

The Eurasia Proceedings of Science, Technology, Engineering and Mathematics (EPSTEM), 2026

Volume 41, Pages 234-240

ICRETS 2026: International Conference on Research in Engineering, Technology and Science

Ovarian Histological Alterations and Fetal Developmental Effects Induced by A Novel Anticancer Phosphoramidate Nucleotide in Rats

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Abstract: The present study evaluated the reproductive toxicity of a novel compound, SH-PAN-19, in pregnant rats. To assess ovarian toxicity, SH-PAN-19 was administered at 25 and 100 mg/kg through a single dose, while fetal outcomes were examined following drug administration on gestational day 16. Fetal development and ovarian morphology were assessed on gestational day (GD18), with additional ovarian recovery analysis performed 21 days post-treatment. Administration of 25 mg/kg intraperitoneally caused a little yet significant reduction in fetal weight and crown–rump length, whereas 100 mg/kg via oral gavage resulted in complete fetal resorption. Histological analysis demonstrated dose-dependent ovarian damage. Control ovaries exhibited normal follicular development, whereas exposure to 25 mg/kg resulted in moderate follicular depletion and a reduced number of corpora lutea. In contrast, the 100 mg/kg dose severely disrupted folliculogenesis, leading to extensive ovarian deterioration. Recovery studies indicated that ovarian damage at 25 mg/kg was largely reversible, while exposure to 100 mg/kg produced persistent and irreversible injury. Collectively, these findings demonstrate that SH-PAN-19 induces pronounced dose-dependent embryotoxicity and ovarian toxicity, underscoring the substantial risk associated with high-dose exposure, while suggesting that the recommended 25 mg/kg dose during the third trimester may still pose potential risks to female fertility and pregnancy outcomes.

Keywords: Anticancer drugs, Phosphoramidate nucleotide, Rat, Fertility

Introduction

Pregnancy-associated Cancer is a serious and complicated medical disease. Breast cancer, cervical cancer, melanoma, lymphoma, and acute leukaemia are the most common types of malignancies that women might get while pregnant (Puzzi-Fernandes et al., 2020). A pregnant woman with cancer usually undergoes various treatments, including chemotherapy, radiation therapy, Surgery, and hormonal therapy. However, many treatments are associated with adverse reproductive results, including diminished fertility in females and adverse effects on embryonic development (Wolters et al., 2021). Pregnancy-associated Cancer is considered a rare condition, with an estimated incidence of approximately 1 in 1,000 pregnancies (Dalmartello et al., 2020). In recent years, more women have been diagnosed with cancer during pregnancy. This issue is becoming more significant in the clinic (Zhang et al., 2017). When a woman has cancer and is pregnant at the same time, it makes treatment more difficult and often leads to a worse outcome for the mother. It is imperative to devise

therapeutic strategies that proficiently enhance maternal health while minimizing dangers to fetal welfare (Zhang et al., 2017).

Developmental Toxicity and Reproductive Impact

Organ developmental toxicity refers to structural and functional impairments in the offspring's organs resulting from exposure to harmful intrauterine circumstances during gestation. Adverse conditions include environmental stressors (physical, chemical, and biological agents) as well as maternal factors, including illness, starvation, and mental stress. Drug exposure has been identified as a contributor to developmental toxicity among these factors (Lu et al., 2023). The effects of cancer and its treatment on female reproductive function have become increasingly evident in recent years. Advances in cancer therapy have improved survival rates; however, these treatments are often accompanied by long-term adverse effects on reproductive health. Epidemiological studies indicate that, compared with the general population, women diagnosed and treated for cancer are approximately 38% less likely to achieve pregnancy, regardless of cancer type. This consistent reduction in post-treatment fertility highlights the significant reproductive burden associated with cancer and underscores the need for improved understanding and management of treatment-related gonadotoxicity (Anderson et al., 2018).

The adverse effects of chemotherapy on ovarian function have been recognized for decades. Accumulating clinical and experimental evidence has increasingly clarified the impact of different chemotherapeutic regimens on female reproductive health. These effects extend beyond immediate disruptions in ovarian activity and include alterations in short-term markers of ovarian reserve, such as hormonal profiles and follicular dynamics, as well as long-term consequences for fertility potential. Moreover, exposure to certain chemotherapeutic agents has been strongly associated with an elevated risk of premature ovarian insufficiency and early menopause. Collectively, these findings underscore the substantial reproductive burden imposed by chemotherapy and highlight the importance of evaluating ovarian toxicity when selecting and optimizing anticancer treatments (Anderson et al., 2018).

Chemotherapy Exposure and Formulation Considerations

During pregnancy, doctors employ a number of chemotherapeutic medications to treat cancer. These are alkylating drugs, anthracyclines, and a taxane. These medications might aid pregnant women, but they could potentially hurt the mother's reproductive health and the baby's growth (Struys et al., 2024). Organic solvents used as formulation vehicles can influence embryonic development, and their concentrations must therefore be carefully controlled in developmental studies. Within experimentally defined tolerable ranges, several vehicles, including PEG 400, corn oil, DMSO, and poloxamer 188, can be employed without overt adverse effects on early development (Chen et al., 2023; Maes et al., 2012).

Novel Therapeutic Candidate and Study Aim

A novel therapeutic drug candidate, SH-PAN-19, currently under patent, is a modified molecule derived from phosphoramidate acyclic nucleotide, exhibiting anticancer efficacy across various cell lines (Huh-7, HepG-2, Caco-2, A549 cells). It demonstrates a promising effect in the HCC rat model, exhibiting a great safety profile and significant potency. Compound code: (9a) , compound name: Isopropyl[(S)-{2-(9-(4-bromophenyl)-3,3,6,6-tetramethyl-1,8-dioxo2,3,4,5,6,7,8,9-octahydroacridin-10(1H)-yl)ethoxy}(phenoxy)phosphoryl]-L-alaninate (Hammad, 2024).

In this study, more preclinical studies to investigate the effect of this drug candidate on pregnancy and female fertility. The assessment was performed through histological examination of maternal ovaries, aiming to detect any reproductive and developmental toxicities associated with maternal exposure.

Method

Drug Synthesis and Solubilization

SH-PAN-19 was synthesized and characterized as previously described [8]. The crude product was initially characterized by thin-layer chromatography (TLC) using silica gel plates and a 1:1 ethyl acetate: hexane solvent system, visualized under UV light, and characterized by observing its characteristic UV–visible spectrogram. For purification, the main TLC band was carefully scraped, and the compound was extracted using methanol. The purified **SH-PAN-19** was further characterized by UV–visible spectroscopy, showing a maximum absorption at 350 nm. The purified compound was then formulated for in vivo studies.

The medication exhibited significant insolubility and encountered challenges in dissolving within acceptable parameters during pregnancy. Multiple experiments were conducted at 25 ± 3 °C to attempt its dissolution. After numerous tests, we identified the most suitable vehicle for intraperitoneal injection during pregnancy (Table 1). The medication was evaluated for its impact on ovarian tissues and fetal development at a high dose of 100 mg/kg and a low dose of 25 mg/kg.

Experimental Animals and Housing Conditions

Fifteen Adult virgin female rats ($n = 5$ per group) were used in this study. Animals were housed under standard conditions with a room temperature of 22 ± 3 °C, relative humidity of 30–70%, and a 12-h light/12-h dark cycle. Rats had free access to standard laboratory chow and water ad libitum. Rats were fed for eight days to adapt to the laboratory environment, and then mated (female: male = 2:1) overnight from 7 p.m. to 9 am during their estrous cycle. On the second day at 9 am, the females were examined for vaginal plug, which was designated as day 0 (Choi et al., 2020). SH-PAN-19 was evaluated for its effects on female fertility and fetal development. Pregnant rats were randomly assigned to control or treatment groups. The drug was administered via two dose levels: High dose: 100 mg/kg via Oral gavage and Low dose: 25 mg/kg via intraperitoneal.

Experimental Design and Drug Administration.

SH-PAN-19 was administered to rats at doses of 25 mg/kg or 100 mg/kg. A Single dose was administered on gestation day 16 only. After 48 hours of the treatment (on gestation day 18), the animals were sacrificed by decapitation, and their weights were determined. Upon sacrificing the animals, the ovarian tissue was fixed by immersing them in 10% formalin. After fixation, both tissues were dehydrated with ascending ethanol concentrations for 15 min each, followed by embedding in paraffin wax as reported earlier. Afterward, the tissues were sliced (5 μ m thickness). After that, the H&E sections were observed and examined using a light microscope (Olympus CX31, Tokyo, Japan). The two horns of the uterus were excised and washed with cold PBS, and fetuses were obtained, and their weights and crown–rump length were determined. The fetuses were examined for any external abnormalities. The fetal and the mother's organs were kept at -80 °C for biochemical analysis. For the high-dose group, animals received a single oral dose of 100 mg/kg of the drug, while the corresponding control group received the vehicle only. In the low-dose group, animals received a single intraperitoneal dose of 25 mg/kg of the drug.

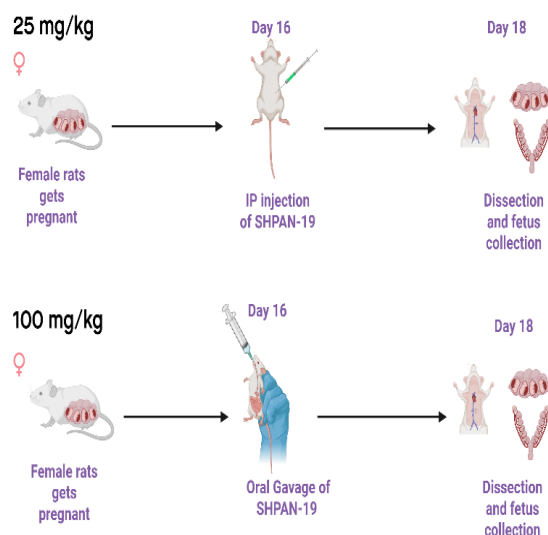


Figure 1. Schematic overview of experimental design. Created using BioRender.com.

Histological Analysis

Ovarian tissues were embedded in paraffin, sectioned 5 μm , and stained with hematoxylin and eosin (H&E). Sections were examined under a light microscope to assess follicular development, corpus luteum formation, and overall ovarian morphology. Histological comparisons were made between control and Treated groups at both 25 mg/kg and 100 mg/kg doses (Figure 1).

Results and Discussion

To solubilize the drug, organic solvents used were employed without overt adverse effects on early development, as illustrated by the tolerated concentrations summarized in **Table 1**, including PEG 400, corn oil, DMSO, and poloxamer 188. The drug was successfully dissolved in a mixture of DMSO and sunflower oil at a concentration higher than 4 mg/ml.

Table 1. Solubility of SH-PAN-19 in different formulations. Various solvents and excipient combinations were tested to identify suitable solubilization strategies for *in vivo* administration.

COMPONENTS			SOLUBILITY
A)	SALINE		Insoluble
B)	CORN OIL		Insoluble
C)	PEG 400	5%	Insoluble
	DMSO	1%	
	POLOXMAR188	12.5%	
	SALINE	81.5%	
D)	DMSO	+ SUNFLOWER OIL	Soluble ~ 4mg/ml
	SUNFLOWER OIL		

To investigate the effect of SH-PAN-19 on pregnancy and female fertility, pregnant rats were administered the drug at concentrations of 25 or 100 mg/kg as explained in Fig 1. The rats were sacrificed and dissected at gestational day 18. Morphological examination of the animals revealed a minimal reduction in weight and length of fetuses in animals administered 25mg/kg. On the other hand, administration of 100mg/kg resulted in resorption of the fetuses completely, as shown in Fig 2. This suggests that SH-PAN-19 shows a dose-dependent effect on fetal development. At 25 mg/kg, fetal weight and crown–rump length were reduced, indicating impaired growth. At 100 mg/kg, complete fetal resorption occurred, demonstrating severe embryotoxicity. These results suggest that SH-PAN-19 disrupts fetal viability in a dose-dependent manner, likely through cytotoxic effects on naturally dividing cells or interference with maternal-fetal support, highlighting the need for careful evaluation of its reproductive safety.

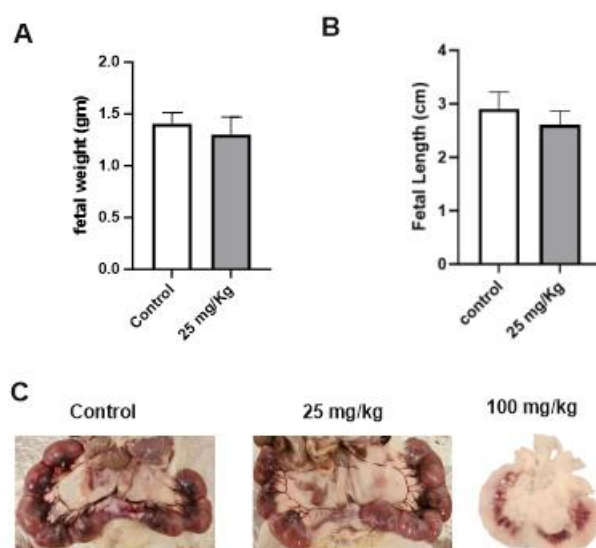


Figure 2. Comparison of fetal weight (A) and fetal crown–rump length (B) in control, 25 mg/kg, and 100 mg/kg treatment groups, with representative photographs of fetuses shown (C). The 100 mg/kg group resulted in complete fetal resorption and was excluded from quantitative analysis.

To further assess SH-PAN-19–induced ovarian toxicity in pregnant rats, ovarian tissues were examined by H&E staining (Figure 3). Ovaries from control pregnant rats exhibited normal histoarchitecture, characterized by an intact ovarian cortex, well-organized stromal tissue, and prominent corpora lutea consistent with pregnancy. In contrast, treatment with SH-PAN-19 at a dose of 100 mg/kg resulted in marked disruption of ovarian organization, including degeneration of the cortical region, stromal disintegration, vascular congestion, and extensive cellular degeneration, while in rats administered 25 mg/kg intraperitoneally, considerable follicular depletion and a reduced number of corpora lutea were noted, alongside partial preservation of stromal architecture.

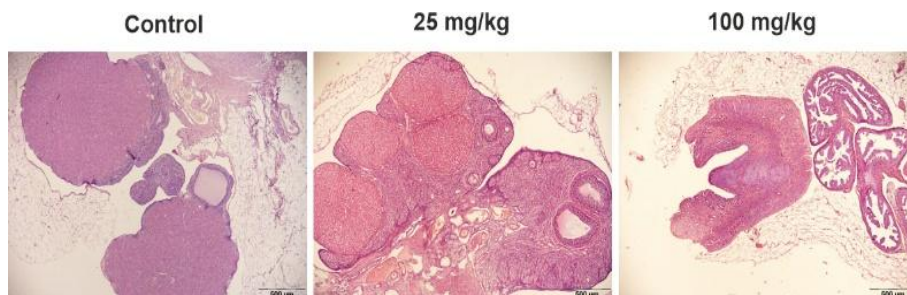


Figure 3. Representative H&E-stained ovarian sections: Photomicrographs of rat ovaries illustrating dose-dependent histological alterations. Ovaries from control pregnant rats show preserved architecture with well-developed corpora lutea, along with normal gestational activity. In rats exposed intraperitoneally to 25 mg/kg, moderate follicular depletion and fewer numbers of corpora lutea are observed, with partial preservation of stromal architecture. At the 100 mg/kg dose, ovaries display a significant reduction in corpora lutea, accompanied by diffuse stromal thinning, indicating pronounced impairment of normal ovarian cyclic and gestational function. n = 5 per group. Scale bar = 500 µm.

The control of non-pregnant ovaries exhibits preserved architecture with follicles at various developmental stages and well-organized stroma, indicating normal folliculogenesis. low-dose exposure (25 mg/kg), ovaries show partial structural restoration with reappearance of growing follicles and corpora lutea, with mild stromal alterations, and atretic follicles. In contrast, high-dose exposure (100 mg/kg) demonstrates incomplete regeneration, characterized by fewer developing follicles and corpora lutea with persistent stromal thinning and follicular atresia in Figure 4.

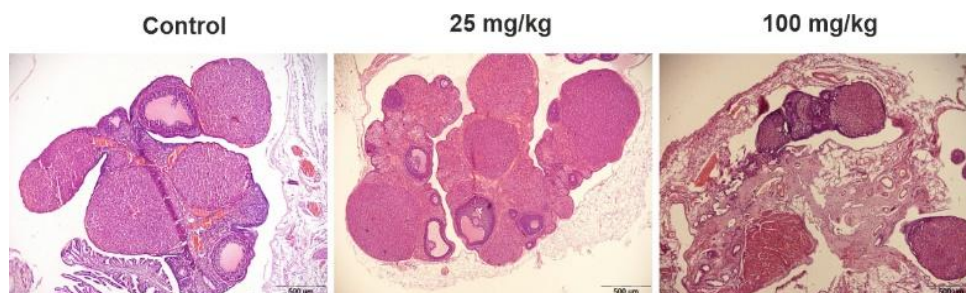


Figure 4. Representative H&E-stained ovarian sections after 21-day recovery. Photomicrographs showing recovery-associated histological changes in rat ovaries. Control, 25mg/kg and 100 mg/kg. n = 5 per group. Scale bar = 500 µm.

Conclusion

SH-PAN-19 exhibited clear, dose-dependent reproductive and developmental toxicity in pregnant rats. A single intraperitoneal dose of 25 mg/kg on gestation day 16 shows a minimal reduction in fetal weight and crown–rump length at gestation day 18, indicating impaired intrauterine growth without gross external malformations. In contrast, a single oral dose of 100 mg/kg resulted in complete fetal resorption, demonstrating severe embryotoxicity and loss of pregnancy at the higher exposure level. These functional outcomes were accompanied by marked histopathological alterations in the ovary, including cortical disruption, stromal disorganization, vascular congestion, and extensive cellular degeneration in pregnant animals, as well as incomplete structural recovery and persistent follicular atresia in non-pregnant ovaries at 100 mg/kg. Collectively, these findings indicate that SH-PAN-19 has a narrow or absent safety margin in pregnancy and exerts substantial, dose-related adverse effects on both ovarian structure and fetal development.

Recommendations

Future studies should incorporate detailed molecular analyses, including markers of apoptosis, oxidative stress, DNA damage, and endocrine disruption in ovarian and fetal tissues, to clarify the mechanisms underlying SH-PAN-19–induced reproductive and developmental toxicity.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

Conflict of Interest

* The authors declare that they have no conflicts of interest.

Funding

* The first author is supported by a scholarship from the Egyptian Ministry of Higher Education (MoHE), which is gratefully acknowledged.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on Research in Engineering, Technology and Science (www.icrets.net) held in Batumi/Georgia on July 09-12, 2026.

* This work was supported by the Egyptian Ministry of Higher Education (MoHE), by providing financial support through MoHE scholarship, and the Egypt-Japan University of Science and Technology (E-JUST) supported the research by providing the facilities and labs required to conduct this study.

References

- Anderson, R. A., Brewster, D. H., Wood, R., Nowell, S., Fischbacher, C., & Kelsey, T. W. (2018). The impact of cancer on subsequent chance of pregnancy: A population-based analysis. *Human Reproduction*, 33(7), 1281–1290.
- Chen, K., Lu, X., Xu, D., Guo, Y., Ao, Y., & Wang, H. (2023). Prenatal exposure to corn oil, CMC-Na or DMSO affects physical development and multi-organ functions in fetal mice. *Reproductive Toxicology*, 118, 108366.
- Choi, S.-H., Cho, Y.-S., Kim, M.-J., Lee, C.-H., Seong, H.-H., & Baek, S.-H. (2020). Studies on the induction of pregnancy and the number of fetuses during pregnancy in rats. *Journal of Animal Reproduction and Biotechnology*, 35(3), 232–238.
- Dalmartello, M., Negri, E., La Vecchia, C., Scarfone, G., Buonomo, B., & Peccatori, F. A. (2020). Frequency of pregnancy-associated cancer: A systematic review of population-based studies. *Cancers*, 12(6), 1356.
- Hammad, S. F. A. M. (2024). *Discovery of a highly selective and potent pro-apoptotic acyclic phosphoramidate nucleotide for hepatocellular carcinoma*. International patent application PCT/EG2024/000018. Retrieved from <https://patentscope.wipo.int/search/en/WO2025146241>
- Lu, Z., Guo, Y., Xu, D., Xiao, H., Dai, Y., & Liu, K. (2023). Developmental toxicity and programming alterations of multiple organs in offspring induced by medication during pregnancy. *Acta Pharmaceutica Sinica B*, 13(2), 460–477.
- Maes, J., Verlooy, L., Buenafe, O. E., De Witte, P. A., Esguerra, C. V., & Crawford, A. D. (2012). Evaluation of 14 organic solvents and carriers for screening applications in zebrafish embryos and larvae. *Neurotoxicology and Teratology*, 34(6), 627–635.
- Puzzi-Fernandes, C., Surita, F. G., Schettini, C. S., Parpinelli, M. A., Guida, J. P., & Costa, M. L. (2020). Awareness towards an increasing concern during pregnancy: Maternal and perinatal outcomes of women with cancer. *American Journal of Obstetrics & Gynecology MFM*, 2(3), 100168.

- Struys, I., Song, J. Y., Velazquez, C., Boadum, T., Posch, M. J., & van de Ven, M. (2024). Compound-dependent fetal toxicity after in utero exposure to chemotherapy in a pregnant mouse model. *Environmental Toxicology and Pharmacology*, 112, 104594.
- Wolters, V., Heimovaara, J., Maggen, C., Cardonick, E., Boere, I., & Lenaerts, L. (2021). Management of pregnancy in women with cancer. *International Journal of Gynecological Cancer*, 31(3), 314–322.
- Zhang, X., Meng, X., Chen, Y., Leng, S. X., & Zhang, H. (2017). The biology of aging and cancer: Frailty, inflammation, and immunity. *The Cancer Journal*, 23(4), 201–205.

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To cite this article:

Mabrouk, E.T., Hammad, S. F., & Osman, A. (2026). Ovarian histological alterations and fetal developmental effects induced by a novel anticancer phosphoramidate nucleotide in rats. *The Eurasia Proceedings of Science, Technology, Engineering and Mathematics (EPSTEM)*, 41, 234-240.