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Evaluation of reproductive indices of chronic mild-stress depressed rats treated with *Solanum aethiopicum*

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Abstract

Stress in various forms leads to a range of adverse effects on the male reproductive system. *Solanum aethiopicum*, a local plant rich in nutrients, is used to treat certain diseases due to its phytochemical composition. The aim of this study was to assess the effect of *Solanum aethiopicum* on the reproductive indices of chronic mild stress depressed rats. Twenty-five male Wistar rats were divided into five groups (A, B, C, D, E). Group A (control) was administered distilled water only. Group B was subjected to chronic mild stress for 28 days. Groups C and D were exposed to chronic mild stress for 28 days and administered 400 and 800 mg/kg of *Solanum aethiopicum* orally. Group E received only 800mg/kg of *Solanum aethiopicum* orally. An increase in sperm motility, viability and normal sperm morphology was observed for groups C, D and E while a decrease was observed in group B. Groups C, D, and E had a significant decrease ($p < 0.05$) in the level of cortisol and malondialdehyde while group B had a significant increase ($p < 0.05$). A significant decrease ($p < 0.05$) of catalase was observed in Group B while a significant increase ($p < 0.05$) was observed in Groups C, D and E. The levels of FSH decreased in Group B but increased in Groups C and D, respectively. The levels of testosterone decreased in Group B but increased in Group E. Degeneration of seminiferous tubules and congestion of blood vessels occurred in Group B. Group C and D, respectively revealed mild regeneration of seminiferous tubules while normal testicular cytoarchitecture was observed in Group E. The results of this study show that *S. aethiopicum* has the potential to mitigate the adverse effects of chronic stress on male reproductive parameters.

Keywords: Reproductive, Antioxidant, Stress, Depressed, Mitigate.

Introduction

Stress is characterized as a real or perceived danger to an organism's equilibrium (Del Giudice et al., 2018). Stress, both acute and chronic, can interfere with man's ability to reproduce (Dutta et al., 2021). Long-term exposure to stress has been reported to have a deleterious effect on tissues, which inhibits bodily functions (Yaribeygi et al., 2017).

Numerous studies have documented changes in the morphology and function of the testes as a result of long-term stress. The outcomes reported included decreased serum testosterone levels and sperm production impairments (Ramya et al., 2023). A major percentage of infertility due to a decline in spermatogenesis (Ilacqua et al., 2018). Various studies on the effect of different form of stress on male reproductive parameters have been

conducted. Studies on the effects of chronic stress on reproductive parameters focused on glucocorticoid receptors (GRs) in Leydig, Sertoli and Germ cells (Maeda et al., 2015; Hazra et al., 2014; Yazawa et al., 2000). Kumar et al. (2022) and Sangishetti et al. (2017) studied the effect of stress and caffeine on male infertility and the effect of stress on the histopathology of the male reproductive system. Collodel et al. (2018) studied the effect of emotional stress on sperm quality. A cross-sectional study conducted by Bräuner et al. (2020), examined the relationship between male factor infertility, testicular function and psychological stress. The effect of prenatal stress on reproductive function of male offspring was studied (Gong et al., 2024). Valcarce et al. (2023) conducted research on the effect of chronic stress on male reproductive parameters in zebrafish. A percentage of the various studies on stress and male reproductive parameters reported effects on serum testosterone, follicle stimulating hormone, luteinizing hormone, and testicular histology (Bariweni et al., 2019). Some other others reported on sperm concentration, motility, morphology, GAT1 expression (Demirci & Sahin, 2019). The current study focused on the effect of chronic mild stress and a natural plant extract on sperm parameters, testicular histology, and serum hormone assays (FSH and testosterone) in Wistar rats. Studies have gone on further to highlight the relationship between the reproductive parameters and oxidative stress. An association between oxidative stress and a surplus production of reactive oxygen species (ROS) has been linked to impaired sperm motility, concentration and morphology (Agarwal et al., 2014). ROS can be transformed under unfavorable conditions in the body to agents that are highly reactive which leads to the dysregulation of diverse signaling pathways. This results in damage to nucleic acids, proteins and lipids leading to impaired sperm motility, loss of membrane integrity etc. (Ritchie & Ko, 2021; Du Plessis et al., 2015).

The application of medicinal plants with antioxidant properties have been utilized for the potential to treat or avert various human pathologies (Sharifi-Rad et al., 2020). The perception that medicinal plants are more affordable, nutrient-dense, and have less adverse effects may be the reason they are used (Nwakanma et al., 2022). *S. aethiopicum* is such a plant that demonstrates high antioxidant properties due to the various phytochemicals it contains (Eletta et al., 2017) especially phenolic compounds and flavonoids (Faraone et al., 2022).

Various studies have reported the ability of *S. aethiopicum* to improve semen parameters Atukpa et al. (2023), increase serum levels of follicle-stimulating hormone, luteinizing

hormone and testosterone (Queen et al., 2021). The aim of this study was to evaluate the reproductive indices of chronic mild stressed depressed rats treated with *S. aethiopicum*.

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Materials and methods

Plant material

Fresh leaves of *S. aethiopicum* were purchased from Obomazia, Ihiala Local Government Area, Anambra State, Nigeria. The leaves were identified and authenticated at the herbarium section of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Agulu, Anambra State, Nigeria. With identification number PCG/474/S/032.

Extract preparation

The identified leaves were destalked, washed thoroughly and air-dried at room temperature for eight (8) days. The dried *S. aethiopicum* leaves were homogenized using an auto-milling machine into coarse form. A 50g of the fine powder form of eggplant leaves was macerated in 100 ml of 95% ethanol. It was exposed to a temperature of 78°C and allowed to stay for 72 hours. The solution was filtered, and the filtrate was concentrated using a rotatory evaporator, to evaporate the extracting solvent (ethanol). It was later transferred to a thermostat oven, to release any trace of the solvent. After evaporation, the filtrate was steam-dried and weighed.

Phytochemical Analysis

The extract was subjected to preliminary qualitative phytochemical screening. Tests were undertaken to establish the constituents (Harborne, 1973).

Experimental Animals

Twenty-five (25) male Wistar rats with an average weight of 150g were kept under standard conditions and allowed to acclimatize for 2 weeks before the start of the experiment. Experimental ethics were in accordance with the guidelines for animal research, as stated in the NIH guidelines for the care and use of laboratory animals (Guide for the care and use of laboratory animals).

Experimental Design

The rats were randomly divided into five groups (Groups A, B, C, D, E) based on two classifications: stressed and naïve groups. The naïve subjects stayed in their social housing condition (5 rats per cage) while the stressed group were moved to individual housing (Yusha'u et al., 2021). The naïve groups consisted of Group A (control) and Group E (unstressed group). Group A received distilled water, group B was subjected to chronic mild stress for 28 days. Groups C and D were subjected to chronic mild stress for 28 days and also administered *S. aethiopicum* (400 and 800 mg/kg) from the 15th to 35th day of the experiment. Group E was not subjected to stress but was administered *S. aethiopicum* (800 mg/kg) for 14 days. The extract was administered orally.

The chronic mild stress regimen, employed in this investigation was derived from a Willner approach that Ducottet et al. (2003) applied to rats. The following stressors were briefly applied to rats: empty cages, empty cages with 100 ml of water, water deprivation, clean cages, dirty cages, tilted cages at an angle of 45°, social stress, predator sounds for 60 minutes (meowing sound), overnight illumination, and white noise (static radio sound). The exposure to stress was random and unpredictable for the first 14 days of the experiment. The application of chronic mild stress regimen was repeated for another 14 days after the initial exposure to stress for 14 days. On the 29th day of the experiment, the animals were anesthetized using ketamine. Blood was collected via ciliary puncture. Blood was centrifuged at 3000 rpm for 10 minutes and the blood serum was evaluated for testosterone and follicle stimulating hormone (FSH) levels. Blood was analysed for oxidative stress markers (cortisol, malondialdehyde and catalase). The rats were dissected and the semen from the testes was collected for semen analysis. The testes of all the rats were harvested through abdominal incision and fixed in 10% formalin. A section 5 µm thick was stained with hematoxylin and eosin for histopathological examination.

Sperm Motility and Count Assay

A drop of semen was placed on a glass slide to assess sperm motility. 2.9% sodium citrate was added. This was covered with a glass slip and viewed under the light microscope to determine the motile and non-motile spermatozoa (Zemjanis, 1970). Sperm count was conducted with use of a hemocytometer. The supernatant containing the epididymal sperm was diluted with normal saline, filtered and an aliquot

of 10µl was placed on the hemocytometer. The improved Neubauer chamber (Deep 1/10m, LABART, Munich, Germany) was used for the count (Pant & Srivastava, 2003). Concentration was expressed in millions of sperm per ml.

Sperm morphology and viability assay

The semen was placed on the slide, and Wells and Awa stain (composed of 0.2g eosin and 0.6g fast green dissolved in distilled water and ethanol in ratio 2:1) was added (Wells and Awa, 1970); Isaac & Ebenezer (2018). In determining the viability of sperm, a drop of semen was placed on the slide, and a drop of Eosin-nigrosin stain (which is 1% eosin with 5% nigrosin in 3% sodium citrate solution) was added. It was air dried and observed under the microscope for viable sperms. They were counted as described by Wells & Awa (1970) and Isaac & Ebenezer (2018).

Measurement of Oxidative Stress Levels

Testicular tissue was removed and used for the measurement of oxidative stress markers (cortisol, MDA and catalase). Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (Aboul-Soud et al., 2011). Cortisol was measured by the method used by Uzoma et al., (2021). Catalase levels were measured by the method of Sinha (1972).

Serum hormonal assay

The rats were euthanized. Blood samples were collected by retro-orbital bleeding and stored in sterile test tubes. The blood samples were left to clot at room temperature for at least 30 minutes and centrifuged at 1000–2000 per gram for 10 minutes to separate the serum. The serum samples were analyzed to determine the level of testosterone and Follicle Stimulating Hormone (FSH) using enzyme-linked immunosorbent assay (ELISA) kits that specifically measured the testosterone and FSH levels. The testosterone and FSH levels were recorded, and statistical analysis was carried out to determine the mean and standard deviation values.

Tissue Processing

The testes were collected, stored in 10% neutral buffered formalin and processed for histological analysis using H & E. The testes were embedded in paraffin wax, sectioned and mounted on glass slides. Histological alterations were observed by placing the glass slides under the microscope.

Statistical Analysis

The statistical analysis was done using the standard package for social sciences (SPSS version 20.0). The results were analyzed using the one-way analysis of variance (ANOVA) with a significant difference at $p < 0.05$. LSD comparison was used to test for significant differences between the groups. The results were presented as mean \pm standard error of mean.

Result

Result of Phytochemical Analysis of *S.aethiopicum*

Results from the phytochemical analysis of *S. aethiopicum* is shown in Table 1. Qualitative phytochemical analysis revealed the presence of alkaloids, steroids, terpenoids, saponins, tannins, glycosides and flavonoids.

Effect of *S. aethiopicum* leaf extract on sperm parameters

Result for the effect of the *S. aethiopicum* leaf extract on semen parameters is shown in Table 2. The semen parameters tested were sperm motility, total sperm count, normal and abnormal sperm cells. In this study rats in Group B had a decrease in sperm count while group C had an increase in sperm count. In Group D there was an increase in sperm count which was also observed in group E. There was a decrease in the viability of the sperms in Group B, while there was an increase in viability of sperms in groups C, D and E. There was a decrease in the normal sperm morphology of group B while an increase in the normal sperm morphology was observed for groups C, D and E.

Table 1: Phytochemical analysis of *S. aethiopicum*

Phytochemicals	Inference
Alkaloids	++
Steroids	+
Terpenoids	+
Tannins	+
Saponin	++
Glycosides	++
Flavonoids	+++

Key: + low ++ moderately present +++ highly present

Table 2: Effect of *Solanum aethiopicum* leaf extract on Sperm Parameters

Groups	Sperm Count X10 ⁶ mls	Activity Motility (%)	Sluggish Motility (%)	Normal Sperm Cells	Abnormal Sperm Cells
A	490	80	20	85	15
B	270*	60	40*	55*	45*
C	564	70	30	75	25
D	597	80	20	85	15
E	640	85	15	90	10

Key: Values are expressed as mean \pm SD. **P < 0.05 group B when compared with group A. Group A= Control; Group B= Chronic mild stress; Group C=Chronic mild stress +*S. aethiopicum* (400 mg/kg); Group D=- Chronic mild stress + *S. aethiopicum* (800 mg/kg); Group E-(unstressed group) *S. aethiopicum* (800 mg/kg).

Effect of *S.aethiopicum* on Cortisol, MDA and Catalase

Results of oxidative stress revealed a significant increase in the level of cortisol in-group B ($p < 0.05$). Groups C, D, and E had a significant decrease ($p < 0.05$) when compared to group B (Figure 1). The MDA result revealed significant increase in-group B ($p < 0.05$). Groups C, D, and E had a

significant decrease ($p < 0.05$) compared to B (Figure 2). A significant decrease ($p < 0.05$) of catalase levels in-group B occurred. Groups C, D and E had a significant increase ($p < 0.05$) in catalase compared to Group B (Figure 3).

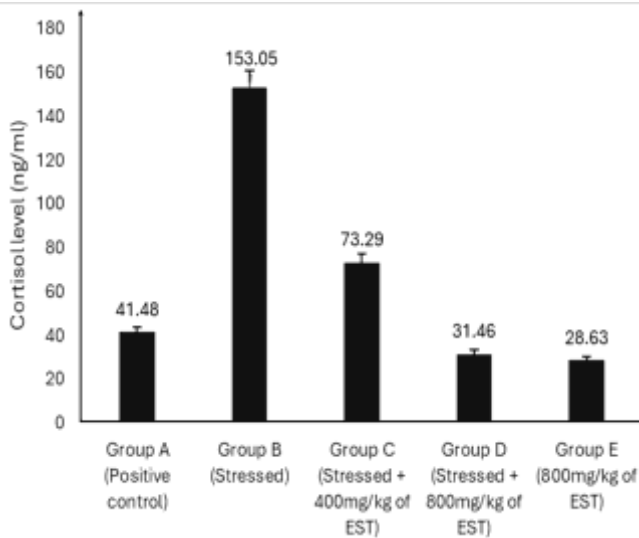


Figure 1: Comparison of cortisol level in different experimental groups.

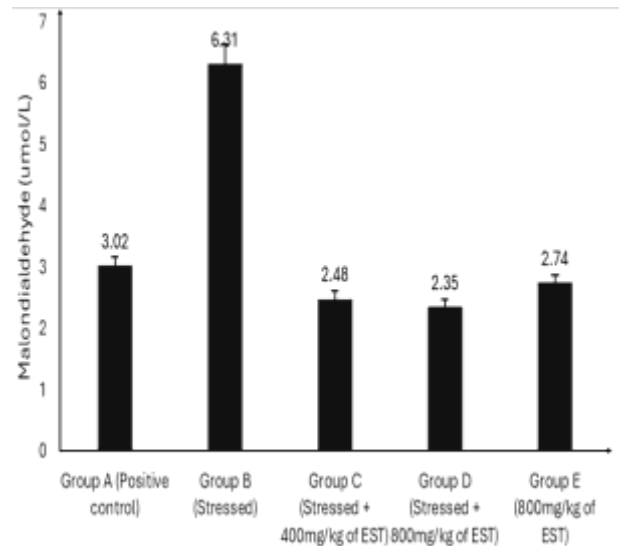


Figure 2: Comparison of Malondialdehyde level in different experimental groups.

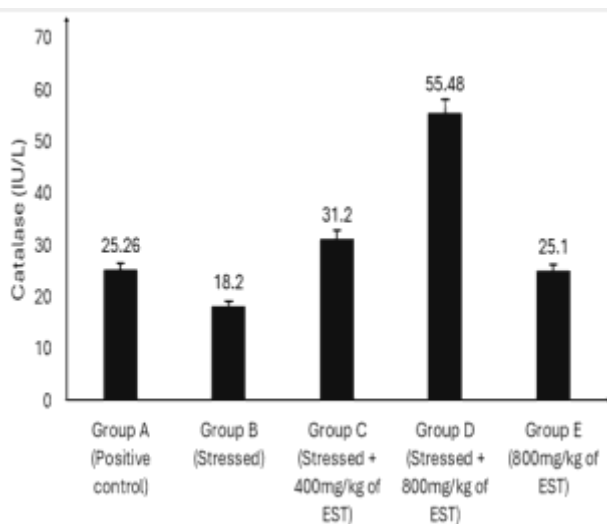


Figure 3: Comparison of catalase level in different experimental groups

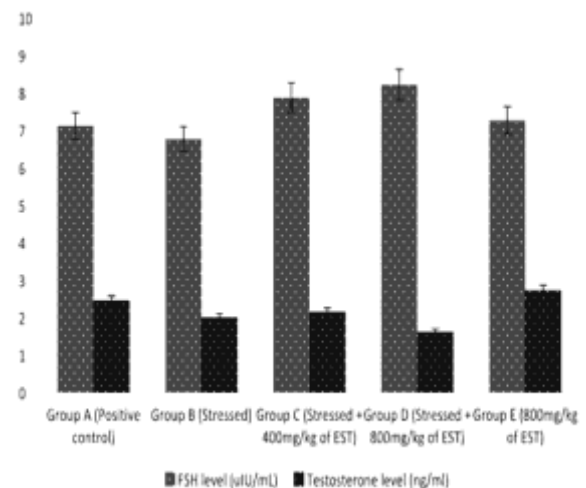


Figure 4: Effect of ethanolic extract of *S. aethiopicum* on FSH and testosterone level following exposure to stress. Group A= Control; Group B= Chronic mild stress; Group C= Chronic mild stress +*S. aethiopicum* (400 mg/kg); Group D= - Chronic mild stress.

Effect of *S.aethiopicum* leaf extract on the hormones

Figure 4 shows the result for the effect of *S. aethiopicum* leaf extract on FSH and testosterone levels following stress-induced toxicity. Group B had a decrease in the FSH and testosterone levels. Groups C and D had an increase in the

FSH level and a decrease in the testosterone level which was not statistically significant in Group E there was an increase in the level of testosterone that was not statistically significant.

Effect of *S. aethiopicum* Leaf Extract on the Histology of the Testes

The testicular cytoarchitecture of Wistar rat (Figure 5), revealed normal testicular architecture with no degeneration of the seminiferous tubules for the control group. The testes in Group B rats revealed degeneration of the seminiferous tubules and basement membrane, congestion of blood

vessels. Testes of rats in Group C revealed mild regeneration of seminiferous tubules, congestion of blood vessels. Testes of rats in Group D revealed mild regeneration of seminiferous tubules, congestion of blood vessels and loss of interstitial cells. Testes of rats in Group E showed normal testicular cytoarchitecture.

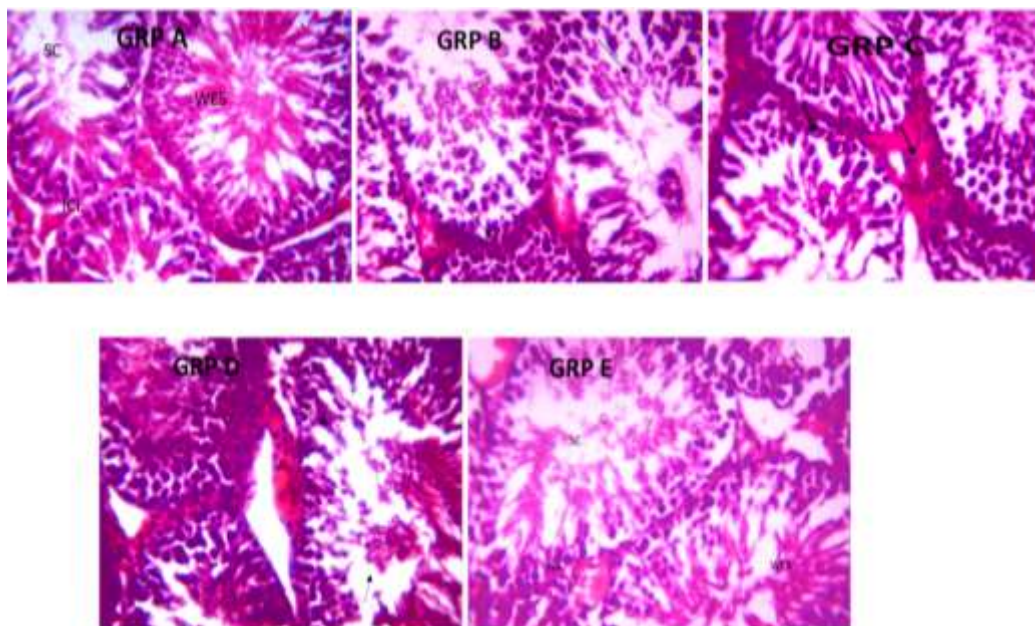


Figure 5: Photomicrograph of testis section from Group A= Control; Group B= Chronic mild stress; Group C= Chronic mild stress +*S. aethiopicum* (400 mg/kg); Group D= Chronic mild stress + *S. aethiopicum* (800 mg/kg); Group E- (unstressed group) *S. aethiopicum* (800 mg/kg). Shows reduced count in interstitial cells of the Leydig (ICL) in B compared to C and D, Sertoli cell (SC), well enhanced spermatogenesis (WES), congestion of blood vessels (↓). Mag X 400; H&E

Discussion

S. aethiopicum has a lot of essential nutrients such as carbohydrates, fats, proteins, fibers and vitamins (A, B1, B2, B6, B12, C, and D) that are beneficial to human health (Chinedu et al., 2011). Studies conducted scientifically have upheld the use of *S. aethiopicum* in the improvement of male reproductive parameters (Atukpa et al., 2023; Queen et al., 2021). In this study the decrease in sperm count observed in rats in group B is an indication that chronic mild stress hinders spermatogenesis. This is consistent with research which found that exposure to erratic, mild chronic stress caused spermatogenesis to stop at the G0/G1 phase of the cell cycle and increased the number of apoptotic spermatids (Zou et al., 2019). Male semen quality is distorted by psychological stress, according to another study (Fischer et al., 2023). Spermatogenesis has been documented to be suppressed by long-term, intermittent

stress and also reported to decrease the rat testis' Johnsen score (Meng et al., 2022).

In this study, treatment with 400 mg/kg and 800 mg/kg of *S. aethiopicum* increased the sperm count as seen in groups C, D, and E. Hence, this indicates that the extract demonstrated the ability to mitigate the damage to the sperm count caused by chronic mild stress. *S. aethiopicum* is a powerful antioxidant and radical scavenger known to ameliorate radicals generated by both natural and experimental stress, which in turn results in a notable increase in spermatogenic activity (Faraone et al., 2022). It has been stated that antioxidants occurring naturally are the secondary metabolites of phytochemicals (Krishnaiah et al., 2007). In this study the phytochemical analysis revealed the presence of various phytochemicals beneficial to reproductive parameters in males. A higher sperm count could suggest that *S. aethiopicum* affected the mitochondria, located in the spermatozoon's body responsible for the synthesis of ATP

(Adelakun et al., 2020). The standard of semen is largely dependent on sperm motility (Chakraborty & Saha, 2022). According to a number of studies, psychological stress increases the amount of ROS in seminal plasma. This causes the antioxidant defense mechanisms to decline, which ultimately lowers the quality of the sperm (Kowalczyk et al., 2022). By reducing sperm motility and fertility, oxidative stress can have an effect on the quality of semen (Kaltsas et al., 2023). Stress-related increases in ROS may be connected to the decline in sperm motility in group B after exposure to stress. The increase in the levels of cortisol and MDA as well as the decrease in catalase reported in this study, is a clear sign that oxidative stress occurred. It has been reported that major phases of spermiogenesis are particularly vulnerable to ROS induced damages (Nowicka-Bauer & Nixon, 2020). This has been stated to occur due to lack of DNA repair mechanisms and susceptible periods of sperm chromatin condensation (Agarwal et al., 2008). Increase in the levels of reactive oxygen species in this study led to oxidative stress, which as reported by Juan et al (2021) culminated in damage to lipids, proteins and DNA. The increase in sperm motility in group E agrees with the report from a previous study by Tunwagun et al. (2020). Administration of *S. aethiopicum* extract following stress exposure in Groups C and D, respectively, reversed the decline in sperm motility.

The reduction in sperm morphology observed in Group B is consistent with an earlier study, (Rahimipour et al., 2014) which found that exposure to an environment that increased ROS generation resulted in a decrease in the proportion of sperm with normal morphology. The treated groups had higher percentages of sperm with normal morphology, which was consistent with the findings of a previous study (Agarwal et al., 2008). The outcome suggests that *S. aethiopicum* showed the ability to diminish the damage that stress had on sperm parameters. Hence, the result may be due to the strong antioxidant and free radical scavenger claims of *S. aethiopicum* (Faraone et al., 2022). Flavonoids one of the antioxidants found in *S.aethiopicum* has the ability to suppress problems related to the male reproductive system (Mishra et al., 2024).

Stress-related factors are attributed to have led to lower FSH and testosterone levels in group B. Research has demonstrated that stress triggers the activation of the hypothalamo-pituitary adrenal axis, which subsequently suppresses the adrenal axis. The suppression results in a decrease in testosterone levels (Gandhi et al., 2017).

The decline in FSH levels may possibly be due to the suppression of hypothalamo-pituitary-adrenal axis, as

reported by an earlier study (Zabbey et al., 2021). *S. aethiopicum* administration is suggested to have stimulated the hypothalamic-pituitary axis, which ultimately led to an increase in the concentration of FSH. This increase can also be linked to the flavonoids and saponins reported in the phytochemical analysis of *S. aethiopicum* in this study. The reduction in the level of testosterone in Group D may be due to the short duration (14 days) of extract administration in this study. It was reported that there was an increase in the serum levels of testosterone and FSH after administration of *S. aethiopicum* for 30 days (Muthuselvi & Meena, 2019).

Histological analysis of the testes revealed that the testes of stress-exposed rats had alterations. The effect of prepubertal immobilization stress on the testis' histology showed a substantial decrease in the quantity of secondary spermatocytes, according to a previous study (Queen et al., 2021). Regeneration of testicular tissue treated with *S. aethiopicum* was observed following stress exposure. According to a previous study, (Sadraie et al., 2021) testicular histopathology in rats given *S. aethiopicum* for 30 days was normal. Spermatogonia cells were found to have well-defined interstitial niches. This result implies that *S. aethiopicum* has a protective effect on the testis.

It has been observed that flavonoids play a role in free radical scavenging and lesion prevention (Maalhigh et al., 2016). Flavonoids have anti-oxidant properties that are beneficial to health (Čižmárová et al., 2023). The flavonoids in *S. aethiopicum* may be responsible for the moderate regeneration effect seen in the treatment groups. A decline in FSH and testosterone levels in experimental group B after 14 days of induced stress alone was observed in this study. This suggests that stress inhibits male testosterone and FSH production (Whirledge & Cidlowski, 2010).

Conclusion

The benefits of *S. aethiopicum* on various sperm parameters was reflected in this study as shown in the hormonal and histological analysis. Results in this study affirm the ability of *S. aethiopicum* to mitigate the adverse effects of chronic stress on male reproductive parameters through its antioxidant properties.

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Ethical Clearance

Ethical approval was obtained from the Research Ethics Committee of Faculty of Basic Medical Sciences Chukwuemeka Odumegwu Ojukwu, University, Uli Campus (COOU/FBMS/012).

Conflict of Interest

The authors declare no conflict of interest.

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