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

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Research Article

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Abstract

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. This study was designed to evaluate the impact of Pumpkin Seed Oil (PSO) on gestation using Wistar rats as a model. Thirty Wistar rats (15 females, 15 males for mating) were used. Proestrus females were paired with males at 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, laparotomy was done to assess foetal weight, foetal crown-rump length (FCRL), number of live pups/litter size, total implantation sites, placental weights, post-implantation loss, and the foeto-placental weight ratio. 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. It is therefore concluded that PSO supports healthy gestation at the doses used in this study.

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 foetus (Soma-Pillay et al., 2016). This period is critical because it directly affects the health and well-being of both the mother and the foetus (Rahayu et al., 2014). Throughout gestation, females encounter some issues and challenges, including an increased need for nutrition to sustain the developing foetus (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 becomes significant, as it can provide essential nutrients, including fatty acids, proteins, vitamins, and antioxidants (Shaban & Sahu, 2017), which are vital for the health of both the mother and the developing foetus (Hart et al., 2025; Qin & Xie, 2023).

Pumpkin seed oil is derived from the seeds of the *Cucurbita pepo* (pumpkin) plant (Nishimura et al., 2014) and has been traditionally used for its medicinal properties. *Cucurbita pepo* is the species with the greatest monetary value and is among the top ten leading vegetable crops grown worldwide (Gong et al., 2012). Mature pumpkin fruits contain flat, oval-shaped, dark green seeds (Gavril et al., 2024), and the

seed content of pumpkin fruit varies from 3.5% to 4.27% of fruit weight (Devi et al., 2018). The medicinal properties of pumpkin seeds largely stem from their mineral composition and the phytochemical composition of the pumpkin seed oil (Bardaa et al., 2016; Perez Gutierrez, 2016).

Pumpkin seeds are the most important component of the plant due to their high protein and low-fat content (Gavril et al., 2024). According to Karanja et al. (2013), pumpkin seeds contain high levels of crude protein, crude fiber, and crude oil. Pumpkin seed meal has been reported to contain approximately 54% protein, and the protein has a balanced content of essential amino acids and possesses many important biological activities. Hence. pumpkin seed meal qualifies as a premium source of protein and has great potential to be added to other food products or used as a dietary supplement (Xie et al., 2025). Pumpkin seed oil, with its fat content of 41.59% and protein content of 25.4%, is a highly abundant source of both protein and edible oil (Gavril et al., 2024). Pumpkin can be regarded as one of the best protein sources for providing many essential amino acids for the growth and maintenance of consumers, especially vulnerable people like children, pregnant women, the elderly, and the sick.

Pumpkin seeds are widely appreciated as a delicacy in several cultures, and the seed oil has culinary and therapeutic uses due to its high concentration of bioactive compounds, including polyunsaturated fatty acids, essential amino acids, lutein, vitamins, phytosterols, γ-tocopherols, and β-carotene pigments, fibers, and substantial amounts of micronutrients (P, Mg, Mn, K, and Ca) (Dotto & Chacha, 2020). In Nigeria, the seeds, which make up a substantial portion of the diet, are eaten as a meal and used as ingredients in local soups (Oloyede et al., 2012). In many countries, pumpkin seeds are used primarily for the production of oil and protein (Raihana et al., 2015). Pumpkin seeds are a high-energy source that contributes significantly to human nutrition in many parts of the world (Bardaa et al., 2016), as they are rich in triterpenes, lignins, phytosterols, antioxidative phenolic compounds, carotenoids, tocopherol, dietary fibre and minerals (Leichtweis et al., 2025).

Pumpkin seed oil has long been considered for the prevention of various ailments, particularly prostate diseases, owing to its nutritional and medicinal properties (Shaban & Sahu, 2017). It holds a special place among edible oils produced in many countries, including Nigeria. It is a good source of essential fatty acids, including omega-3 and omega-6 fatty acids, and contains antioxidants, such as vitamin E and carotenoids (Hasan & Abbas, 2022; Shaban & Sahu, 2017), which can help combat oxidative stress during pregnancy and support overall maternal and foetal health. Given that pumpkins are among the best sources of bioactive compounds, research on functional foods is increasingly focusing 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, 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 effects 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.

Material and Methods

Ethical approval

The research protocols were duly approved by the Research Ethics Committee of the Centre for Research Management and Development, University of Port Harcourt with the Ref. No: UPH/CEREMAD/REC/MM83/038. The animals were humanely handled in accordance with the Ethics and Regulations guiding the use of research animals, as approved by the University.

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. 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. 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 sample size for the study was calculated using the ‘resource equation’ approach, which sets the acceptable range of the error degrees of freedom (DF) in an analysis of variance (ANOVA), and is suitable for exploratory studies of this nature, where the standard deviation and effect size are impossible to assume (Arifin & Zahiruddin, 2017). 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 oestrous cycle of 4-5 days were used for the study. Anaestrus and irregularly cycling females were excluded from the study.

Experimental Animals and Protocol

The experimental protocol is in accordance with the ARRIVE guidelines 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 for this study. They were procured 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 properly housed under standard conditions of 28 °C ambient temperature and approximately 12- hour natural light-dark cycle, and fed *ad libitum* 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 observed under a light microscope at x10, to determine the presence of different cell types, which correspond to the phases of the oestrous cycle.

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.

From gestational day 6 to gestational day 19, the mated female rats were given pumpkin seed oil by oral gavage as follows:

Group A (control) = No Treatment

Group B = 500 mg/kg pumpkin seed oil

Group C = 1000 mg/kg pumpkin seed oil

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.

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, and foetal crown-rump length (FCRL). Post-implantation loss (%) was calculated as [(number of implantation sites - number of live pups) ÷ number of implantation sites] x 100. The placentas were excised, dabbed on filter paper to remove excess fluid, and weighed. The foetoplacental weight ratio was calculated as the foetal weight

divided by the placental weight (Foetal weight ÷ Placental weight). The pups were examined for macroscopic external malformations.

Statistical Analyses

The Statistical analyses were done with SPSS 25, the data were represented as mean ± SEM, and assessed using one-way Analysis of Variance (ANOVA) followed by Least Significant Difference (LSD) post-hoc test and significance was set at $p < 0.05$.

Results

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), and a highly significant ($p<0.01$) decrease in post-implantation loss in rats given 500 and 1000 mg/kg doses (groups B and C) relative to the control (figure 2). There was no significant difference in the mean foetal weight, foetal crown-rump length, total implantation sites, and placental weights from groups B and C treated rats in comparison with the control (group A) as seen in figures 3 – 6). Table 1 shows the foeto-placental weight ratio of rats from groups A, B and C.

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.

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.

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.

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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.

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 consuming pumpkin seeds during pregnancy improves maternal health because the high iron content of these seeds supports the increased blood volume associated with pregnancy and lowers the risk of anaemia. Magnesium, another element found in pumpkin seeds, 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 after supplementing with pumpkin seeds, 79% of mothers reported a strong appetite, and 97% of participants reported experiencing no tingling or numbness, indicating the possible impact of pumpkin seed supplementation on anaemia in pregnant mothers.

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 present in Pumpkin seed oil, 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). Pumpkin seeds are a nutrient-dense food that can provide essential vitamins and minerals to support healthy foetal development during pregnancy. The high protein content aids in the formation and growth of foetal tissues and muscles; iron is essential for the development of the foetal brain and red blood cells; and zinc promotes the immune system development. (Preventive Healthcare, 2025). It has been reported that pumpkin seeds, which are high in zinc 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 finding is in agreement with the report 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 maternal and developmental toxicity induced by Al_2O_3-NPs.

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.

Declarations

Acknowledgement

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Conflict of interest

None is declared

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Figures

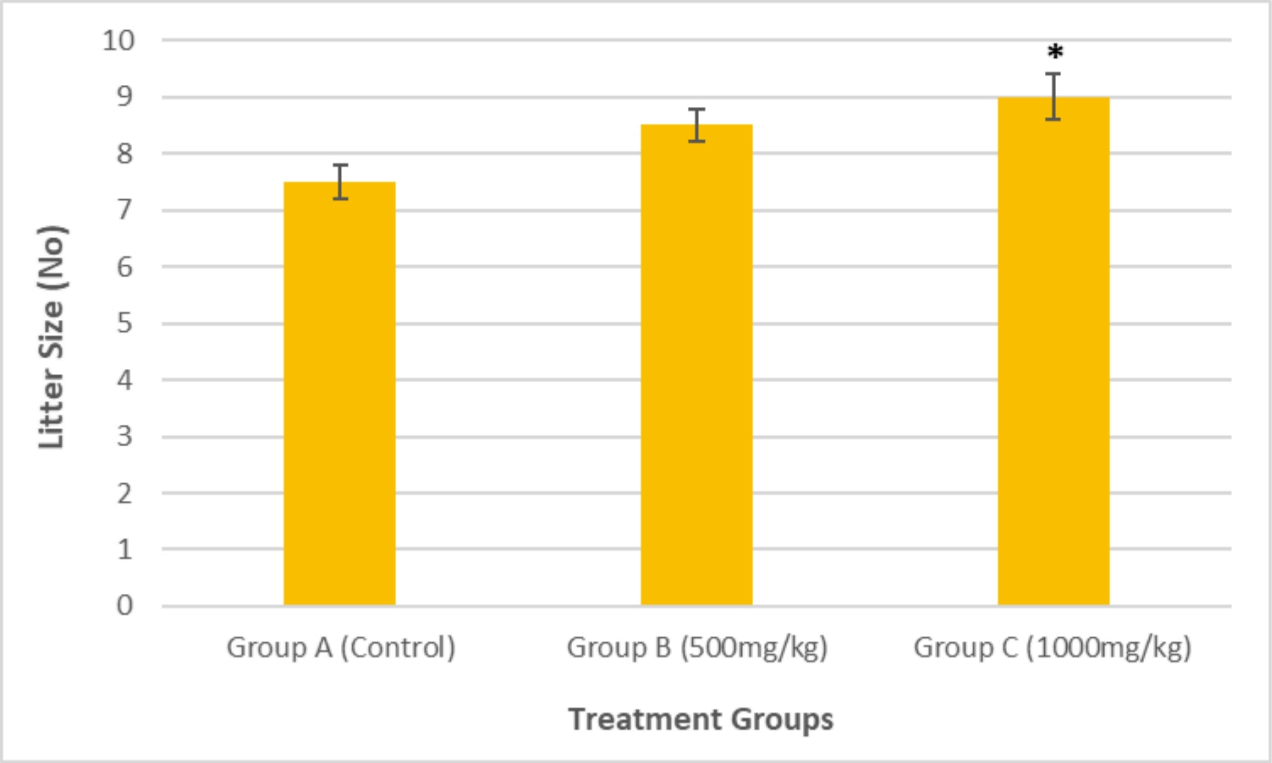

Figure 1

Litter Size (Number of Live Pups) from Pumpkin-Seed-Oil-treated Pregnant Rats.

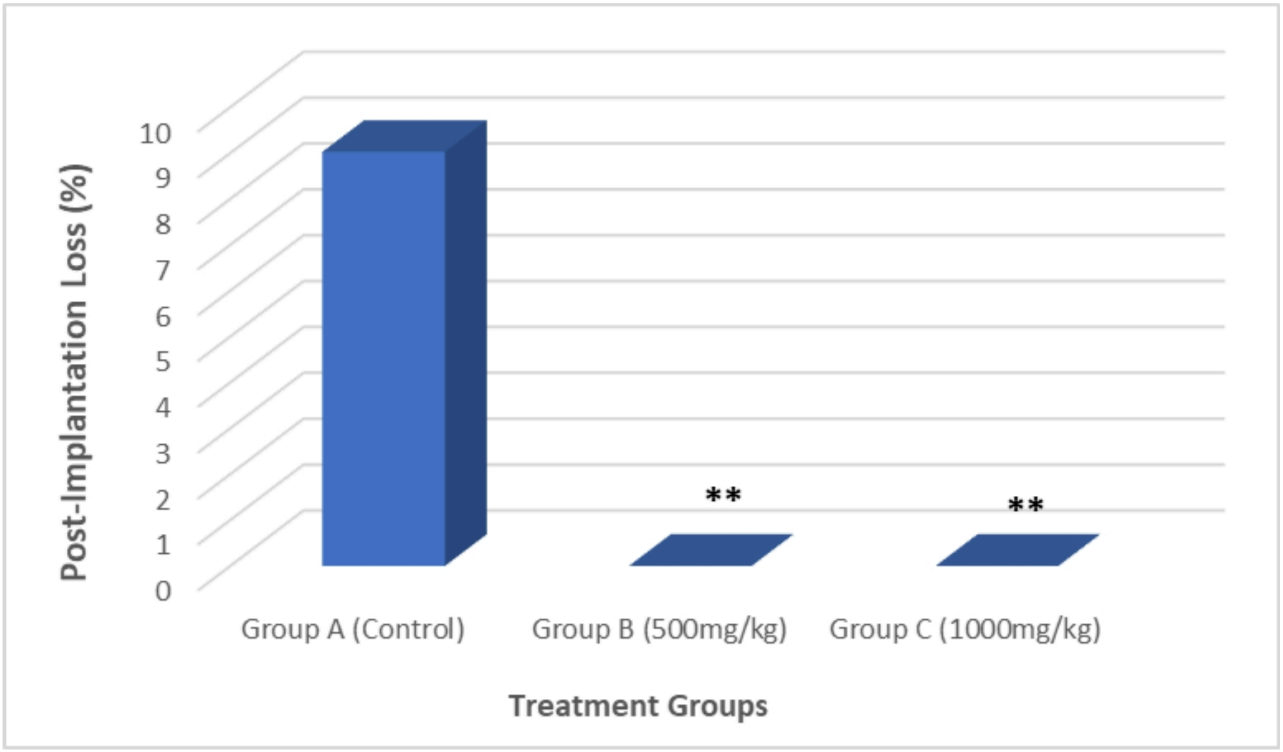

Figure 2

Post-Implantation Loss (%) in Pumpkin-Seed-Oil-treated Pregnant Rats.

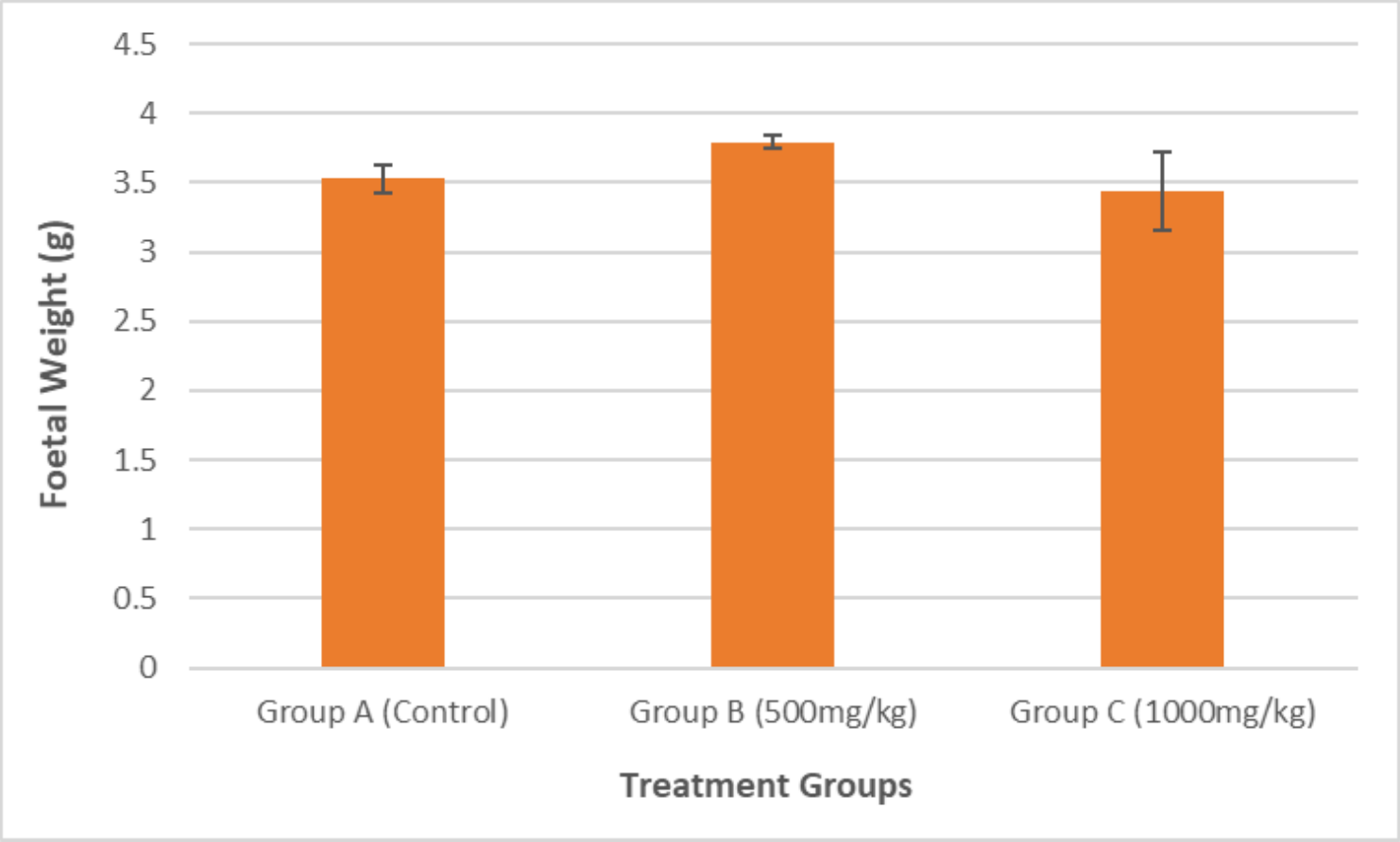

Figure 3

Foetal Weight of pups from Pumpkin-Seed-oil-treated pregnant rats

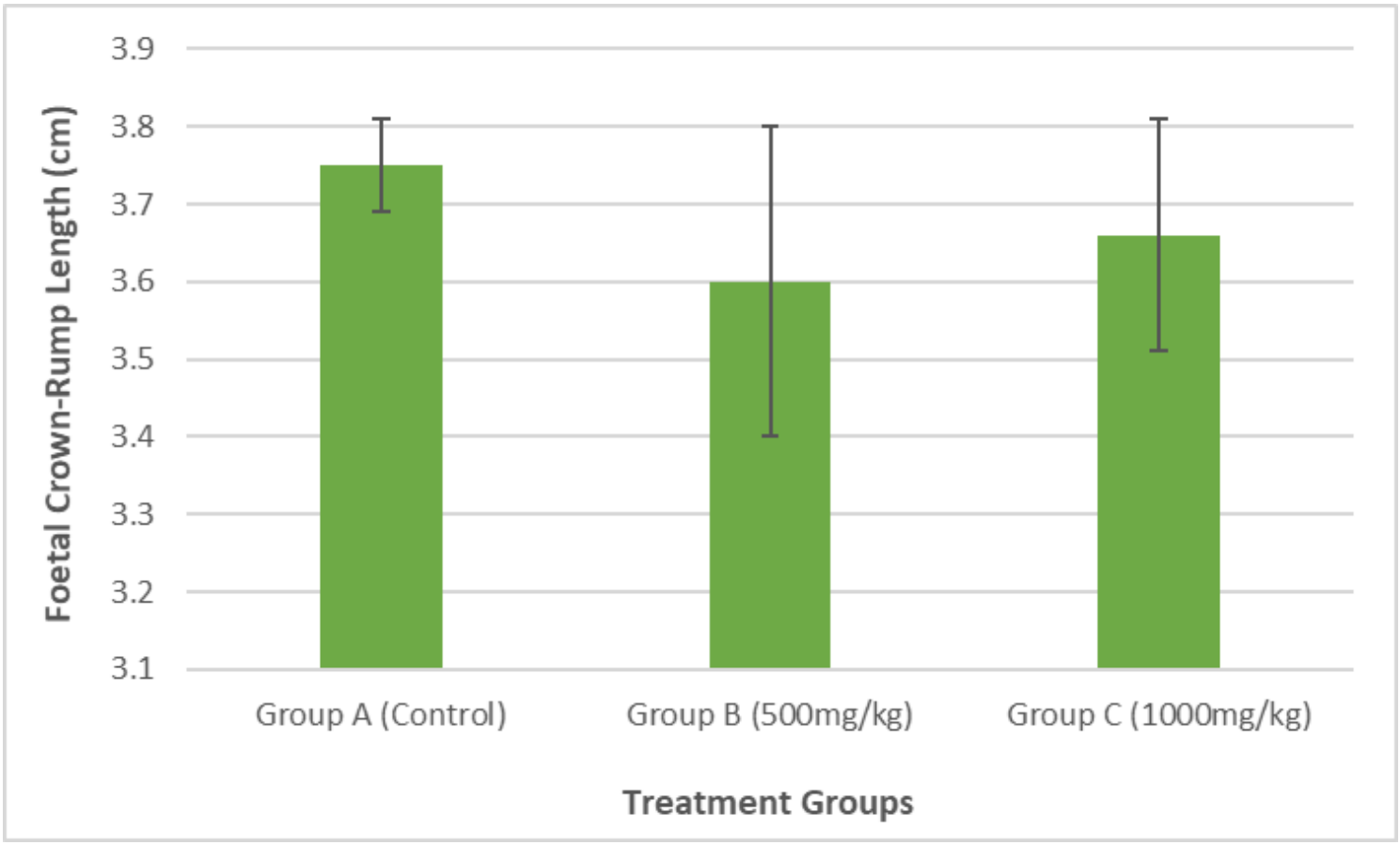

Figure 4

Foetal Crown-Rump Length of pups from Pumpkin-Seed-Oil-treated Pregnant Rats

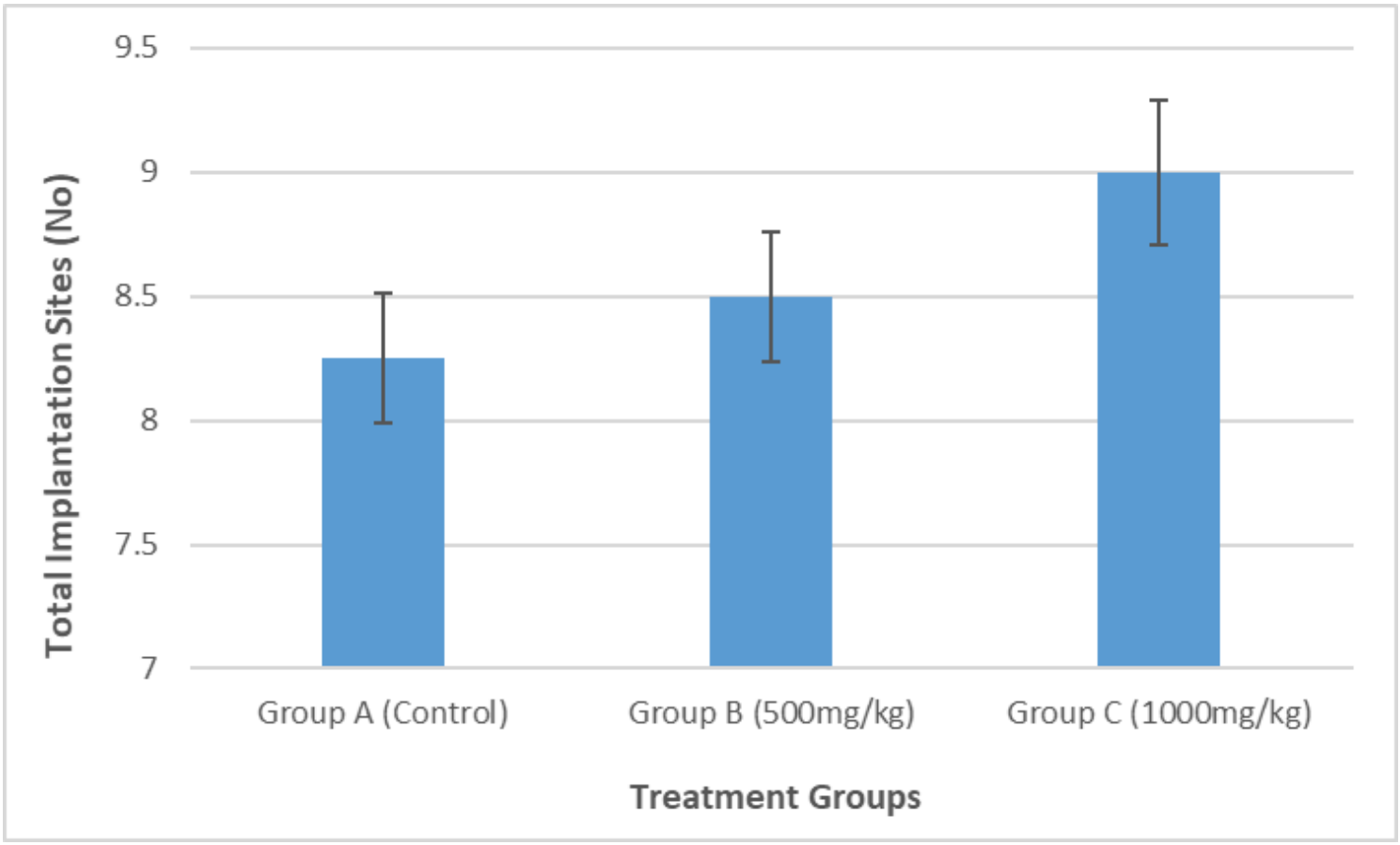

Figure 5

Total Number of Implantation Sites in Pumpkin-Seed-Oil-treated Pregnant Rats.

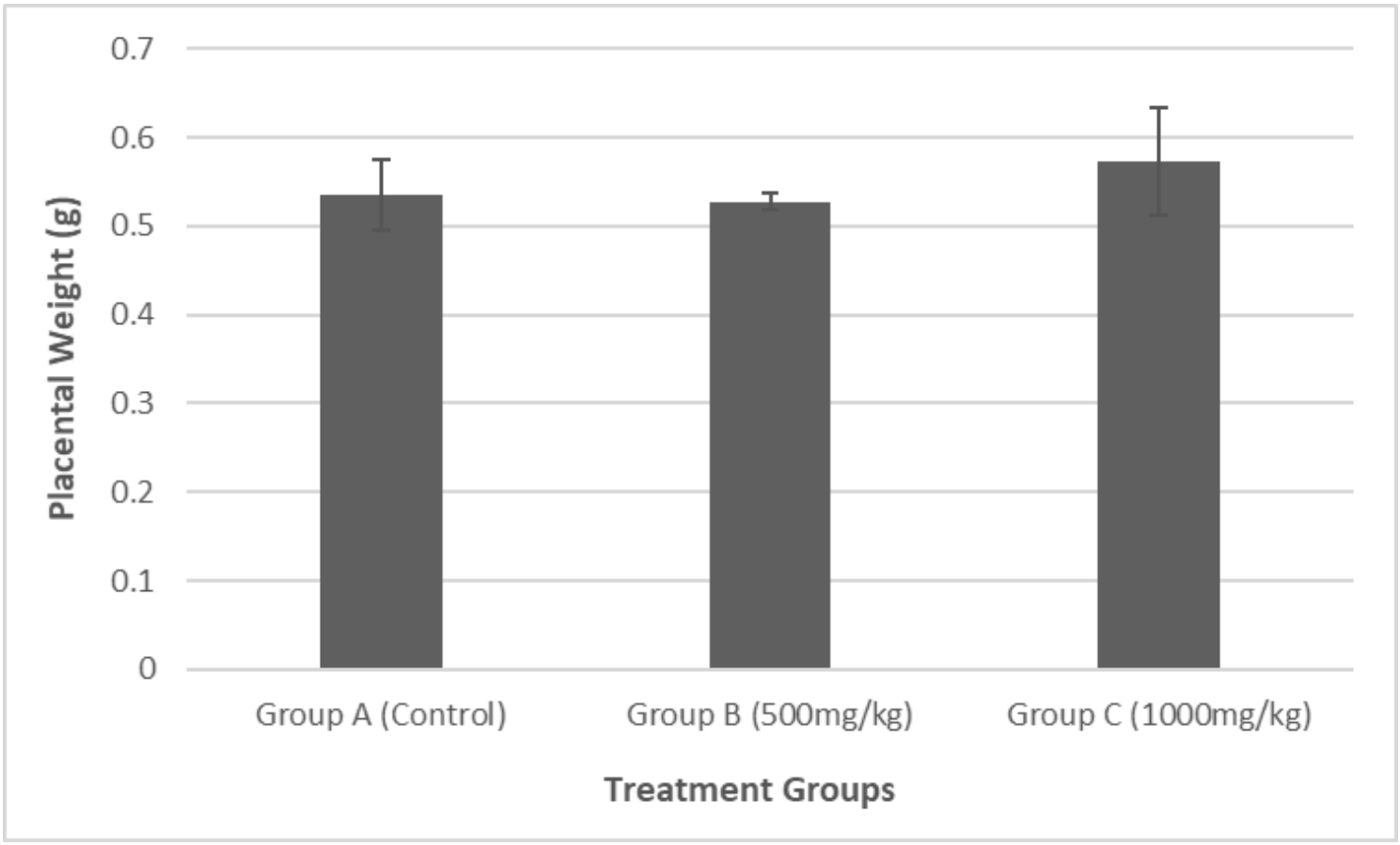

Figure 6

Figure 5: Placental Weight of Pumpkin-Seed-Oil-treated Pregnant Rats.