Journal of Ethnopharmacology and Toxicology

Research Article

Dose-dependent protective effect of *Cucurbita pepo* seed extract on induced reproductive toxicity

***Anyanwu, C. F., Siminialayi, I.M., Kemebirinemughan, T.,**

Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, P.M.B. 5323, Nigeria.

***Correspondence**
Dr. Chinwe F. Anyanwu
Chinwe.anyanwu@uniport.edu.ng

Volume: 4, Issue: 1, Pages: 10-18
DOI: https://doi.org/10.37446/jet/rsa/4.1.2026.10-18
Received: 5 September 2025 / Accepted: 13 December 2025 / Published: 12 January 2026

In recent times, there has been growing interest in the search for natural products as possible remedies for mitigating reproductive toxicity caused by intake of medications. This study aims to investigate the effect of ethanol extract of *Cucurbita pepo (C. pepo)* seed on pregabalin-induced reproductive toxicity in male Wistar rats. Group A (normal control) = 0.5ml 5% Tween 80; B (negative control) = 75mg/kg pregabalin, group C= 500mg/kg of *C. pepo* extract; D = 1000mg/kg of *C. pepo* extract, group E = 75 mg/kg of pregabalin and 500mg/kg of *C. pepo* extract and group F = 75mg/kg of pregabalin and 1000mg/kg of *C. pepo* extract. There was a Significant ($p<0.05$) increase in testosterone, viable sperm cell, and decrease in abnormal sperm cell observed in test group C relative to controls. No significant variation in FSH and LH relative to both controls. A Significant ($p<0.01$) increase in dead sperm cells and significant ($p<0.01$) decrease in normal and viable sperm cells, in group F compared to controls. *Cucurbita pepo,* at lower doses may possess beneficial effects on male reproductive parameters. Higher doses in combination with pregabalin demonstrate potential reproductive toxicity.

Keywords: *Cucurbita pepo Seed, Testosterone, luteinizing hormone, Pregabalin, Reproductive Toxicity*

Introduction

According to the World Health Organization (WHO, 2019), reproductive health is a vital component of human welfare. It encompasses the physical, mental, and social well-being that is linked with a person's reproductive system during their entire life. Those who experience disruptions in their reproductive health may face substantial repercussions for themselves, their families, and society as a whole. According to (Krzastek et al., 2021; Mascarenhas et al., 2024), reproductive function can be affected by a number of factors, including environmental toxins, lifestyle choices, and pharmaceutical treatments. These factors can lead to infertility, difficulties during pregnancy, and other reproductive disorders. It is possible for pharmaceutical drugs, despite their intended purpose of treating a variety of medical conditions, to occasionally have unintended effects on the reproductive health of the patient (Carey et al., 2017). There have been concerns raised regarding the potential impact that pregabalin may have on male reproductive function (Hamed, 2018). Pregabalin is a medication that is licensed for the treatment of neuropathic pain, epilepsy, and generalized anxiety disorder. Despite the fact that the underlying mechanism by which it operates is well-known, the impact that it has on reproductive health is nevertheless constantly being researched. According to the findings of studies carried out on animals and observations made on humans, pregabalin has the potential to produce adverse effects on the reproductive system (de Fátima Mestre et al., 2023; Shokry et al., 2020). These adverse effects include alterations in the properties of sperm, hormonal irregularities, and damage to the testicles. In recent years, there has been a growing interest in the investigation of natural products as possible remedies for decreasing the negative effects that pharmaceuticals have on reproduction (Elshafie et al., 2023; Noh et al., 2020). Aqueous ethanol extract that is obtained from the seeds of *Cucurbita pepo*, which are more often known as pumpkin seeds, is an example of a natural product. The use of extract from the seeds of the

Cucurbita pepo plant in traditional medicine for the treatment of a variety of illnesses, including the purported benefits it offers for reproductive health, has been documented for a considerable amount of time (Hashemi & Dadkhah, 2014; Salehi et al., 2019; Mudau et al., 2022). The *Cucurbita pepo* plant, more often referred to as pumpkin, is a member of the *Cucurbita genus*, which is classified under the *Cucurbitaceae* family. The reproductive health benefits related to the extract that obtained from *Cucurbita pepo* seeds have been shown by a number of researches (Anyanwu et al., 2025; Anyanwu et al., 2024; Oh et al., 2024; Ivoh et al., 2023; Theil et al., 2022). An investigation that was carried out by Abarikwu et al. (2022) demonstrated that the administration of pumpkin seed oil as a supplement resulted in an increase in the levels of testosterone and sperm parameters in diabetic rats. The seed extract was also reported to be beneficial in reducing the severity of testicular injury induced by bisphenol-A, as reported by (Fawzy et al., 2018). It was reported that the antioxidant and anti-inflammatory properties of the extract were responsible for this improvement.

Materials and Methods

Plant parts and authentication

Cucurbita pepo (Pumpkin) was obtained from Ogbete Lane, Artisan Market, Enugu. The University of Port Harcourt Department of Pharmacology received it in a plastic bag. The plant species was verified using University Herbarium voucher specimens (UPH/PSB/2021/071).

Sample preparation (extraction):

The Cucurbita pepo was dried to remove moist, deshelled and grinded using electric grinder. The extraction process was done according to the method of Harborne (1998). Aqueous ethanol extract of Cucurbita pepo seed was obtained by exhaustive extraction for a period of 72 hours with fresh replacement of solvent after 24 hours. 2kg of the grinded C. pepo seed was macerated in 80% ethanol for 72 hours, at room temperature in macerating glass jar and filtered after 24 hours, with the solvent being replaced every 24 hours. At the end of three days, the mixture was poured through double layered muslin cloth to get rid of the debris and the fluid portion, filtered through Whatman No. 1 filter paper. The collected filtrate was then concentrated at 45°C with a rotary evaporator and later transferred to an evaporating dish and dried over a water bath. The dried extract was stored in a desiccator.

Animals

Male Wistar rats, aged 8-10 weeks, weighing between 180-220 grams, were obtained from the Animal House of the Department of Pharmacology, University of Port Harcourt. Before the commencement of the experiment, the animals were acclimatized to the laboratory environment for two weeks to minimize stress and ensure stable physiological conditions. They were housed in clean plastic cages lined with wood shavings, with a 12-hour light/dark cycle and constant room temperature (22 ± 2°C). The rats had unrestricted access to standard commercial rodent feed and clean drinking water throughout the duration of the study.

Drugs

The Pregabalin tablets 75mg were supplied by Alpha Pharmacy, Ogbunabali, Port Harcourt, Rivers State, Nigeria. (Manufacturer: Celon Laboratories PVT, Ltd).

Experimental design and protocol

Anyanwu et al., 2025) reported a 5000 mg/kg fatal dosage (LD50) for Cucurbita pepo seed extract acute toxicity. This result led us to choose 20% (1000 mg/kg) as the maximum dose and 10% (500 mg/kg) as the medium dose for our study.

In particular, these doses caused no mortality, morbidity, or adverse effects.

Group 1: (Normal Control) Tween 80
Group 2 (negative control): 75mg/kg pregabalin tablets
Group 3: 500mg/kg *C. pepo* seed extract
Group 4: 1000mg/kg *C. pepo* seed extract.
Group 5: 75mg/kg pregabalin and 500mg/kg *C. pepo* seed extract.
Group 6: 75mg/kg pregabalin and 1000mg/kg *C. pepo* seed extract.

Sacrifice, sample collection

After treatment, the animals were anaesthetized and blood was drawn from the jugular vein in simple bottles. After 10 minutes of centrifugation at 4000 RPM, serum was collected and kept in Cryovials. The testis was then excised and kept in 10% formalin for histological evaluation using Rieger et al. (2020) 's technique with Hematoxylin and Eosin.

Tissue processing and preparation

After fixation, the testicular tissues were dried with ethanol and then embedded in paraffin wax to make it easier to get cells out. After being soaked in paraffin and embedded, sections that were 5 µm thick were made and stained with haematoxylin and eosin (H&E) dyes. We put the treated tissues on glass slides, put a coverslip on top, and looked at them using a light microscope (Olympus CX23, Olympus Corporation, Japan) at a magnification of x400. An Olympus CX23 digital camera took the photomicrographs.

Spermatozoa characteristics analyses

An examination of sperm characteristics was performed on the spermatozoa obtained from the caudal epididymis. The caudal epididymis was cut up, mashed, and put onto a clean glass slide. Two drops of normal saline were added to make a suspension. The suspension was made less strong by adding formal saline at a 1:20 ratio. A drop of diluted sperm was put into the new and improved Neubauer counting chamber (Hemocytometer). The counting chamber was placed on a light microscope slide stage and magnified to ×40 so that we could view it. The count was taken in million/ml suspension (Cheesbrough, 2006). To check sperm motility, 10 ul of sperm suspension was put on a slide and looked at under a microscope at a magnification of 400. Approximately 100 sperm cells were analysed and categorised as either active, sluggish, or dead, with results presented as a percentage.

The sperm cell count

We looked at the spermatozoa we got from the caudal epididymis to see what their features were. The caudal epididymis was cut open, torn apart, and put onto a clean glass slide. To make a suspension, two drops of normal saline were added. We mixed formal saline with the suspension at a 1:20 ratio. An updated Neuberger counting chamber (hemocytometer) was filled with a drop of diluted sperm. To look at the counting chamber, it was mounted on a light microscope slide stage and blown up to ×40. The count was taken in million/ml suspension (Cheesbrough, 2006).

Sperm cell motility

This was comparable to the findings published by Kumar et al. (2017). Sperm motility was assessed by depositing 10 µl of sperm suspension on a slide for microscopic examination at a magnification of ×400. We looked at about 100 sperm cells and put them into three groups: active, sluggish, or dead.

Sperm cell morphology

The eosin and nigrosine stain was used to find out the shape of the sperm. Briefly, 10 ul of eosin and nigrosine were mixed with 40 ul of sperm suspension. The sperm suspension was kept at 40°C for 5 minutes before being resuspended with a micro-pipette. Approximately 100 sperm cells per rat were subjected to morphological examination under ×400 magnification microscopy. Morphological abnormalities were categorized as normal, abnormal, and viable. After that, the testicular tissues were taken out and stored in 10% formalin for histological analysis using the method described by Bilinska et al. (2018) and stained with hematoxylin and eosin.

Sperm cell viability

To make a suspension, one drop of sperm from the caudal epididymis was put on a clean glass slide, and two drops of normal saline were added. One drop of 0.5% eosin solution was added to the suspension. A light microscope with a ×40 magnification was utilized to look at the slide a few seconds later. Eosin-stained non-viable sperm exhibited differences from viable sperm.

Testosterone assay

We used Accu-bind ELISA Microwells (Testosterone Test System Product Code: 3725-300) from Monobind, USA, to collect blood samples and measure testosterone levels.

Histological examination of the tissues

The testicular samples were subjected to histological processing according to the methodology outlined by Anyanwu et al. (2024). After fixation, the tissues were dried with ethanol and then embedded in paraffin wax to make it easier to get cells out of them. After the paraffin infiltration and embedding, sections 5 µm thick were made and stained with haematoxylin and eosin (H&E) dyes. The treated tissues were placed on glass slides, covered with a cover slip, and looked at under a light microscope (Olympus CX23, Olympus Corporation, Japan) at a magnification of x 400. An Olympus CX23 digital camera was used to take photomicrographs.

Statistical analyses

The data collected during the study will be represented as mean ± SEM. The statistical level of significance will be determined by one-way analysis of variance (ANOVA) and the Turkey post Hoc test. The significance level was set at $P<0.05$. Table 1 and 2 respectively shows the effect of C. pepo seed extract on reproductive hormones and sperm characteristics in male Wistar rats intoxicated with pregabalin. There is a significant ($p<0.01$) increase in serum concentration levels of testosterone in rats treated with 500mg of C. pepo seed extract relative to both controls, as shown in table 1. However, there is no significant variation in serum concentration levels of FSH and LH relative to both controls. Table 2 shows that there is a significant decrease in abnormal sperms as well as sperm viability in treatment group C, relative to the negative control. In contrast, rats treated with combination of 75mg/kg of pregabalin as well as 1000mg/kg of C. pepo seed extract (Group F treated rats) were shown to have significantly elevated active and dead sperm cells as well as a significantly decreased number of normal sperm cells when compared to both controls (as shown in table 2). However, there is no significant variation in these parameters in all other treatment groups relative to both controls. Figure 1 shows the Photomicrographs of testicular sections of 4 rats each from groups A (Normal Control), B (Negative Control), C (500mg/kg of *C. pepo* seed extract), D (1000mg/kg of *C. pepo* seed extract) E and F (75mg/kg Pregabalin plus 500 mg/kg *C. pepo* seed extract and 75 mg/kg pregabalin plus 1000mg/kg C. pepo seed extract respectively) treated for 60 days. Findings show that there is no obvious change in the histoarchitecture of the testicular sections of rats treated with C. pepo seed extract when compared with the negative control.

Table 1. Effect of *Cucurbita pepo* seed extract on male Reproductive Hormones following ingestion of Pregabalin for 60 days

GROUPS	TET	FSH	LH
A	5.79±0.26	1.48±0.31	0.64±0.13
B	3.30±0.08	1.00±0.32	0.58±0.10
C	9.95±0.40** ββ	1.61±0.12	0.69±0.05
D	5.09±1.06	1.72±0.50	0.74±0.21
E	6.06±065	1.67±0.28	0.72±0.12
F	5.99±0.89	0.74±0.10	0.33±0.04

Values are given as mean ± SEM for each group. Experimental groups are compared with group A (Normal Control) and group B (Negative Control). *p<0.05, **p<0.01 was considered as significant versus the Normal control (Group A); ᵝp < 0.05, ᵝᵝp<0.01 was considered significant versus the Negative Control (Group B). Statistical evaluation was done by one-way ANOVA, followed by Tukey's post-hoc test.

Key: A= Normal control (Tween 80), B= Negative control (75mg/kg pregabalin), C = 500 mg/kg of *C. pepo* seed extract, D = 1000 mg/kg of *C. pepo* seed extract, E = 500 mg/kg of *C. pepo* seed extract plus 75mg/kg pregabalin, F = 1000 mg/kg of *C. pepo* seed extract plus75mg/kg pregabalin

Table 2. Effect of *Curcurbita pepo* seed extract on Sperm Cell Characteristics following ingestion of Pregabalin for 60 days

Groups	Spermatozoa Morphology (%)		Sperm Cell Parameters (%)				Sperm Cell Count ($X10^6$)
	Normal	Abnormal	Active	Sluggish	Dead	Viability	
A	84.00±3.67	16.00±3.67 ββ	82.00±3.39	7.00±1.23	11.00±2.45	83.00±2.21ᵝ	530.00±86.02
B	81.00±4.00	38.00±3.39**	78.00±3.39	8.00±1.23	14.00±2.45	65.00±3.54*	360.00±50.99
C	84.00±2.45	12.00±2.00 ββ	81.00±1.87	8.00±1.23	11.00±1.00	86.00±2.92ββ	620.00±51.48
D	72.00±3.39	28.00±3.39	68.00±4.06	10.00±0.00	22.00±4.06	76.00±2.92	460.00±81.24

E	72.00±5.61	28.00±5.61	66.00±7.31	9.00±1.00	25.00±6.52	81.00±4.00	580.00±64.42
F	62.00±3.39**$^{\beta}$	19.00±4.00	54.00±4.30** $^{\beta\beta}$	10.00±0.00	37.00±3.39** $^{\beta\beta}$	77.00±4.90	440.00±81.24

Values are given as mean ± SEM for each group. Experimental groups are compared with group A (Normal Control) and group B (Negative Control). *p<0.05, **p<0.01 was considered as significant versus the Normal control (Group A); $^{\beta}$p < 0.05, $^{\beta\beta}$p<0.01 was considered significant versus the Negative Control (Group B). Statistical evaluation was done by one-way ANOVA, followed by Tukey's post-hoc test.

Key: A= Normal control (Tween 80), B= Negative control (75mg/kg pregabalin), C = 500 mg/kg of *C. pepo* seed extract, D = 1000 mg/kg of *C. pepo* seed extract, E = 500 mg/kg of *C. pepo* seed extract plus 75mg/kg pregabalin, F = 1000 mg/kg of *C. pepo* seed extract plus75mg/kg pregabalin.

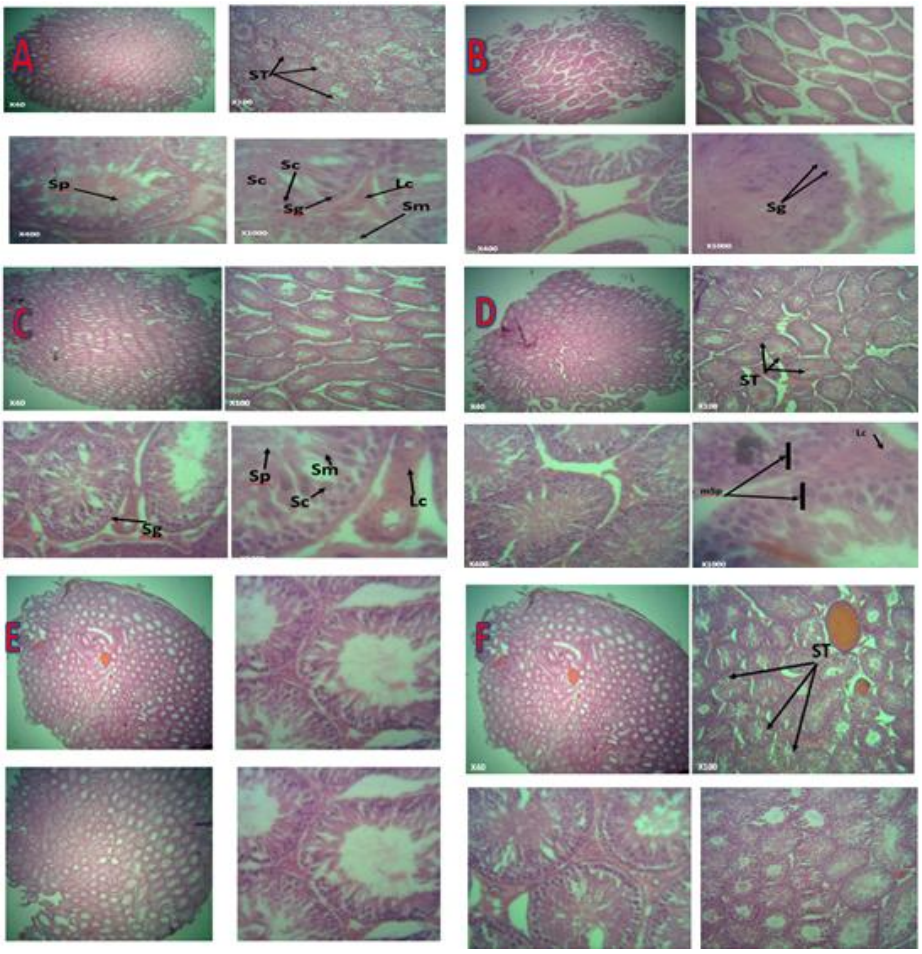

Figure 1. Photomicrographs of testicular sections of 4 rats each from groups A (Normal Control), B (Negative Control), C (500mg/kg of *C. pepo* seed extract), D (1000mg/kg of *C. pepo* seed extract) E and F (75mg/kg Pregabalin plus 500 mg/kg *C. pepo* seed extract and 75 mg/kg pregabalin plus 1000mg/kg *C. pepo* seed extract respectively) treated for 60 days; stained with H&E (×400). Testis shows normal seminiferous tubules (ST) with spermatogonia (Sg), spermatocytes (Sm), spermatids (Sp), and sustentacular cells (Sc), in the testis of rats treated with 500 and 1000mg/kg of *C. pepo* seed extract alone. No obvious change in the histoarchitecture of the testicular sections of rats treated with *C. pepo* seed extract when compared with the negative control.

DISCUSSION

Cucurbita Pepo seed (pumpkin) has been reported to be rich in antioxidants as well as phenolics and carotenoids, which may boost overall health and well-being (Mohammadi et al., 2023; Oyetayo et al., 2020). Substances such as caffeic acid, apigenin, ferulic acid, luteolin, kaempferol, quercetin, protocatechuic acid, vanillic acid, and sinapinic acid are found in pumpkin seeds, and some have been associated with reproductive enhancement (Akomolafe, 2021; Ivoh et al., 2023; Anyanwu et al., 2024). Our study therefore, assessed the reproductive effect of ethanol extract of *Cucurbita pepo* seed in pregabalin- ingested rats. Results of our study show that group C (500 mg/kg of *C. pepo*) treated rats had significantly

elevated serum concentration levels of testosterone relative to both controls. This finding is in tandem with results from Akomolafe et al. (2025), who reported similar testosterone-enhancing effects in rats treated with roasted *C. pepo* seeds. However, our observation of no significant changes in serum concentration levels of FSH and LH levels in all treatment groups contrasts with the findings of (Akomolafe et al., 2025; Anyanwu et al., 2024; Ivoh et al., 2023), who noted substantial variations in these hormones with similar treatments. These discrepancies may be as a result of differences in experimental design, dosage, or specific extracts used. Our study's findings may possibly indicate that while *C. pepo* extracts boost testosterone effectively, it may not disrupt the hypothalamic-pituitary-gonadal axis. However, from our results, there was no significant variation in serum concentration levels of testosterone in other treatment groups relative to both controls. This plausible demonstrable negative impact in the treatment group at the highest dose of *C. pepo* seed as well as the combination groups (Groups D, E and F) highlights the importance of using herbal products at appropriate doses thus, aligning with findings of Wei et al. (2022), who emphasized the possibility of hormonal imbalances resulting from intake of herbal supplements either excessively, or at large doses. The insignificant variation in serum levels of testosterone and other reproductive hormones in Group F may suggest possible interactions between pregabalin and high doses of *C. pepo* seed extract. Pregabalin has been reported to decrease testosterone as well as elevate LH and FSH via disruption of calcium channel-mediated gonadotropic regulation (Salem Hareedy et al., 2020). This interplay may likely be as result of modulation of 5α-reductase or androgen receptors, alongside influences on LH/FSH release that regulate testosterone production, thereby buffering pregabalin's suppressive effects on Leydig cell steroidogenesis. These findings, consistent with Wei et al. (2022) on hormonal imbalances from excessive herbal intake, emphasize the need for further research on receptor-level pathways, optimal dosing, and hypothalamic-pituitary-gonadotropin dynamics to ensure safe combinational therapies and prevent unintended endocrine disruptions. Our study also demonstrated improvement in sperm cell characteristics such as increased sperm viability and reduction in abnormal sperm cells in group C treated rats relative to the control. This finding may not be unconnected with reports by (Aghaei et al., 2014; Akomolafe et al., 2025; Elakhdar et al., 2020) of the presence of bioactive compounds in *C. pepo* seed extracts which improve sperm health via its antioxidant properties, thus reducing oxidative stress. In contrast, this improvement was not observed in rats treated with maximum dose of *C. pepo* seed extract (1000 mg/kg) and thus may have been as a result of detrimental effects resulting from excessive or combined dosages of supplements which tend to induce oxidative stress as well as cellular damage (Mehda et al., 2025; Odigie et al., 2025; Tao et al., 2020). Overall, the plausible beneficial effects of low dose (500mg/kg) *C. pepo* seed extract on the reproductive hormones and sperm cell characteristics in treated male Wistar rats as observed in our study aligns with research studies highlighting the possible use of herbal supplements in the management of reproductive health problems (Anyanwu et., 2025; Ivoh et al., 2023; Malan & Neuba, 2011). However, the insignificant variations in the serum concentration levels of FSH and LH observed in our research does not align with some scientific literature demonstrating significant spikes in hormonal levels associated with herbal supplements (Almohaimeed et al., 2021; Anyanwu et al., 2025; Anyanwu et al., 2024; Lestari & Meiyanto, 2018; Matus et al., 1993; Ofoego et al., 2017). These differences may be as a result of discrepancies arising from experimental design, dosing thus emphasizing the deer need for standardizing herbal formulations in future experiments.

CONCLUSION

In conclusion, from our study, it can be deduced that *Cucurbita pepo* seed extract, especially at lower doses (500 mg/kg), may possess beneficial effects on male reproductive parameters. However, higher doses (1000 mg/kg) in combination with pregabalin demonstrate potential reproductive toxicity. Further research is essential to evaluate the underlying mechanisms of these effects and to assess the extract's potential for clinical applications in safeguarding male reproductive health.

Acknowledgments

None.

Author contributions

A.C.F: Conceptualization, supervision, Data analysis and manuscript draft; S.I.M: supervision, experimentation, and manuscript editing; K.T: Experimentation, project management, data collection, and visualization. All authors read and approved the final manuscript for submission.

Funding

No funding.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

Ethical approval for this study was obtained from the University of Port Harcourt Research Ethics Committee (Reference No.: UPH/CEREMAD/REC/MM103/023). All procedures were conducted in accordance with established ethical guidelines.

AI tool declaration

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

References

Abarikwu, S. O., Oleribe, A. L., Mgbudom-Okah, C. J., Onuah, C. L., Chikwendu, C. S., & Onyeike, E. N. (2022). The protective effect of fluted pumpkin seeds against atrazine-induced testicular injury. *Drug and Chemical Toxicology, 45*(2), 799–809.

Aghaei, S., Nikzad, H., Taghizadeh, M., Tameh, A. A., Taherian, A., & Moravveji, A. (2014). Protective effect of pumpkin seed extract on sperm characteristics, biochemical parameters and epididymal histology in adult male rats treated with cyclophosphamide. *Andrologia, 46*(8), 927–935.

Akomolafe, S. F. (2021). Effects of roasting on the phenolic phytochemicals and antioxidant activities of pumpkin seed. *Vegetos, 34*(3), 505–514.

Akomolafe, S. F., Ogundare, M. A. B., Akinlua, I., Ajayi, O. O., Odesanmi, E. O., & Adewale, O. B. (2025). Comparative effect of dietary fortification of raw and roasted seeds of pumpkin (*Cucurbita pepo* L.) on fertility biomolecules in cisplatin-induced spermatotoxic rats. *Journal of Trace Elements and Minerals, 12*, 100236. https://doi.org/10.1016/j.jtemin.2025.100236.

Almohaimeed, H. M., Hamed, S., Seleem, H. S., Batawi, A. H., Mohammedsaleh, Z. M., Balgoon, M. J., … Ayuob, N. (2021). An ethanolic extract of *Cucurbita pepo* L. seeds modifies neuroendocrine disruption in chronically stressed rats and adrenal expression of inflammatory markers and HSP70. *Frontiers in Pharmacology, 12*, 749766. https://doi.org/10.3389/fphar.2021.749766.

Anyanwu, C. F., Georgewill, O. A., & Georgewill, U. O. (2024). Effect of *Cucurbita pepo* (Pumpkin) seed extracts on the cyclicity and reproductive hormones of female Wistar rats. *Journal of Herbmed Pharmacology, 13*(4), 620-629.

Anyanwu, C. F., Georgewill, O. A., & Obinna, V. C. (2025). Reproductive outcome in female Wistar rats following treatment with *Cucurbita pepo* (pumpkin) seed extracts. *British Journal of Healthcare and Medical Research, 12*(02), 01–12.

Anyanwu, C. F., Georgewill, U. O., Onuama, V. I., & Phil-Ashiri, N. U. (2024). Potential reproductive toxicity of Lacatomtom drink in male Wistar rats. *Tropical Journal of Natural Product Research, 8*(6).

Bilinska, B., Hejmej, A., & Kotula-Balak, M. (2018). Preparation of testicular samples for histology and immunohistochemistry. In *Sertoli cells: Methods and protocols* (pp. 17–36). Springer.

Carey, J. L., Nader, N., Chai, P. R., Carreiro, S., Griswold, M. K., & Boyle, K. L. (2017). Drugs and medical devices: Adverse events and the impact on women's health. *Clinical Therapeutics, 39*(1), 10–22.

Cheesbrough, M. (2006). *District laboratory practice in tropical countries* (Part 2, 2nd ed.). Cambridge University Press.

de Fátima Mestre, V., Martins, C. C. N., de Brito, L. V., Zeffa, A. C., Sestário, C. S., & Salles, M. J. S. (2023). Pregabalin alters reproductive performance in male mice and causes congenital anomalies in offspring. *Reproduction, Fertility and Development, 35*(18), 750–759.

ElAkhdar, N., Bawab, R., & Borjac, J. (2020). Effect of *Ceratonia siliqua* and *Cucurbita pepo* seed extracts on spermatogenesis in male mice. *BAU Journal – Health and Wellbeing, 3*(1), 7.

Elshafie, H. S., Camele, I., & Mohamed, A. A. (2023). A comprehensive review on the biological, agricultural and pharmaceutical properties of plant-based secondary metabolites. *International Journal of Molecular Sciences, 24*(4), 3266.

Fawzy, E. I., El Makawy, A. I., El-Bamby, M. M., & Elhamalawy, H. O. (2018). Improved effect of pumpkin seed oil against bisphenol-A adverse effects in male mice. *Toxicology Reports, 5*, 857–863.

Hamed, S. A. (2018). Sexual dysfunctions induced by pregabalin. *Clinical Neuropharmacology, 41*(4), 116–122. https://doi.org/10.1097/WNF.0000000000000286.

Harborne, A. J. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis*. Springer Science & Business Media.

Hashemi, B., & Dadkhah, T. (2014). Properties of *Cucurbita pepo* L. in Islamic Persian medicine. *Health, Spirituality and Medical Ethics, 1*(3), 24–28.

Ivoh, G., Anyanwu, C. F., & Obinna, V. C. (2023). Oestrogenic activity of aqueous ethanol extract of Cucurbita pepo (pumpkin) seed in female Wistar rats. *Asian J. Res. Rep. Endocrinol*, *6*(1), 170-178.

Krzastek, S. C., Farhi, J., Gray, M., & Smith, R. P. (2021). Impact of environmental toxin exposure on male fertility potential. *Translational Andrology and Urology, 9*(6), 2797–2813. https://doi.org/10.21037/tau-20-685.

Kumar, P., Srivastava, N., Pande, M., Prasad, J. K., & Sirohi, A. S. (2017). Evaluating sperm cell viability and membrane integrity. In Protocols in semen biology (comparing assays) (pp. 57–71). Springer.

Lestari, B., & Meiyanto, E. (2018). The emerging nutraceutical potential of pumpkin seeds: A review. *Indonesian Journal of Cancer Chemoprevention, 9*(2), 92–101. https://doi.org/10.14499/indonesianjcanchemoprev9iss2pp92-101.

Malan, D. F., & Neuba, D. F. (2011). Traditional practices and medicinal plants use during pregnancy by Anyi-Ndenye women (Eastern Côte d'Ivoire). African journal of reproductive health, 15(1), 85-93.

Mascarenhas, C., Sousa, A. C., & Rato, L. (2024). Effects of pharmaceutical substances with obesogenic activity on male reproductive health. *International Journal of Molecular Sciences, 25*(4), 2324. https://doi.org/10.3390/ijms25042324.

Matus, Z., Molnár, P., & Szabó, L. G. (1993). Main carotenoids in pressed seeds (*Cucurbitae semen*) of oil pumpkin (*Cucurbita pepo* convar. *pepo* var. *styriaca*). *Acta Pharmaceutica Hungarica, 63*(5), 247–256.

Mehda, S., Laib, I., Diab, F., Attia, R., Benaissa, Y., Bellabidi, M., … Croun, D. (2025). Protective effects of *Calligonum comosum* against pregabalin-induced toxicity. *Food Science & Nutrition, 13*(7), e70681.

Mohammadi, N., Shirian, S., Gorji, A., Roshanpajouh, M., Ahmadi, E., Nazari, H., & Arezoomandan, R. (2023). The potential protective effect of melatonin and N-acetylcysteine alone and in combination on opioid-induced testicular dysfunction and degeneration in rat. Reproductive Toxicology, 120, 108453.

Mudau, F. N., Chimonyo, V. G. P., Modi, A. T., & Mabhaudhi, T. (2022). Neglected and underutilised crops: a systematic review of their potential as food and herbal medicinal crops in South Africa. Frontiers in Pharmacology, 12, 809866.

Noh, S., Go, A., Kim, D. B., Park, M., Jeon, H. W., & Kim, B. (2020). Role of antioxidant natural products in management of infertility: a review of their medicinal potential. Antioxidants, 9(10), 957.

Odigie, J. O., Anyanwu, C. F., & Ameja, C. N. (2025). Protective Effect of Ethanol Extract of Cucurbita pepo (Pumpkin) Seed on Pregabaline Induced Hepatorenal Toxicity. Journal of Complementary and Alternative Medical Research, 26(8), 35-43.

Ofoego, U. C., Nwachukwu, O., Ekwujuru, E. U., Mbagwu, S., Nwakanma, A., Nweke, E. O., & Ofoego, A. N. (2017). Ethanolic Seed Extract of Cucurbita Pepo (Pumpkin) Protects the Testis from Azadirachta Indica (Neem) Induced Damage. Journal of Natural Sciences Research, 7(24), 120-131.

Oh, J., Hong, S., Ko, S. H., & Kim, H. S. (2024). Evaluation of antioxidant effects of pumpkin (*Cucurbita pepo* L.) seed extract on aging-and menopause-related diseases using Saos-2 cells and ovariectomized rats. *Antioxidants*, 13(2), 241.

Oyetayo, F. L., Akomolafe, S. F., & Osesanmi, T. J. (2020). Effect of dietary inclusion of pumpkin (*Cucurbita pepo* L.) seed on cisplatin-induced nephrotoxicity in rats. *Journal of Food Biochemistry, 44*(10), e13439.

Oyetayo, F. L., Akomolafe, S. F., & Osesanmi, T. J. (2020). Effect of dietary inclusion of pumpkin (*Cucurbita pepo* L.) seed on nephrotoxicity occasioned by cisplatin in experimental rats. *Journal of Food Biochemistry*, 44(10), e13439.

Rieger, J., Pelckmann, L.-M., & Drewes, B. (2020). Preservation and processing of intestinal tissue for the assessment of histopathology. In *Animal models of allergic disease: Methods and protocols* (pp. 267–280). Springer.

Salehi, B., Capanoglu, E., Adrar, N., Catalkaya, G., Shaheen, S., Jaffer, M., ... & Capasso, R. (2019). Cucurbits plants: A key emphasis to its pharmacological potential. *Molecules*, 24(10), 1854.

Salem Hareedy, M., Mohamed Tawfik, K., Badary, D. M., Ahmed Mahmoud, W., & Mohamed Mohamed, E. (2020). Pregabalin administration and withdrawal affect testicular structure and functions in rats. *Andrologia*, 52(11), e13808.

Shokry, D. A., El Nabrawy, N., Yassa, H. D., Gaber, S. S., Batiha, G. E. S., & Welson, N. N. (2020). Pregabalin induced reproductive toxicity and body weight changes by affecting caspase3 and leptin expression: protective role of wheat germ oil. *Life Sciences, 260*, 118344.

Tao, G., Dagher, F., Moballegh, A., & Ghose, R. (2020). Role of oxidative stress in the efficacy and toxicity of herbal supplements. *Current Opinion in Toxicology, 20*, 36-40.

Theil, G., Richter, M., Schulze, M., Köttig, T., Patz, B., Heim, S., … Fornara, P. (2022). Extract from *Cucurbita pepo* improves BPH symptoms without affecting sexual function. *World Journal of Urology, 40*(7), 1769–1775.

Wei, L. S., Goh, K. W., Abdul Hamid, N. K., Abdul Kari, Z., Wee, W., & Van Doan, H. (2022). Co-supplementation of probiotics and medicinal herbs in aquaculture: A mini-review. *Frontiers in Veterinary Science, 9*, 869564.

World Health Organization. (2019). *WHO consolidated guideline on self-care interventions for health: sexual and reproductive health and rights*. World Health Organization.