was 60 days.[32].

The body weights of the rats were measured using a digital weighing scale before sacrifice. The rats were euthanized by cervical dislocation. With the sacrificed rats in dorsal recumbence, a midline incision was made to provide access to the abdominal and thoracic cavities. The surrounding tissues were removed to expose the testes on each side. The testes were retrieved, the spermatic cords severed, and the testes removed. Testicular weight was measured using a digital weighing scale. They were then stored in tissue containers with Davidson fixative and transported for testicular histological processing.[33]

The cauda epididymides were macerated and minced in 0.8 mL of 1% trisodium citrate solution for 7–8 minutes using anatomical scissors to make up 8 mls. More of the 1% trisodium citrate solution was added and mixed thoroughly for 1 min. The suspension was diluted to 1:1 in 10% buffered formalin.

The spermatozoa were subsequently counted using a Cell Counter (double-chambered counting slides) loaded with 10 µL epididymal sperm solution. Sperm motility and concentration were assessed at room temperature (24–28°C). The microscopic field was systematically scanned, and each sperm cell encountered was evaluated. For this study, motility was categorized simply as either motile or non-motile and their percentages estimated.[33] This involved the use of standard hemocytometer and light microscope. This process was repeated to obtain the average readings.[33]

Relative Testicular Weight Measurement

The testes were carefully dissected and immediately weighed using an digital weighing scale. The relative organ weight of each animal was calculated as follows:[34]

$$\text{Relative organ weight (\%)} = \frac{\text{Absolute testicular weight (g)}}{\text{Bodyweight of rat on sacrifice day (g)}} \times 100$$

Quantitative Biochemical Assay of Glutathione Peroxidase

Glutathione peroxidase (GPx) levels were measured in testicular tissue, as described by.[35] Briefly, 10% trichloroacetic acid (TCA) was added to the homogenate prior to centrifugation. In 100 mL of 0.1% sodium nitrate, 1.0 mL of supernatant was combined with 0.5 mL of Ellman's reagent (19.8 mg of 5, 50-dithiobisnitro benzoic acid (DTNB) (Sigma-Aldrich Inc., St. Louis, MO, USA Batch D8130-5G) and 3.0 mL of phosphate buffer (0.2 M, pH 8.0). Absorbance was read and recorded at 412 nm.

Quantitative Biochemical Assay of Catalase Activity

Catalase activity was colorimetrically measured at 620 nm and represented as l mol of H_2O_2 per minute.[35] Briefly, the reaction mixture (1.5 mL) included 1.0 mL of 0.01 M pH 7.0 phosphate buffer, 0.1 mL a tissue homogenate, and 0.4 mL of 2M H_2O_2. The reaction was stopped by adding 2.0 mL of dichromate-acetic acid reagent −1:3 mixture of 5% potassium dichromate and glacial acetic acid).

Semen Analysis

After the testes were removed, a longitudinal incision was made along the caudal epididymides using a scalpel blade and the epididymal contents were emptied into the test tube. A dilution was made by adding 19 mL of distilled water to 1 mL

of the epididymal content to obtain a dilution factor of 20. Using a 20-microliter micropipette, part of this solution was introduced onto a hemocytometer with a coverslip. A hemocytometer was placed under a microscope for sperm analysis. Sperm count, viability, and motility were done at × magnification of X20. The motility of epididymal sperm was determined by calculating motile spermatozoa per unit area and expressing the results as percentage motility. The epididymal sperm count was expressed in milliliters per milliliter (mL) of suspension. Sperm viability was expressed as a percentage of viability.[36]

Determination of Sperm Count

A Neubauer enhanced hemocytometer was used to determine the sperm count. A dilution ratio of 1:20 was created from each well-mixed sample by diluting 50 ll of epididymal spermatozoa suspended in physiological saline with 950 ll diluent. The average count was obtained after scoring both chambers of the hemocytometer and ensuring that the difference between the two counts did not exceed 1/20 of their sum (ie, less than 10% difference). If the two counts were not within 10%, the sample dilution was remixed, and another hemocytometer was prepared and counted. To reduce errors, the count was repeated three times for each epididymal sample. The observations were the average of all six counts, three from each side, from a single rat.[33]

Determination of Sperm Morphology

Sperm cell suspensions were prepared, dried, fixed (three volumes of absolute methanol and one volume of glacial acetic acid), stained with hematoxylin for 15 min, washed, stained with 1% eosin for 10 min, washed, and left to dry at room temperature for 90 min for sperm morphology evaluation.

To examine sperm morphology, smears were prepared on slides and dried for 90 min before examination under a phase-contrast microscope at 400 × magnification. One hundred spermatozoa were analyzed for each animal.

Morphological anomalies were divided into two broad categories: head morphology (without curvature, without characteristic curvature, or isolated form, meaning there was no tail attached) and tail morphology (broken or isolated, meaning there was no head attached or rolled into a spiral). This analysis was performed as described by.[30]

Determination of Sperm Motility

Sperm motility (%) and progressive motility were determined and recorded under a microscope. Sperm motility within the semen was evaluated as quickly as feasible, ideally within 30 minutes. The spermatozoa did not settle out of the suspension when the semen sample was properly mixed, and the semen aliquot was removed promptly after mixing. Before removing the replication aliquot, the semen samples were mixed. A wet preparation of approximately 20 µm depth was prepared for each replication. The slide was examined using phase-contrast optics at x200 or x400 magnification after 60s waiting (for the sample to stop drifting). The procedure was performed at room temperature or 37 °C using a heated microscope stage, and the slides and coverslips were pre-warmed.

Determination of Sperm Viability

The Hypoosmotic Swelling Test (HOS) and dye exclusion test were used to assess cell membrane integrity. The dye exclusion test relies on live sperm with intact cell membranes, thereby rejecting dye absorption. Eosin Y and nigrosin are the two most widely used dyes. Nigrosin is very effective because it darkens the background and provides strong contrast for sperm assessment. If sperm heads are dead, Eosin Y stains them red or dark pink. It discolors live sperm heads to a light pink or white color. The HOS test is based on the ability of live sperm (intact cell membranes) to swell in hypotonic solution. The HOS test is preferred over the dye exclusion test because it can detect necrozoospermia quickly without damaging viable sperm.[23]

Testicular Histomorphological Examination

Following fixation in Bouin's fluid, tissues were dehydrated using ascending grades of alcohol, cleared in xylene, and embedded in paraffin embedding.[37] Thereafter, 4µm tissue sections were obtained using a Reichert-Jung 2050 rotary

microtome, followed by hematoxylin and eosin staining. The slides were examined under a light microscope and photomicrographs were captured using a digital camera.[38]

Statistical Analysis

The obtained data are reported as the mean ± SEM before being subjected to one-way analysis of variance (ANOVA) and Tukey's post-hoc multiple comparison test. All data were analyzed using GraphPad Prism (version 6.0) for Windows (GraphPad Software, San Diego, California, USA). $P < 0.05$ Values were considered statistically significant.

Results

Effect of Vitamin C on Body Weight

The results of body weight from week 1 to week 9 revealed significantly ($P<0.05$) higher body weights in the 100 mg/kg Vitamin C, 20 mg/kg fluoxetine + 50 mg/kg Vitamin C, 20 mg/kg fluoxetine + 100 mg/kg Vitamin C, and 20 mg/kg fluoxetine + 150 mg/kg Vitamin C treated group when compared to the distilled water treated group. Significantly lower mean body weight was observed in the 20 mg/kg fluoxetine treated group when compared to the distilled water treated group at both weeks 8 and 9 (Table 1).

Effect of Vitamin C on Absolute and Relative Testicular Weights

The absolute weight of testes was significantly ($P<0.05$) lower in the 20 mg/kg fluoxetine + 50 mg/kg Vitamin C treated group when compared to the 100 mg/kg Vitamin C treated group (Figure 1A). On the other, the results of the relative weight of testes was significantly higher ($P<0.05$) in the 20 mg/kg fluoxetine + 150 mg/kg Vitamin C group when compared to the 20 mg/kg fluoxetine treated group (Figure 1B).

Effect of Vitamin C on Testicular Catalase Activity of Treated Rats

The results of the mean tissue catalase activity showed significantly ($P<0.05$) higher levels in the 20 mg/kg fluoxetine + 150 mg/kg Vitamin C treated group when compared to the distilled water treated group. Significantly higher values of tissue catalase activity ($P<0.05$) was also observed in the 100 mg/kg Vitamin C, 20 mg/kg fluoxetine + 100 mg/kg Vitamin C, and 20 mg/kg fluoxetine + 150 mg/kg Vitamin C treated groups when compared to the 20 mg/kg fluoxetine treated group (Figure 2A).

Effect of Vitamin C on Testicular Glutathione Peroxidase Levels of Treated Rats

The mean levels of glutathione peroxidase was significantly lower in the 20 mg/kg fluoxetine group ($P<0.05$) when compared to the distilled water treated group. Significantly higher glutathione peroxidase levels were observed in the 100 mg/kg Vitamin C, 20 mg/kg fluoxetine + 100 mg/kg Vitamin C, and 20 mg/kg fluoxetine + 150 mg/kg Vitamin C treated groups when compared to the distilled water treated group ($P<0.05$), as shown in Figure 2B.

Effect of Vitamin C on Fertility Parameters of Treated Rats

A significantly ($p<0.05$) lower sperm count, sperm progressive motility, and viability was observed in the 20 mg/kg fluoxetine treated group when compared to the distilled water treated group. On the other hand, a significantly ($p<0.05$)

Table 1 Change in Body Weights

Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Dist. Water	133.6±2.34	141.1±0.93	148.9±1.24	154.5±3.18	160.4±2.00	170.0±1.84	178.8±1.10	189.3±2.69^{b,c}	197.2±4.37^{b,c}
Flx 20mg/kg	132.9±3.63	132.8±1.11	139.0±1.70	139.7±0.78	141.5±0.96	161.7±2.20	169.5±0.71	176.2±1.81^{a,c}	181.2±2.44^{a,c}
Vit.C 100mg/kg	159.9±12.97	165.8±3.40	168.4±1.99	171.9±0.93	176.1±1.10	180.4±2.26	187.5±0.44	196.7±1.72^{a,b}	209.5±8.51^{a,b}
Flx20mg/kg +VitC 50mg/kg	164.4±3.42	153.2±4.18	158.3±1.64	164.9±1.24	168.8±1.08	177.0±1.34	174.9±0.77	175.4±2.03^{a,b,c}	179.6±7.04^{a,b,c}
Flx20mg/kg +VitC 100mg/kg	173.2±6.74	181.0±6.48	172.8±1.82	180.0±1.72	183.4±0.93	186.6±1.41	183.5±3.15	198.4±2.97^{a,b,c}	203.7±6.71^{a,b,c}
Flx20mg/kg +VitC 150mg/kg	174.6±4.23	177.5±4.38	179.6±1.95	182.6±1.11	190.1±1.61	196.2±2.05	200.4±1.78	214.5±2.29^{a,b,c}	226.5±2.97^{a,b,c}

Notes: Values are expressed as mean ± SEM. n=5, ^{a,} ^{b,} and ^{c,} indicate significance difference when compared to the distilled water, and Flx20mg/kg, Vitamin C 100mg/kg treated group respectively at a $p \leq 0.05$.

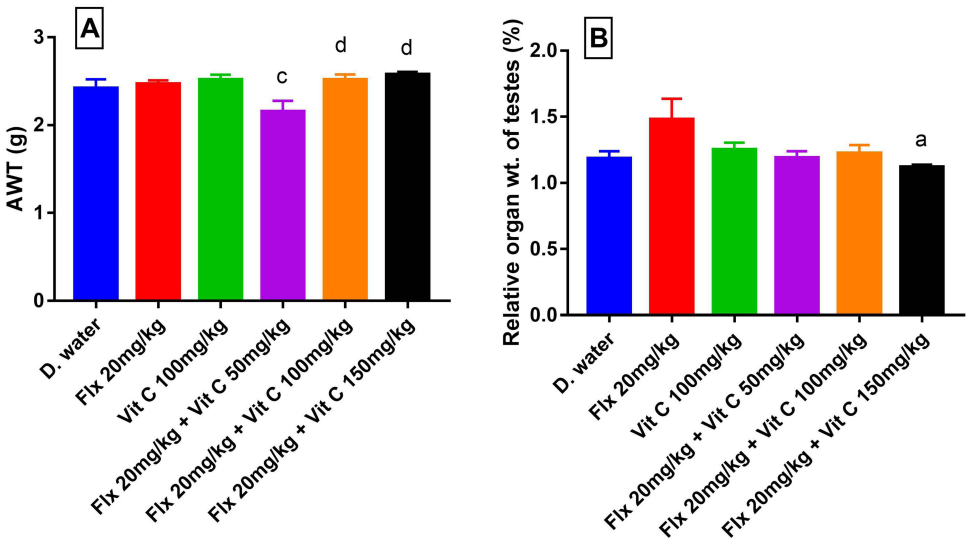

Figure 1 The mean absolute weight of testes (AWT) in grams (**A**) and relative organ weight of the testes in percentage (%) (**B**) after sacrifice. Values are expressed as mean ± SEM. n=5. [a, c,] and [d] indicate significance difference when compared to the distilled water, Vit.C 100mg/kg and Flx20 mg/kg+Vit.C50mg/kg treated group respectively at a $p \leq 0.05$.

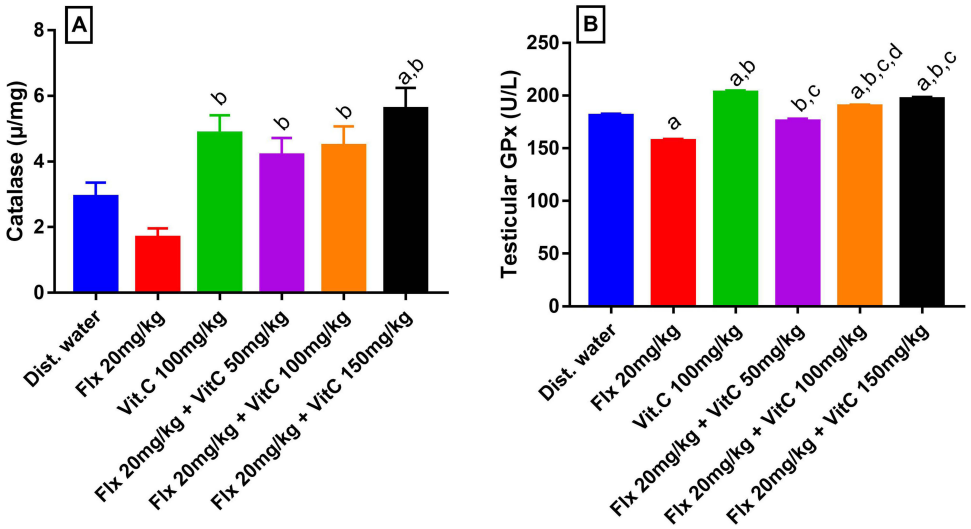

Figure 2 The mean catalase in µ/mg (**A**) and testicular glutathione peroxidase (GPx) in U/L (**B**). Values are expressed as mean ± SEM. n=5. [a, b, c,] and [d] indicate significance difference when compared to the distilled water, Flx20 mg/kg, Vit.C 100mg/kg and Flx20 mg/kg+Vit.C50mg/kg treated group respectively at a $p \leq 0.05$.

higher sperm count, sperm progressive motility, and viability was observed in the 100 mg/kg Vitamin C treated group when compared to the distilled water treated group (Table 2). There was a marked improvement in sperm count, motility, progressive motility, viability, and morphology in the 20 mg/kg fluoxetine + 100 mg/kg Vitamin C, and 20 mg/kg fluoxetine + 150 mg/kg Vitamin C treated group when compared to 20 mg/kg fluoxetine treated group (Table 2).

Effect of Vitamin C on Testicular Histomorphology

The results of the histological study showed a relatively normal histoarchitecture of the testes in the distilled water treated group (Figure 3A and B). The 20 mg/kg fluoxetine treated group showed evidence of pathological changes, including vacuolation (green arrows), with a region of disrupted germ cell layer (DC), and reduced cellularity (Figure 4A and B). The 100 mg/kg Vitamin C treated groups (Figure 5A and B) showed relatively normal histoarchitecture of the testes. There was a marked improvement in the cytoarchitecture of the testes in the 20 mg/kg fluoxetine + 50 mg/kg,

Table 2 Fertility Parameters of Treated Rats

Fertility Parameters	Distilled Water	Flx. 20mg/kg	Vit.C 100mg/kg	Flx. 20mg/kg + VitC 50mg/kg	Flx. 20mg/kg + VitC 100mg/kg	Flx. 20mg/kg + VitC 150mg/kg
Sperm count (10^6/mL)	109.4±5.56[b]	51.71±8.91[a]	148.3±4.94[a,b]	89.57±8.77[b]	128.7±0.87[b]	103.6±6.38[b]
Sperm motility (%)	68.43±1.84[b]	27.29±2.21[a]	89.43±2.24[a,b]	56.43±2.33[b]	71.86±1.14[b]	81.57±2.30[a,b]
Sperm progressive motility (%)	57.86±2.20[b]	14.43±1.72[a]	71.14±2.02[a]	48.57±2.84[b]	55.86±2.09[b]	64.29±2.60[b]
Sperm non progressive motility (%)	10.00±2.51	12.29±3.26	17.57±3.98[a]	8.27±1.97	14.14±0.74	17.29±3.01[a,b]
Sperm immotility/dead (%)	29.43±1.73[b]	71.71±1.73[a]	5.71±1.58[b]	40.86±2.13[b]	25.71±1.19[b]	11.71±3.30[b]
Sperm viability (%)	69.29±0.97[b]	34.86±1.81[a]	87.71±1.86[a,b]	63.86±1.82[b]	72.86±1.74[b]	79.57±2.20[b]
Sperm morphology (%)	9.57±0.75[b]	2.27±0.29[a]	19.00±1.6[a,b]	7.71±0.75[b]	11.71±1.19[b]	15.14±1.53[a,b]

Notes: Values are expressed as mean ± SEM. n=5, [a] and [b] indicate significance difference when compared to the distilled water and Flx20mg/kg treated group respectively at a $p \leq 0.05$.

100 mg/kg, and 150 mg/kg Vitamin C treated groups (Figure 6A and B, 7A and B, 8A and B, respectively) compared to the 20 mg/kg mg/kg fluoxetine-treated group.

Discussion

The comprehensive analysis of the data collected in our study reveals significant insights into the protective effects of Vitamin C against the adverse impacts of Fluoxetine (Flx) on both physiological and biochemical parameters in treated rats. The findings underscore the multifaceted benefits of Vitamin C supplementation. The results of this study showed a reduction in the absolute testicular weight in the animals treated with fluoxetine and a low dose of Vitamin C (Vitamin C 50 mg/kg); however, there was an increase in the relative organ weight of the testes in the fluoxetine 20 mg/kg treated group. The mean absolute weight of testes indicates that Vitamin C at 100 mg/kg significantly mitigates the testicular weight loss associated with Fluoxetine treatment. The statistical significant ($P<0.05$) difference when compared to both the Fluoxetine + Vitamin C 50 mg/kg group and the Fluoxetine group suggests that higher doses of Vitamin C can effectively preserve testicular mass, a critical indicator of reproductive health. The significant increase in relative testicular weight in the Vitamin C groups compared to the Fluoxetine group ($P<0.05$) indicates that Vitamin C not only supports absolute testicular weight but also enhances its proportion relative to overall body weight, suggesting a protective role in maintaining reproductive organ health. Relative organ weight is one of the major factors considered

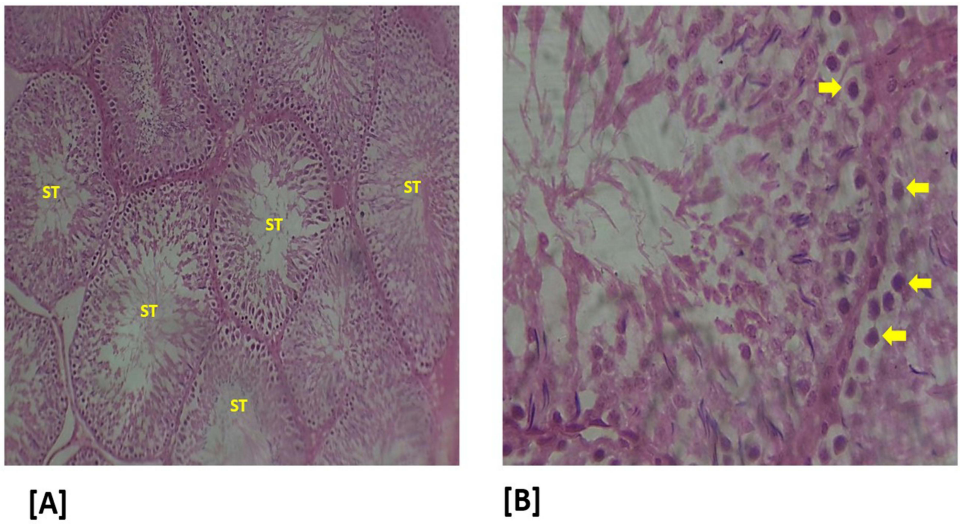

Figure 3 Photomicrograph of the testes from the distilled water treated group; showing relatively normal seminiferous tubule (ST), spermatogonia (yellow arrows) (H&E; (**A** and **B**) represent ×250 and ×400 magnifications respectively).

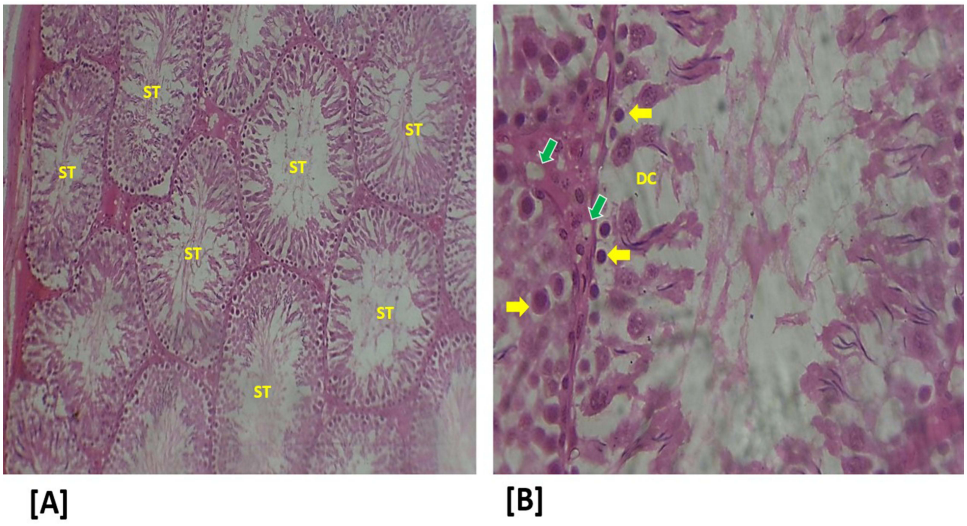

Figure 4 Photomicrograph of the testes from the 20mg/kg of fluoxetine treated group; showing seminiferous tubule (ST), spermatogonia (yellow arrows), vacuolation (green arrows), with region of disrupted germ cell layer (DC) and reduce cellularity (H&E; (**A** and **B**) represent ×250 and ×400 magnifications respectively).

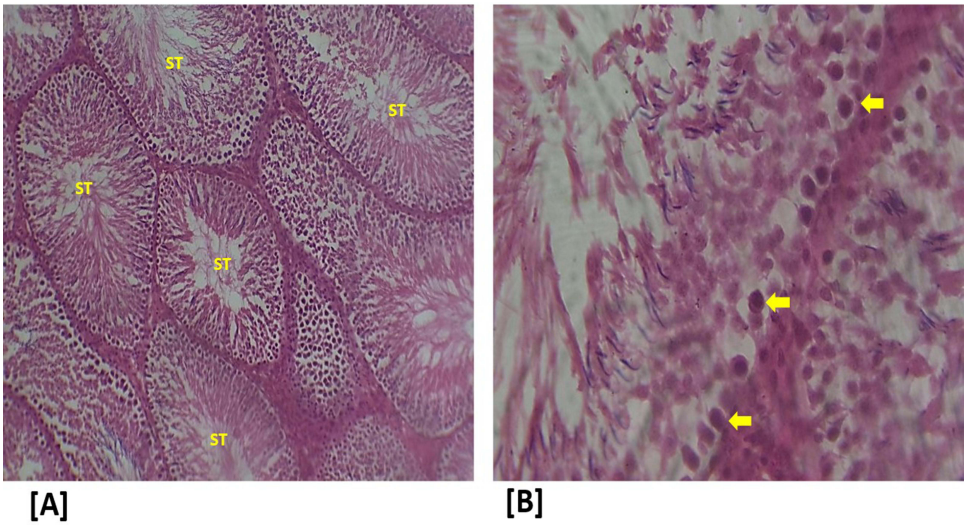

Figure 5 Photomicrograph of the testes from the 100mg/kg of Vitamin C treated group; showing relatively normal seminiferous tubule (ST), spermatogonia (yellow arrows) (H&E; (**A** and **B**) represent ×250 and ×400 magnifications respectively).

when conducting toxicity testing and safety assessments of new drugs or chemicals.[39] The changes in relative organ weight are an indicator of organ damage from chemicals and reflect a chemically induced change in overall body weight.[40–42] The changes in the relative organ weight of the testes could be indicative of the toxic or pathological effects of fluoxetine, leading to interstitial tissue inflammation, fibrosis, and atrophy of Leydig cells due to a decrease in blood supply, which is also observed in the histology and cytoarchitecture of the testes.[11,19]

Biochemical evaluation of oxidative stress biomarkers showed a decrease in testicular Glutathione peroxidase (GPx) levels in the fluoxetine-treated group. This observation is consistent with the findings of.[43] However, there was a significant increase ($P<0.01$) in GPx levels in the Vitamin C-treated groups when compared to the fluoxetine-treated group. The elevation of GPx levels, particularly in the groups receiving Vitamin C, points to an antioxidant effect that may counteract oxidative stress induced by Fluoxetine. The significant differences ($P<0.001$) between the Fluoxetine group and the distilled water control suggest that Vitamin C plays a crucial role in enhancing the antioxidant capacity of testicular tissue, which is vital for sperm function and overall fertility. GPx may have been depleted in the fluoxetine

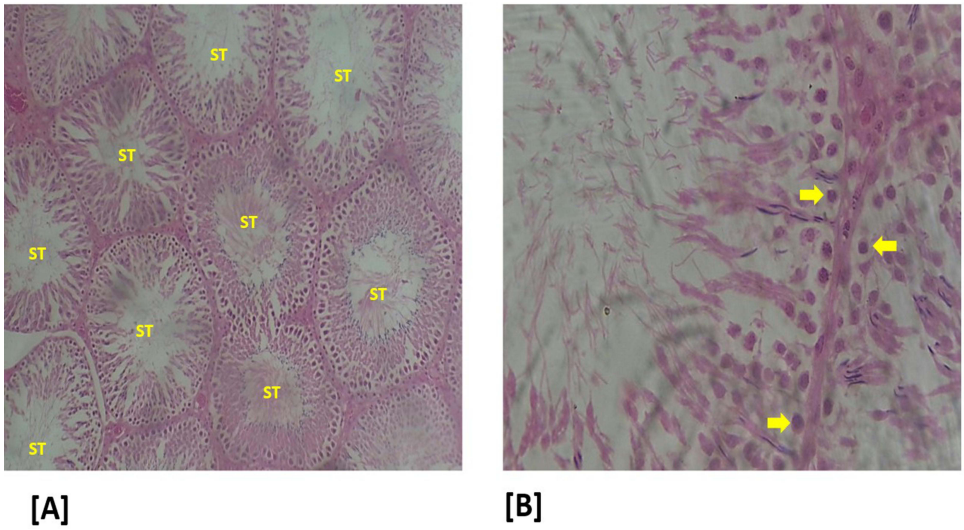

Figure 6 Photomicrograph of the testes from the 20mg/kg of fluoxetine + 50mg/kg of Vitamin C treated group; showing relatively normal seminiferous tubule (ST), spermatogonia (yellow arrows), with a marked reduction in cellularity (H&E; (**A** and **B**) represent ×250 and ×400 magnifications respectively).

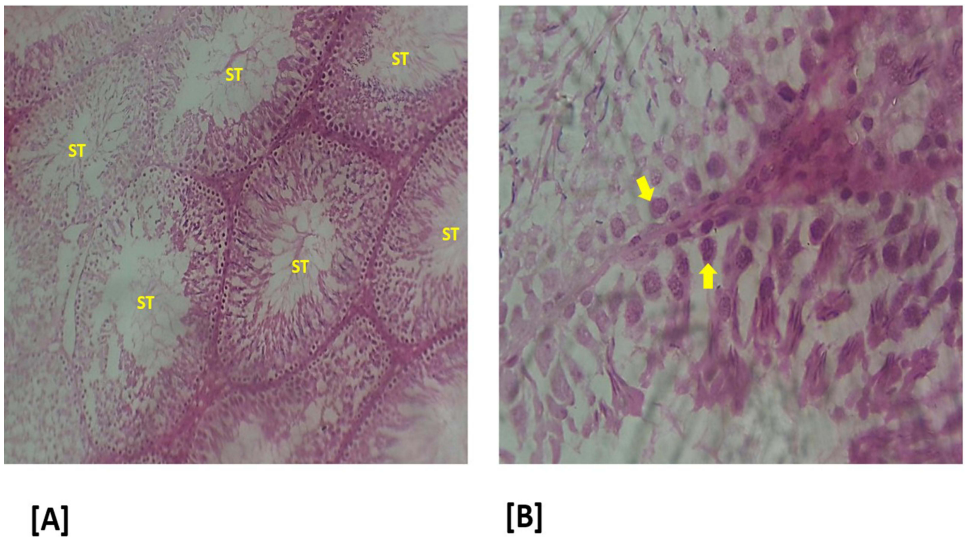

Figure 7 Photomicrograph of the testes from the 20mg/kg of fluoxetine + 100mg/kg of Vitamin C treated group; showing relatively normal seminiferous tubule (ST), spermatogonia (yellow arrows), with observed improved cellularity (H&E; (**A** and **B**) represent ×250 and ×400 magnifications respectively).

20 mg/kg treated group due to its response to oxidative injury. GPx is an endogenous antioxidant enzyme that protects the plasma membrane and cytosol from lipid peroxidation by catalyzing the reduction of both organic hydroperoxidases and H_20_2 produced at the membrane level of water, as shown by.[44]

The study showed higher activity of testicular tissue catalase (CAT) in the fluoxetine 20 mg/kg + Vitamin C 100 mg/kg and fluoxetine 20 mg/kg + Vitamin C 150 mg/kg groups than in the distilled water-treated group. There was also a higher activity of tissue catalase in the group treated with Vitamin C 100 mg/kg. Our results as highlighted above indicate that fluoxetine treatment reduces testicular Glutathione Peroxidase (GPx) levels, likely due to oxidative stress, which depletes this key antioxidant enzyme. In contrast, Vitamin C treatment, especially at higher doses, enhanced Catalase (CAT) activity, another crucial antioxidant defense. This conformed with the results of,[45] where increased concentrations of Vitamin C protected tissues and cells against the activities reactive oxygen species. This is further supported by the findings of,[26,27] highlighting that Vitamin C treatment decreased Citalopram, Lead, and Arsenic testiculotoxicity by reducing oxidative stress and lipid peroxidation while increasing GPx, SOD, and CAT activity as

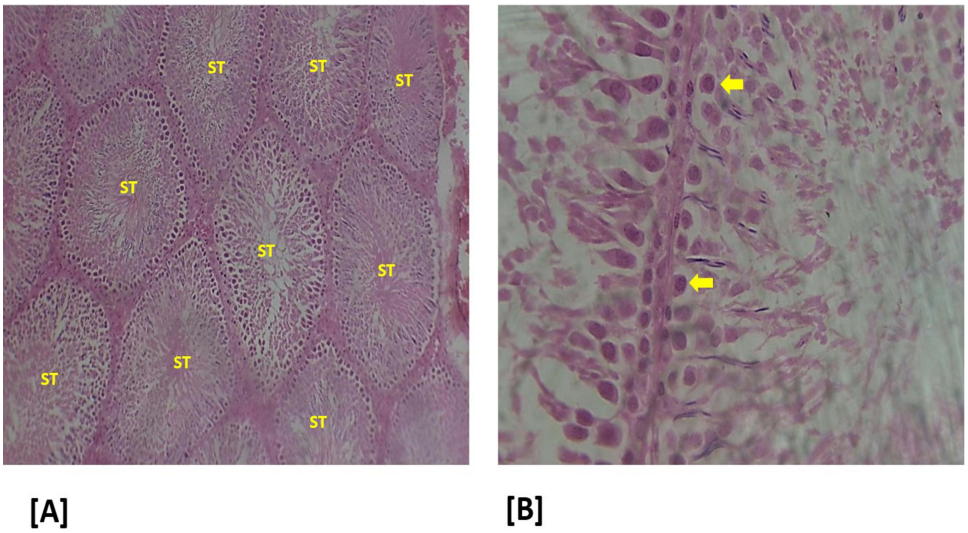

Figure 8 Photomicrograph of the testes from the 20mg/kg of fluoxetine + 150 mg/kg of Vitamin C treated group; showing relatively normal seminiferous tubule (ST), spermatogonia (yellow arrows), with observed improved cellularity (H&E; (**A** and **B**) represent ×250 and ×400 magnifications respectively).

well as the total antioxidant capacity in the testis. CAT is present in all aerobic organisms and is located in the mitochondria, cytosol, and chloroplasts,[46] which are the major sites that produce hydrogen peroxide (H_20_2) in the cellular environment of higher plants,[47] CAT is an antioxidant enzyme that catalyzes H_20_2 to water and oxygen in an energy-efficient manner in cells exposed to environmental stressors.[48] The higher concentration of catalase observed in the fluoxetine + Vitamin C treated groups may be partly due to the antioxidant activity of Vitamin C, which mitigates the pro-oxidant effect of fluoxetine and accelerates its protection and removal. This suggests that Vitamin C helps counteract oxidative damage in the testes, potentially protecting against testicular toxicity associated with fluoxetine-induced oxidative stress. Thus, Vitamin C may mitigate the harmful effects of fluoxetine on testicular function by bolstering the antioxidant defense system.

Concerning sperm fertility analysis, our results showed that treatment of Fluoxetine 20 mg/kg exposure with Vitamin C 50 mg/kg, 100 mg/kg, and 150 mg/kg (low, medium, and high dose) offered protection from Fluoxetine-induced low sperm count. This may be due to the antioxidant activity of Vitamin C, which reduces the load of free radicals acting on the testes, thereby improving testosterone and gonadotropin production, as well as tissue injury, thereby creating a conducive environment for spermatogenesis. Sperm motility is an important parameter for predicting the fertilization capability of sperm cells. Low motility greatly affects the fertilization ability of spermatozoa.[29] The study showed lower percentages of motility in the fluoxetine 20 mg/kg and fluoxetine 20 mg/kg + Vitamin C 50 mg/kg (low-dose) groups than in the other groups. The result favors.[29] Therefore, Fluoxetine causes oxidative cell injury in sperm cells,[16] resulting in a high frequency of abnormalities in sperm morphology.[34] A decrease in antioxidant enzymes in the testes, caused by high ROS levels, may damage the sperm membrane, resulting in altered morphology. There was a relative decrease in sperm morphological abnormalities when fluoxetine was administered in combination with Vitamin C at low, medium, and high doses. The results showed an increased number of dead and immotile sperm cells in the fluoxetine 20 mg/kg group and an increased number of visible sperm in groups treated with fluoxetine 20 mg/kg + Vitamin C 50 mg/kg, 100 mg/kg, and 150 mg/kg. This is consistent with the findings of,[34] who showed that Vitamin C is protective and reduces ROS-induced cellular abnormalities, damage, and death caused by the effect of ROS.

Histomorphological examination of testicular tissues revealed pathological changes such as vacuolation, disrupted germ cell layer, and cellularity in the group treated with fluoxetine 20 mg/kg. The pathological changes observed, such as vacuolation, disrupted germ cell layers, and altered cellularity in the fluoxetine-treated group, indicate that this antidepressant has detrimental effects on testicular cytoarchitecture. These findings align with previous research indicating that treatment with antidepressants including fluoxetine treatment caused negative changes such as disorganized and shrunken seminiferous tubules, thickening of the tunica propria, vacuolization of the germ cells, and diffused Leydig

cells in mice testicular tissue, thereby negatively impacting male reproductive health.[11,49] There was a marked improvement in the histomorphology and cytoarchitecture of the testes when fluoxetine 20 mg/kg + Vitamin C 50 mg/kg, 100 mg/kg, and 150 mg/kg were administered in comparison with the fluoxetine 20 mg/kg-treated group. This microscopic histomorphological examination of testicular tissues revealed the testiculoprotective role of ascorbic acid (Vitamin C) against testicular toxicity induced by fluoxetine in male Wistar rats.

Conclusion

This study demonstrated that fluoxetine administration led to adverse reproductive outcomes in rats, primarily due to oxidative damage, as evidenced by increased body and relative testicular weight, decreased glutathione levels and catalase activity, and significant histopathological changes such as vacuolation and disrupted germ cell layers. Sperm fertility parameters were negatively impacted, with reduced sperm count, motility, and viability, alongside increased immotility and abnormal morphology. In contrast, Ascorbic Acid (Vitamin C) administration at doses of 100 mg/kg showed the most potent testiculo-protective potential, effectively reducing oxidative damage and improving both histomorphological and sperm parameters. These findings suggest that Ascorbic Acid may serve as a valuable adjunct therapy for enhancing male reproductive health in individuals undergoing Fluoxetine treatment.

Institutional Review Board Statement

Ethical approval was obtained from Kampala International University Ethical Review Committee and Uganda National Council for Science and Technology Ref No: HS3533ES.

Data Sharing Statement

The data for the present study can be assessed by the corresponding author upon reasonable request.

Author Contributions

All authors contributed significantly to the work, participated in drafting, revising, and critically reviewing the article, gave final approval, agreed on the journal, and agreed to be accountable for all aspects. JOA, EME, SAB, and KO conceptualized and drafted the study; SAB, KO, NT, TGK, OAG, TP, and IMU designed and critically reviewed the methodology used in the study; SAB, KO, NT, TGK, OAG, TP, and IMU critically review and supervised the work and final draft of the article; JOA, EME, NT, TGK, OAG, TP, and IMU collected the data; JOA, EME, SAB, KO, NT, TGK, OAG, TP, and IMU conducted data analysis and interpretation. EME drafted the initial version and made input in methodology and result sections. JOA, EME, SAB, KO, NT, TGK, OAG, TP, and IMU critically revised the manuscript for important intellectual content and approved the final version to be published.

Funding

This study did not receive any external funding.

Disclosure

The authors declare no conflicts of interest.

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