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

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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* seed on pregabalin-induced reproductive toxicity in male Wistar rats. Group A (normal control) = 0.5ml and 5% Tween 80; B (negative control) = 75mg/kg pregabalin, group C= 500 mg/kg of *C. pepo* extract; D = 1000 mg/kg of *C. pepo* extract, group E = 75 mg/kg of pregabalin and 500 mg/kg of *C. pepo* extract and group F = 75 mg/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 A: (Normal Control) Tween 80
Group B: (negative control): 75 mg/kg pregabalin tablets
Group C: 500 mg/kg *C. pepo* seed extract
Group D: 1000 mg/kg *C. pepo* seed extract
Group E: 75 mg/kg pregabalin and 500 mg/kg *C. pepo* seed extract
Group F: 75 mg/kg pregabalin and 1000 mg/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 X40 so that we could view it. The count was taken in million/ml suspension (Cheesbrough, 2006). To check sperm motility, 10 µl 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 X40. 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 X400. 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 µl of eosin and nigrosine were mixed with 40 µl 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 X400 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 X40 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 X400. 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 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 (500 mg/kg of *C. pepo* seed extract), D (1000 mg/kg of *C. pepo* seed extract) E and F (75 mg/kg Pregabalin plus 500 mg/kg *C. pepo* seed extract and 75 mg/kg pregabalin plus 1000 mg/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** $^{\beta\beta}$	1.61±0.12	0.69±0.05
D	5.09±1.06	1.72±0.50	0.74±0.21
E	6.06±0.65	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); $^{\beta}p < 0.05$, $^{\beta\beta}p<0.01$ 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 75 mg/kg pregabalin, F = 1000 mg/kg of *C. pepo* seed extract plus75 mg/kg pregabalin

Table 2. Effect of *Cucurbita pepo* seed extract on sperm cell characteristics following ingestion of Pregabalin for 60 days

Group	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 $^{\beta\beta}$	82.00±3.39	7.00±1.23	11.00±2.45	83.00±2.21$^{\beta}$	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 $^{\beta\beta}$	81.00±1.87	8.00±1.23	11.00±1.00	86.00±2.92$^{\beta\beta}$	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 (75 mg/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 75 mg/kg pregabalin, F = 1000 mg/kg of *C. pepo* seed extract plus75 mg/kg pregabalin.

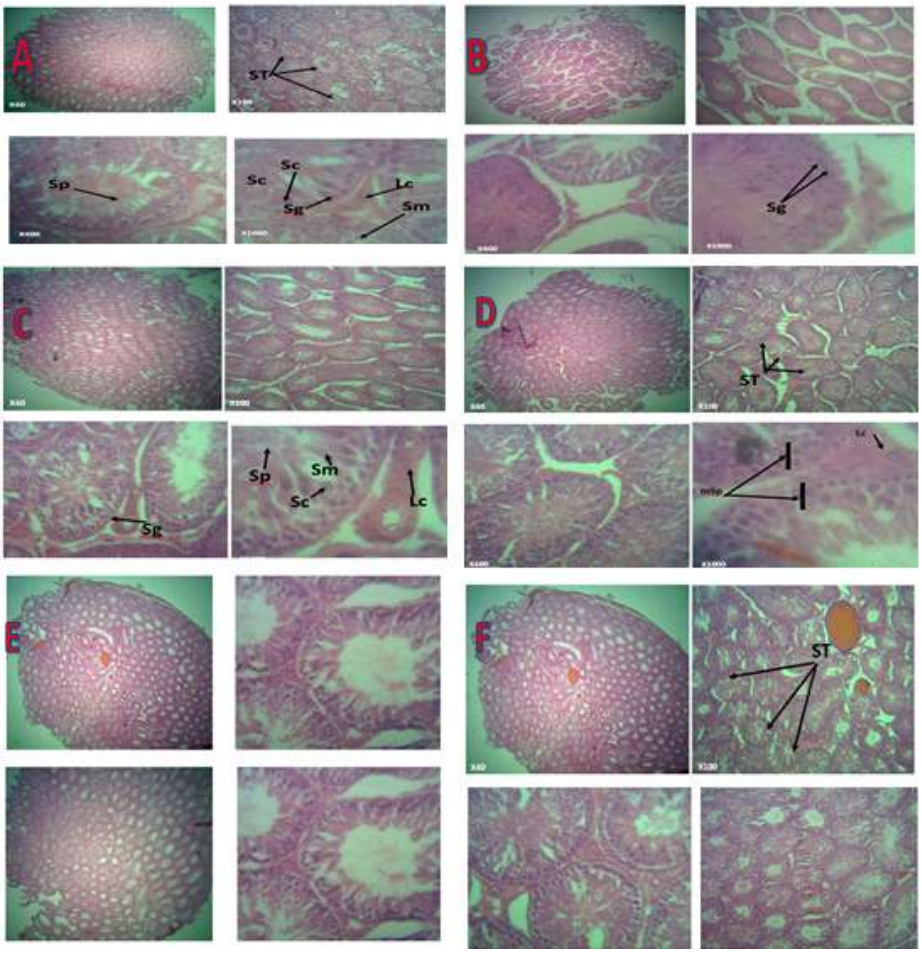


Figure 1. Photomicrographs of testicular sections of 4 rats each from groups A (Normal Control), B (Negative Control), C (500 mg/kg of *C. pepo* seed extract), D (1000 mg/kg of *C. pepo* seed extract) E and F (75 mg/kg Pregabalin plus 500 mg/kg *C. pepo* seed extract and 75 mg/kg pregabalin plus 1000 mg/kg *C. pepo* seed extract respectively) treated for 60 days; stained with H&E (X400).

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 1000 mg/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 al., 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 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.

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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.

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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.

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