JBAAR
Journal of Basic and Applied Research in Biomedicine
ISSN 2413-7014

Original Article

Pumpkin (*Cucurbita pepo*) Seed Oil Supports Healthy Gestation in Wistar Rats

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Received: 06-01-2026
Revised: 11-04-2026
Published: 15-04-2026

FUNDING
The study was not funded by external sources aside the author.

CONFLICT OF INTEREST
The author declares no conflict of interest in this study

DATA AVAILABILITY
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abstract:
Background: Appropriate maternal nutrition is essential during gestation to ensure the optimal health and well-being of both the mother and the developing foetus. Since pumpkins are regarded as one of the best sources of essential bioactive compounds necessary for growth and maintenance, there is a need to evaluate the effect on gestation.
Objectives: This study was designed to evaluate the impact of Pumpkin Seed Oil (PSO) on gestation using Wistar rats as a model.
Methods: Thirty Wistar rats (15 females, 15 males for mating) were used. Proestrus females were paired with males at a 1:1 ratio. Gestational day (GD) 0 = presence of spermatozoa in vaginal lavage of mated proestrus females. Mated rats were randomly divided into three groups: Group A (control) received no treatment, while Group B and Group C received 500 and 1000 mg/kg PSO, respectively, via oral gavage from GD 6 to 19. On GD 20, a laparotomy was performed to assess foetal weight, foetal crown-rump length (FCRL), the number of live pups/litter size, total implantation sites, placental weights, post-implantation loss, and the foeto-placental weight ratio.
Results: Although no significant ($p>0.05$) differences occurred in most of the parameters between the test groups (groups B and C) and Control group (group A), results showed a significant ($p<0.05$) increase in litter size in 1000 mg/kg PSO-treated rats (group C) compared with the control. Post-implantation loss was significantly reduced ($p<0.01$) in test groups relative to the control.
Conclusion: It is therefore concluded that PSO supports healthy gestation at the doses used in this study.

Keywords: *Fetal parameters, Placental weight, Fetoplacental weight ratio*

Cite this article as: Obinna, V.C. (2026) Pumpkin (Cucurbita pepo) Seed Oil Supports Healthy Gestation in Wistar Rats. Journal of Basic and Applied Research in Biomedicine, 12(1): 1-6

INTRODUCTION

Gestation, the period during which a female mammal carries and nourishes her offspring *in utero*, is a crucial and complex phase in the life cycle of both humans and animals. It links conception and childbirth, a period when the female body undergoes significant physiological, hormonal, and metabolic changes to nurture and support the developing fetus (Soma-Pillay et al., 2016). This period is critical because it has a direct impact on maternal and fetal well-being (Rahayu et al., 2014). Throughout gestation, females encounter some issues and challenges, including an increased need for nutrition to sustain the developing fetus (Ahad, 2023). Adequate maternal nutrition is essential during this period to ensure the optimal development of the offspring and the preservation of the health of the mother (Hart et al., 2025; Qin & Xie, 2023). Inadequate maternal nutrition can lead to a myriad of complications, including preterm birth, low birth weight, and developmental abnormalities, which have long-term effects on the health of the offspring (Chaman-Ara et al., 2018). Therefore, supplementation with substances such as pumpkin (*Cucurbita pepo*) seed oil (PSO) becomes significant, as it can provide essential nutrients, including fatty acids, proteins, vitamins, and antioxidants (Shaban & Sahu, 2017), which are essential for maternal and fetal health (Hart et al., 2025; Qin & Xie, 2023).

Curcubita pepo seeds are the source of pumpkin seed oil (Nishimura et al., 2014) and has been traditionally used for its medicinal properties. Among the top ten most important vegetables planted globally, *Cucurbita pepo* has the highest monetary worth (Gong et al., 2012). Ripe pumpkin fruits have dark green seeds that are flat and oval in shape (Gavril et al., 2024), and the seeds make up between 3.5% and 4.27% of the weight of the pumpkin fruit (Devi et al., 2018). According to Bardaa et al (2016) and Perez Gutierrez (2016), the main factors contributing to the medicinal properties of pumpkin seeds are their mineral content and the phytoconstituents of their oil.

The seeds of pumpkin are the most significant part of the plant because they are low in fat and high in protein (Gavril et al., 2024). According to Karanja et al. (2013), they are rich in fibre, oil and protein. The seed meal of pumpkin has been reported to contain approximately 54% protein, which contains numerous significant biological functions and a balanced composition of key amino acids. Therefore, pumpkin seed meal is a high-quality source of protein and has a lot of potential for usage as a dietary supplement or food additive (Xie et al., 2025). Pumpkin seed is a very rich source of edible oil and protein, with a fat level of 41.59% and a protein value of 25.4% (Gavril et al., 2024). Pumpkin is considered one of the best sources of protein since it contains numerous important amino acids that are necessary for the growth and maintenance of consumers, particularly susceptible groups such as infants, expecting mothers, older people, and those with illnesses.

Pumpkin seeds are considered a delicacy in many communities because of their substantial amount of biologically active substances, including essential amino acids, vitamins, polyunsaturated fatty acids, γ-tocopherols, phytosterols, lutein, fibers, β-carotene pigments, as well as large concentrations of microelements - K, P, Mg, Ca and Mn (Dotto & Chacha, 2020). In Nigeria, the seeds are consumed as food and added to indigenous dishes, where they constitute a significant percentage of the meal. (Oloyede et al., 2012). In many countries, they are utilized primarily for the production of oil and protein (Raihana et al., 2015), and they also play a major role in human diets as a rich source of energy (Bardaa et al., 2016), because they are high in trace elements, carotenoids, tocopherol, antioxidative phenolic substances, lignins, phytosterols, triterpenes, and nutritional fiber (Leichtweis et al., 2025).

Pumpkin seed oil has been used for years to prevent different illnesses, especially prostate issues, due to its therapeutic and nutritional benefits (Shaban & Sahu, 2017). It is one of the edible oils produced in several nations, like Nigeria. It is a good source of essential fatty acids, including omega-3 and omega-6 fatty acids, and contains antioxidants (Hasan & Abbas, 2022; Shaban & Sahu, 2017), which can help combat oxidative stress during pregnancy and support overall maternal and foetal health. Oxidative stress is known to be mitigated by nutritional antioxidants such carotenoids and vitamin E (tocopherol) (Lai et al., 2023). Research has demonstrated that carotenoids are crucial for the prevention of numerous pregnancy-related pathologies, which are associated with elevated levels of oxidative stress, and the successful completion of pregnancy (Zielińska et al., 2017). Fatty acids, specifically polyunsaturated fatty acids (PUFAs), play a crucial role in foetal neurodevelopment and during early life stages (Mhatre et al., 2025). Rideout et al. (2015) demonstrated that when compared to pups from non-supplemented mothers, pups born to mothers who were given phytosterol supplements during pregnancy and lactation show beneficial tissue and serum lipid responses such as a reduced liver cholesterol level, reduced serum cholesterol profile, as well as decreased serum triglyceride and very low-density lipid components.

Pumpkin seed contains zinc, a significant nutrient that aids in the production of oestrogen and progesterone, which are essential hormones involved in ovulation and menstruation (Rabbani et al., 2025). The administration of pumpkin seed oil following high salt exposure led to a substantial improvement in the quality of semen, the levels of male sex hormones, and sexual function of adult male Wistar rats. Additionally, the atrophy of testicular cells was significantly ameliorated, and degenerated gonadotropic cells were restored, and hypothalamic neurons were regenerated (Ajibola et al., 2025). A synergistic combination of tocopherols and phytoestrogens in pumpkin seed oil is a valuable natural resource for the hormonal balance of women, as it provides antioxidant protection and hormone-mimetic support (Rabbani et al., 2025).

Given that pumpkins are among the best sources of biologically active substances, research on functional foods has begun to focus on them (Gavril et al., 2024; Sharma et al., 2020). Pumpkin seed oil, with its high concentration of essential fatty acids, antioxidants, and bioactive substances such as carotenoids, tocopherol, phytosterol, and zinc may be a potential dietary supplement for improving prenatal nutrition. However, despite increased interest in the potential benefits of pumpkin seed oil, there has been little systematic research into its impact on gestation using a validated animal model, such as rats. The importance of this work stems from the need to understand the impact of pumpkin seed oil on gestational outcomes in a controlled experimental context, which can pave the way for evidence-based recommendations in maternal nutrition and prenatal care. The study will shed light on the nutritional impact of pumpkin seed oil on foetal development and gestation in general by examining its effects on gestation in a rat model.

MATERIALS AND METHODS

Ethical approval

The Research Ethics Committee, Office of the Research Management and Development, University of Port Harcourt authorized the research protocols on the 14th of December, 2021, with the reference number UPH/CEREMAD/REC/MM83/038. Handling of the animals were done humanely in accordance with the University's Ethics and Regulations for the use of research animals.

Plant Material Collection, Authentication, and Processing

To obtain the pumpkin seeds, fresh pumpkin fruits were procured from Choba Market, near the University of Port Harcourt in Port Harcourt, Rivers State, Nigeria. The fruits were sent to the Department of Plant Science and Biotechnology for authentication, after which a voucher number UPH/PSB/2021/071 was assigned. The seeds were removed from the fruits, deshelled, and allowed to air-dry in the shade for four weeks before being milled into a powder. The powder was macerated with 80% aqueous ethanol through exhaustive extraction for 72 hours at room temperature, with fresh solvent added every 24 hours. The filtrate which contained appreciable quantity of oil (since the *C.pepo* was not de-fatted with a non-polar solvent such as n-hexane prior the maceration), was allowed to settle in separating flask using a physical means. The upper oily phase was separated from the lower aqueous phase. To obtain a pure oil sample, the oil that accumulated on top of the filtrate was collected into crucibles and placed in a water bath at 50 °C. The oil was transferred into a storage bottle and kept at room temperature.

Sample Size Determination

The "resource equation" approach, which establishes the acceptable range of the error degrees of freedom (DF) in an analysis of variance (ANOVA) and is appropriate for exploratory studies of this kind where the standard deviation and effect size cannot be assumed (Arifin & Zahiruddin, 2017) was used to determine the sample size for the study. Using the equation, the total sample size was calculated to be 15 female rats of 5 per group.

Inclusion and Exclusion Criteria

Only nulliparous females with a regular estrous cycle of 4-5 days were used for the study. Anaestrus and irregularly cycling females were not included in the study.

Experimental Animals and Protocol

The procedure for the experiment complied with the ARRIVE criteria as described by Percie et al. (2020). A total of 30 sexually mature Wistar rats (15 females weighing an average of 150g, and 15 males weighing an average of 200g) were used in the research. They were obtained from the Animal House of the Department of Animal and Environmental Biology, where the study was conducted. The males were used strictly for mating. The rats were kept in normal housing with a temperature of 28 °C and a natural light-dark cycle of about 12 hours, and had free access to food and water throughout the study period.

The oestrous cycle of the female rats was monitored daily throughout the mating period using vaginal cytology, a method commonly used to track the different phases of the cycle (Obinna et al., 2021). The diestrus phase is predominantly characterized by leucocytes, the proestrus phase by epithelial cells, the oestrus phase by cornified cells, and the metestrus phase by a mixed population of cornified cells, epithelial cells and leucocytes. Describing the procedure, vaginal lavage was collected from each rat by gently flushing the vagina using a few drops of normal saline with the aid of a dropping pipette. The vaginal lavage was discharged on a grease-free microscope slide and examined using a light microscope at x10, to determine the presence of different cell types, which correspond to the estrous cycle phases.

To achieve mating, proestrus females were paired overnight with the males in a ratio of 1:1. The gestational day 0 was determined by the presence of spermatozoa in their vaginal lavage and/or copulatory plug the following morning (Marcondes et al., 2002; Obinna & Anyanwu, 2024). The mated female rats were assigned to three groups (5 animals each), designated as Groups A, B, and C, using randomization. Treatment commenced from gestational day 6 to gestational day 19 (Early et al., 2025; Fadel et al., 2016; Obinna et al., 2019).

Determination of Administration Volume and Dose Preparation

This was done by determining the specific gravity (density) of the PSO using a pycnometer (density bottle), which measures the density of the oil relative to water. The process entails the weighing of the empty bottle, with the sample and with water to determine the weight of the sample in relation to an equivalent volume of water as described by Siebel & Kott (1937) and Syahadat & Diningsih (2022). The specific gravity of PSO was calculated as follows: -

Specific Gravity (SG) =

$$\frac{\textit{Weight of empty bottle with PSO } (Wf) - \textit{Weight of empty bottle } (Wo)}{\textit{Weight of empty bottle with wate } (Ww) - \textit{Weight of empty bottle } (Wo)}$$

Where;

Weight of empty bottle (Wo) =20.33; Weight of empty bottle with water (Ww) = 45.11; Weight of empty bottle with PSO (Wf) = 43.05

$$SG = \frac{Wf - Wo}{Ww - Wo} = \frac{43.05 - 20.33}{45.11 - 20.33} = \frac{22.72}{24.78} = 0.9g$$

1ml = 0.9g; same as 0.9g/ml = 900 mg/ml (concentration)

$$\text{Administration Volume} = \frac{\text{Dose (mg/kg) x Weight (kg)}}{\text{Concentration (mg/ml)}}$$

The doses of pumpkin seed oil used in this study were adopted from the acute oral toxicity testing by Anyanwu et al. (2025), where the dose of 5000 mg/kg was found to be safe, with no mortality or morbidity. Hence, 1/10 and 1/5 of the dose, 500 and 1000 mg/kg, respectively, were used.

The mated female rats were given pumpkin seed oil by oral gavage as follows:

Group A = Control (No Treatment)

Group B = Lowest Dose (500 mg/kg pumpkin seed oil)

Group C = Highest Dose (1000 mg/kg pumpkin seed oil)

Sample Collection

On Gestation Day 20, the rats were anaesthetised for laparotomy using diethyl ether to evaluate gestational and foetal parameters according to the methods of Hamdi & Hassan (2021) and Obinna et al. (2019). The uterine horns were dissected out and incised at the greater curvature to reveal the pups in the amniotic sacs on the uterine wall. The pups were removed from the uterine wall to determine litter size (number of live pups), number of implantation sites, foetal weight, foetal crown-rump length (FCRL) and Post-implantation loss. The placentas were excised, dabbed on filter paper to remove excess fluid, and weighed. The foetoplacental weight ratio was calculated. The pups were examined for macroscopic external malformations.

Post-implantation loss (%) =

$$\frac{\textit{Number of Implantation Sites} - \textit{Number of live pups}}{\textit{Number of Implantation Sites}} \times 100$$

Foetoplacental weight ratio = Foetal weight ÷ Placental weight

Statistical Analyses

Statistical analyses were performed using SPSS 25. Data were given as mean ± SEM and analyzed using one-way ANOVA followed by Least Significant Difference (LSD) post-hoc test. Significance was set at $p < 0.05$.

RESULTS

No clinical signs or abortion were observed in any of the dams during the gestation period. No external abnormalities were observed in the pups from the control rats and various doses of PSO-treated rats.

The treatment of pregnant Wistar rats with pumpkin seed oil from gestational day 6 to gestational day 19 resulted in a significant ($p<0.05$) increase in the mean litter size (number of live pups) of rats in the group given the highest dose of 1000mg/kg (group C) (figure 1) with a value 9.00±0.41 relative to the control (group A) with the value of 7.50±0.29. PSO caused a highly significant ($p<0.01$) decrease in post-implantation loss in rats given 500 and 1000 mg/kg doses (groups B and C), both with the value of 0.00±0.00 relative to the control with a value of 9.03±12.50 (figure 2). There was no significant difference in the mean foetal weight (group A = 3.53±0.10, group B = 3.79±0.05 & group C = 3.44±0.28), foetal crown-rump length (group A = 3.75±0.06, group B = 3.60±0.20 & group C = 3.66±0.15), total implantation sites (group A = 8.25±0.20, group B = 8.50±0.26 & group C = 9.00±0.29), and placental weights (group A = 0.54±0.04, group B = 0.53±0.01 & group C = 0.57±0.06) across the groups as seen in figures 3 – 6. Table 1 shows the foeto-placental weight ratio of rats from groups A, B and C.

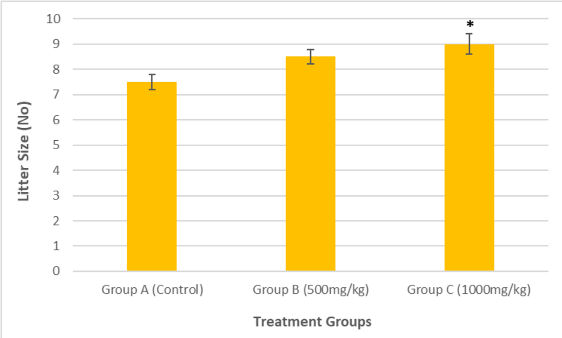

Figure 1: Litter Size (Number of Live Pups) from Pumpkin-Seed-Oil-treated Pregnant Rats. Results are given as Mean ± SEM for each group. *indicate significant difference at $p<0.05$, compared with group A (Control). Statistical evaluation was done by one-way ANOVA, followed by LSD post-hoc test using SPSS. Groups A, B, and C represent the control (non-treated pregnant rats), 500 mg/kg PSO-treated pregnant rats, and 1000 mg/kg PSO-treated pregnant rats respectively.

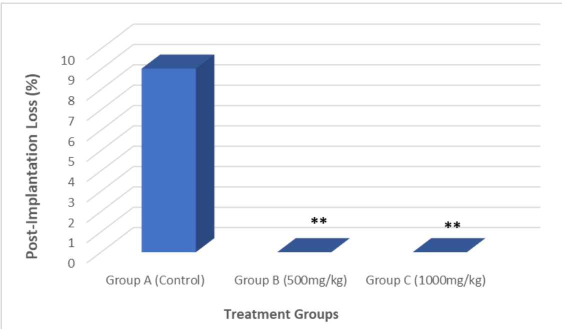

Figure 2: Post-Implantation Loss (%) in Pumpkin-Seed-Oil-treated Pregnant Rats. Results are given as Mean ± SEM for each group. **indicate significant difference at $p<0.01$, compared with group A (Control). Statistical evaluation was done by one-way ANOVA, followed by LSD post-hoc test using SPSS. Groups A, B, and C represent the control (non-treated pregnant rats), 500 mg/kg PSO-treated pregnant rats, and 1000 mg/kg PSO-treated pregnant rats respectively.

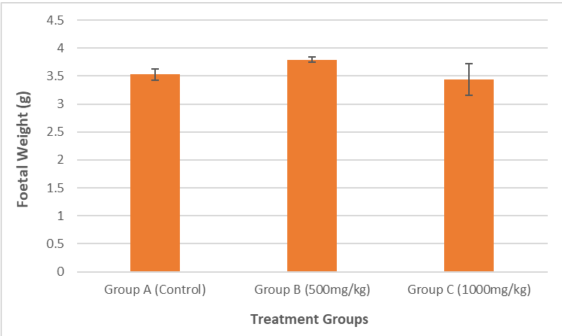

Figure 3: Foetal Weight of pups from Pumpkin-Seed-oil-treated pregnant rats Results are given as Mean ± SEM for each group. No significant difference at a 95% confidence interval ($p >0.05$), compared with group A (Control). Statistical evaluation was done by one-way ANOVA, followed by LSD post-hoc test using SPSS. A, B, and

C represent the control (non-treated pregnant rats), 500 mg/kg PSO-treated pregnant rats, and 1000 mg/kg PSO-treated pregnant rats respectively.

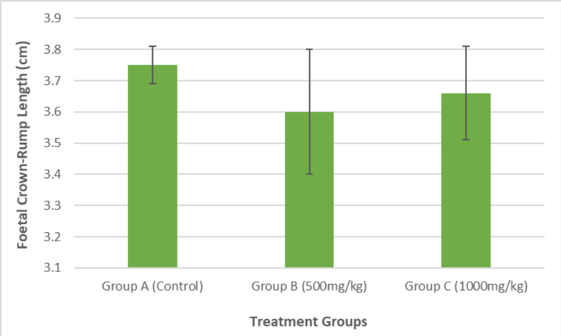

Figure 4: Foetal Crown-Rump Length of pups from Pumpkin-Seed-Oil-treated Pregnant Rats. Results are given as Mean ± SEM for each group. No significant difference at a 95% confidence interval ($p > 0.05$), compared with group A (Control). Statistical evaluation was done by one-way ANOVA, followed by LSD post-hoc test using SPSS. Groups A, B, and C represent the control (non-treated pregnant rats), 500 mg/kg PSO-treated pregnant rats, and 1000 mg/kg PSO-treated pregnant rats respectively.

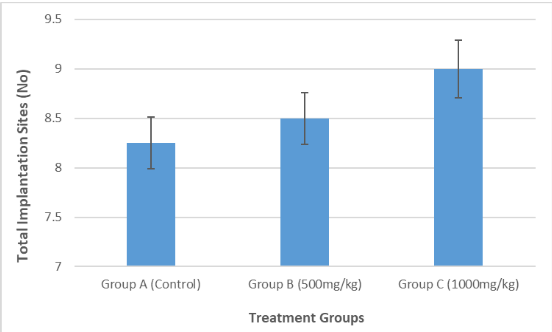

Figure 5: Total Number of Implantation Sites in Pumpkin-Seed-Oil-treated Pregnant Rats. Results are given as Mean ± SEM for each group. No significant difference at a 95% confidence interval ($p > 0.05$), compared with group A (Control). Statistical evaluation was done by one-way ANOVA, followed by LSD post-hoc test using SPSS. Groups A, B, and C represent the control (non-treated pregnant rats), 500 mg/kg PSO-treated pregnant rats, and 1000 mg/kg PSO-treated pregnant rats, respectively.

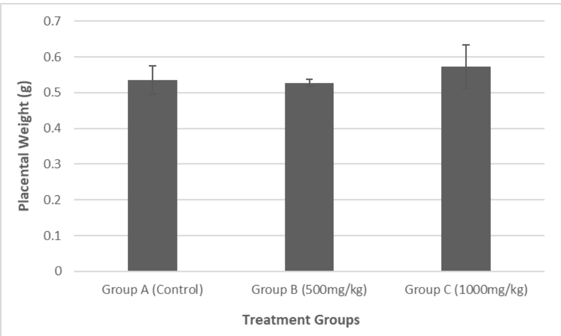

Figure 5: Placental Weight of Pumpkin-Seed-Oil-treated Pregnant Rats. Results are given as Mean ± SEM for each group. No significant difference at a 95% confidence interval ($p > 0.05$), compared with group A (Control). Statistical evaluation was done by one-way ANOVA, followed by an LSD post-hoc test using SPSS. Groups A, B, and C represent the control (non-treated pregnant rats), 500 mg/kg PSO-treated pregnant rats, and 1000 mg/kg PSO-treated pregnant rats, respectively.

Table 1: Impact of Pumpkin Seed Oil on Foeto-Placental Ratio

Groups	Group A (Control)	Group B (500 mg/kg PSO)	Group C (1000 mg/kg PSO)
Foeto-placental ratio	6.59	7.19	6.01

DISCUSSION

Poor maternal nutrition has a detrimental effect on gestation and birth outcomes because gestation is a period when physiological nutrient demands are significantly higher, making a balanced and adequate diet essential (Tsegaye et al., 2021). The search for common indigenous plants that contain essential nutrients required for optimal maternal health and foetal development prompted this study, where varied doses of pumpkin seed oil were given to gestating rats from gestational day 6 to gestational day 19. In rats, gestational day 6 to gestational day 19 involve the critical period of organogenesis and early foetal development, which extends from implantation to the commencement of final maturation immediately preceding delivery. This window is often utilized in developmental toxicology to evaluate the impact of compounds on foetal development because the placenta is fully functional and organ systems develop fast (Desaiah, 1998).

The results of this study showed that pumpkin seed oil at doses of 500 and 1000 mg/kg did not affect the total implantation sites, placental weights, foetoplacental ratio, foetal crown-rump length, or foetal weights. No teratogenicity or malformations of live pups were observed. All pregnant rats in the test groups showed no clear indication of maternal death. This indicates that pumpkin seed oil did not pose any risk to gestation in general or foetal development in particular, and may have contributed to improved maternal health. It was reported that eating pumpkin seeds while pregnant enhances the health of the mother since the elevated iron level of these seeds promotes the elevated blood volume associated with gestation and reduces the chances of anaemia. These seeds also contain Magnesium, which may lower the risk of preterm labour (Preventive Healthcare, 2025). This report is supported by a pre-clinical study conducted by Kumari et al. (2022), who discovered that following pumpkin seed supplementation, 97% of participants reported no sensation of numbness or tingling, and 79% of mothers experienced an improved appetite, suggesting that pumpkin seed administration may have an impact on anemia in pregnant women.

The results of the present study also revealed that there was a dose-dependent increase in litter size of the test groups, which was significant in the 1000 mg/kg PSO-treated group. The pumpkin seed oil prevented post-implantation loss of any type, in contrast to the control group. This further suggests that pumpkin seed oil is beneficial to gestation, and may be associated with the nutritional components contained in PSO, including essential fatty acids, vitamins, and minerals, which are known to play crucial roles in foetal growth and development (Hart et al., 2025; Shaban & Sahu, 2017). The seeds of pumpkin are an excellent source of nutrients that may supply the required minerals and vitamins to help the foetus develop normally throughout gestation. The substantial amount of protein helps with the building and development of foetal tissues and muscles; iron is required for the formation of the foetal brain and red blood cells; and zinc stimulates the development of the immune system (Preventive Healthcare, 2025). It has been reported that pumpkin seeds, rich in Zn and omega-3 fatty acids, directly stimulate progesterone release (AllaraHealth, 2025; Candela & Chellam, 2025) which helps to sustain pregnancy by averting uterine contractions that could cause preterm labour.

In terms of placenta weight, all the test groups displayed uniform placental weight, suggesting a consistency in placental development among the groups, which resulted in non-significant foetal weight. This agrees with the work of Kolluri & Shakunthala (2016) who found that there is a significant relationship between placental weight and foetal weight, and that of Panti et al., (2012) who reported that placental weight is associated with pregnancy outcome. Additionally, the least foetoplacental ratio of 6.01 recorded in the 1000 mg/kg PSO-treated pregnant rats correlated with the increased litter size observed in that group.

In line with this study, Hamdi & Hassan (2021) demonstrated that administration of 4 ml/kg PSO to pregnant rats from gestational day 5 to 19 had no significant effect on total implantation sites, number of corpora lutea, live foetuses, foetal weight, foetal length, placental weight, and post-implantation loss. However, the co-administration with Alumina Nanoparticles (Al_2O_3-NPs) showed a potential preventive effect on the Al_2O_3-NPs-induced maternal and neonatal toxicity.

It is worthy to note that the standard error reported in figure 4 – foetal crown-rump length, which appear to be high, is as a result of the slight variation in the individual data of the animals that made up group B, but had no significant effect in the result.

CONCLUSION

Based on the results of this study, which was designed to evaluate the impact of Pumpkin Seed Oil (PSO) on gestation using Wistar rats as a model, it can be concluded that oral ingestion of pumpkin seed oil supports healthy gestation at the doses used in the study.

ETHICS APPROVAL

Ethical approval for this study was granted by the Research Ethics Committee at the Office of the Research Management and Development, University of Port Harcourt (Ref: UPH/CEREMAD/REC/MM83/038, Date: December 14, 2021). Experimental protocols and animal handling were carried out humanely in accordance with the University's Ethics and Regulations for the Use of Research Animals and complied with the ARRIVE guidelines.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES

The author acknowledges that AI-assisted technologies were partially used in the preparation of this manuscript. Specifically, Grammarly was employed to support language refinement and grammar correction. All content was critically reviewed, edited, and approved by the author to ensure accuracy, originality, and alignment with academic standards. No AI tools were used to generate data, interpret results, or replace human intellectual contribution.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The author declares no conflict of interest in this study

FUNDING

The study was not funded by external sources aside the author.

AUTHOR CONTRIBUTION

The author confirms the sole responsibility for the conception of the study, presented results and manuscript preparation.

ACKNOWLEDGEMENTS

The author thanks Dr. Chinwe F. Anyanwu for her cooperation in providing the plant material for this study. Additionally, the author expresses gratitude to Prof. Sidney O. Nzeako, Head of Department, Animal and Environmental Biology, University of Port Harcourt, for providing the facilities required to complete this study.

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