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ORIGINAL RESEARCH ARTICLE

Investigation of Valacyclovir Oral Administration on Kidney Morphogenesis in Rat Embryo (Histochemical Approach)

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Abstract

Background Viral infections during pregnancy can create a serious threat to fetal development. Herpesviridae viruses are among the most common infectious agents during this period. Valacyclovir, a prodrug of acyclovir, is widely used to treat infections caused by these viruses. Considering its ability to cross the placenta and its potential effects on the fetus, this study aimed to investigate the effects of valacyclovir on the morphogenesis and histogenesis of fetal kidneys in pregnant rats.

Methods In this experimental study, 24 pregnant rats were randomly assigned to four groups. The control group received no treatment, while the experimental groups received valacyclovir at 100, 200, and 300 mg/kg of body weight daily. On gestational day 20, the animals were euthanized. Following blood sampling, fetuses were removed, and their kidneys were collected. Tissue samples were examined using hematoxylin-eosin, periodic acid-Schiff, and Masson's trichrome staining methods.

Results The results showed that increasing doses of valacyclovir significantly reduced crown-rump length and fetal weight. Additionally, kidney size and weight were markedly decreased. Serum levels of creatinine and blood urea nitrogen were significantly elevated. Furthermore, malondialdehyde levels were increased in the third experimental group. Histological assessments revealed structural damage in fetal kidneys, especially in the high-dose treatment groups.

Conclusion These findings indicate that high doses of valacyclovir can adversely affect fetal growth and kidney development. Therefore, its administration during pregnancy should be approached cautiously.

Keywords Fetus, Kidney, Rat Model, Valacyclovir

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1 Introduction

Viral infections during pregnancy are among the most common and high-risk infections.[1] Among these, herpes virus infections are particularly dangerous. Herpes simplex virus type 1 (HSV-1) causes oral and facial infections, affecting approximately 60–95% of adults.[2] Herpes simplex virus type 2 (HSV-2) is responsible for genital herpes, which can be transmitted to neonates and poses a significant risk to them.[3] Varicella-zoster virus causes shingles, a painful skin condition.[4] Cytomegaloviruses not only contribute to infertility but also pose serious risks during pregnancy, as they can lead to congenital infections in neonates.[2] Epstein-Barr virus is one of the common causes of human infections and can cause respiratory disease in newborns.[5] Acyclovir is an antiviral drug used to treat infections caused by herpes viruses. It can be administered orally or intravenously. Acyclovir can cross the placenta and has been detected in the umbilical cord and amniotic fluid. The drug is excreted via the kidneys, and its half-life is approximately three hours.[6] Valacyclovir, marketed as Valtrex, is an antiviral prodrug of acyclovir. It is administered orally (in 500 mg and 1000 mg doses), and after administration, it is converted in the body to acyclovir triphosphate, which can bind to plasma proteins.[7] The drug is excreted through urine and feces, and its half-life ranges from 2.5 to 3 hours. Acyclovir triphosphate inhibits viral DNA polymerase, thereby halting viral replication.[8,9]

From a physiological perspective, the kidneys are among the most complex organs in the human body, playing a critical role in regulating pH, electrolyte balance, and fluid balance. During organogenesis, the intermediate mesoderm gives rise to three excretory organs: pronephros, mesonephros, and metanephros. The metanephric kidney persists postnatally, with the nephron as its functional unit.[10] Given the potential for placental transfer and possible effects on the fetus, this study aimed to investigate the impact of valacyclovir on the morphogenesis and histogenesis of the kidneys in embryos of pregnant mice.

2 Methods

Valacyclovir, with 99% purity and the chemical formula $C_{13}H_{20}N_6O_4$, was purchased from Xi'an Haoze Biotechnology, China. A total of 24 adult female mice, 11 weeks old, with an average weight of 200–220 g, were obtained from the Animal Breeding and Maintenance Center of the Yazd Reproductive Sciences Research Institute. Pregnancy in mice was confirmed by vaginal plug examination. The mice were then weighed and randomly divided into four groups of six animals each. The control group received only distilled water, while the three experimental groups (1, 2, and 3) received oral doses of valacyclovir at 100, 200, and 300 mg/kg, respectively, administered daily until the 20th day of pregnancy.[11,12]

On day 20, six hours after the last drug administration, the mice were anesthetized with chloroform. Blood was collected from the heart following euthanasia. For serum separation, the blood samples were centrifuged at 6000 rpm for 5 minutes to obtain clear, hemolysis-free serum. The serum was then transferred to microtubes using a sampler and stored at −70°C until further analyses, including measurements of malondialdehyde (MDA), glutathione peroxidase (GPx), and renal function markers,blood urea nitrogen (BUN) and creatinine (Cr).

After opening the abdominal cavity, the embryos were removed from the uterus. The body weight, crown-rump length, and abdominal circumference of the embryos were measured, and then the embryos were fixed in 10% formalin. After 48 hours, the embryonic kidneys were prepared for histological processing. The samples were embedded, sectioned, and stained using three methods: hematoxylin-eosin (H&E), PAS, and Masson's trichrome. Photomicrographs were taken using an Olympus microscope (Japan). Serum Cr levels were measured using a diagnostic kit from Darman Kav Company via the Jaffe method with a spectrophotometer at 520 nm. BUN levels were determined using a colorimetric method with a kit from Darman Kav Company, and absorbance was measured at 520 nm. MDA levels were measured using a kit from Zellbio, Germany, with absorbance read at 532 nm. GPx activity was measured using a Zellbio kit, and the absorbance of samples and standards was recorded at 340 nm over 2 minutes. All measurements were performed using a UNICO UV-Vis 2150 spectrophotometer. Statistical analyses were conducted using SPSS software, version 26. Data were analyzed using one-way analysis of variance (one-way ANOVA) followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

3 Results

Morphological Study of the Embryos

Morphological examination of the embryos revealed that all embryos in all groups appeared healthy, with no visible malformations or deformities. No fetal deaths were observed (Figure 1).

Body Weight, Crown-Rump Length, and Abdominal Circumference of the Embryos

Comparison of body weight and crown-rump length between the control group and the experimental groups showed that the mean body weight and crown-rump length of embryos in experimental groups 2 and 3 were significantly decreased ($p < 0.05$) compared to the control group (Table 1). The mean abdominal circumference of

Table 1 Mean changes in body weight, crown-rump length, and abdominal circumference of embryos in different experimental groups. All data are presented as Mean ± SD. Values with different letters (a, b, c) indicate statistically significant differences ($p < 0.05$)

Groups	Control	Experimental 1	Experimental 2	Experimental 3
Embryo weight (g)	5.69 ± 0.47 a,c	5.57 ± 0.54 a,c	5.6 ± 0.5 b,d	4.94 ± 0.57 b,d
Crown-rump length (CRL) (mm)	39.42 ± 2.28 a,c	39.15 ± 2.55 c,a	36.6 ± 2.55 b,d	35.57 ± 2.88 b,d
Abdominal circumference (AC) (mm)	14.88 ± 0.79 a	14.92 ± 0.95 a,b	14.24 ± 1.3 a	13.92 ± 1.28 a,c

the embryos decreased in the experimental groups with increasing doses of valacyclovir compared to the control group, although this reduction was not statistically significant (Table 1 and Figure 1).

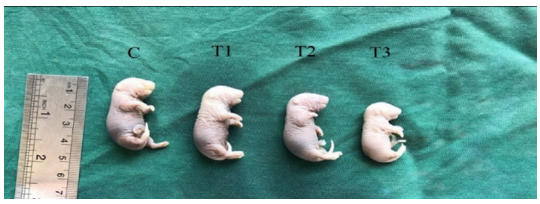

Figure 1 Comparison of crown-rump length and abdominal circumference of embryos across all experimental groups

Morphological Study of the Embryonic Kidneys

Macroscopic examination of the embryonic kidneys showed that the mean kidney weight in experimental group 3 was significantly reduced ($p < 0.05$) compared to the control group. Additionally, the mean kidney length and width in experimental groups 2 and 3 were significantly decreased ($p < 0.05$) compared to the control group (Table 2 and Figure 2)

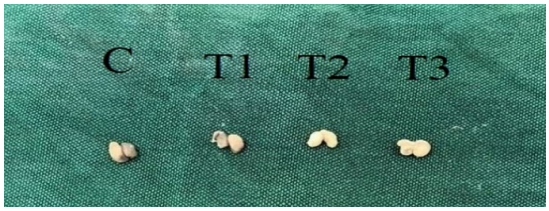

Figure 2 Morphological comparison of embryonic kidneys across all experimental groups

Assessment of Renal Indicators

Serum Cr and BUN, measured as renal function indices in pregnant rats, showed that the mean Cr and BUN levels in experimental groups 2 and 3 were significantly higher than those in the control group ($p < 0.05$) (Figure 3).

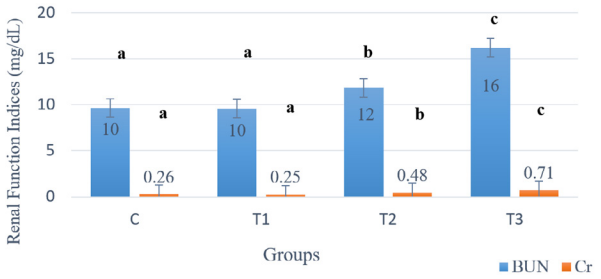

Figure 3 Changes in renal indicators of maternal rats across the experimental groups. All data are presented as Mean ± SD. Columns marked with different letters (a, b, c) indicate statistically significant differences ($p < 0.05$)

Assessment of Biochemical Indices (MDA and GPx)

Serum lipid peroxidation, evaluated through MDA measurement, demonstrated a significant increase in the mean MDA level in experimental group 3 compared with the control group ($p < 0.05$). A significant decrease in this index was also observed in experimental group 1 relative to the control group ($p < 0.05$) (Figure 4).

The results of GPx assessment in maternal rat serum indicated a significant reduction in the mean enzyme level in experimental groups 2 and 3 compared with the control group ($p < 0.05$). In addition, the mean GPx level in experimental group 1 showed a significant increase relative to the control group ($p < 0.05$) (Figure 5).

Table 2 Results of changes in mean fetal weight, kidney length, and kidney width across the experimental groups. All data are presented as Mean ± SD. Values marked with different letters (a, b, c) indicate statistically significant differences ($p < 0.05$)

Groups	Control	Experimental 1	Experimental 2	Experimental 3
Kidney weight (g)	0.21 ± 0.02 a,c	0.21 ± 0.01 a,c	0.20 ± 0.01 a,b,c	0.19 ± 0.01 b
Kidney length (mm)	3.9 ± 0.09 a,c	3.88 ± 0.2 a,c	3.64 ± 0.17 b,d	3.59 ± 0.19 b,d
Kidney width (mm)	2.21 ± 0.17 a,c	2.18 ± 0.19 a,c,d	2.04 ± 0.15 b,d	2.01 ± 0.15 b,d

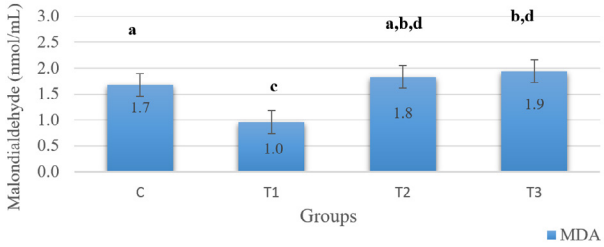

Figure 4 Results of MDA assessment in maternal rats across the experimental groups. All data are presented as Mean ± SD. Columns marked with different letters (a, b, c) indicate statistically significant differences ($p < 0.05$)

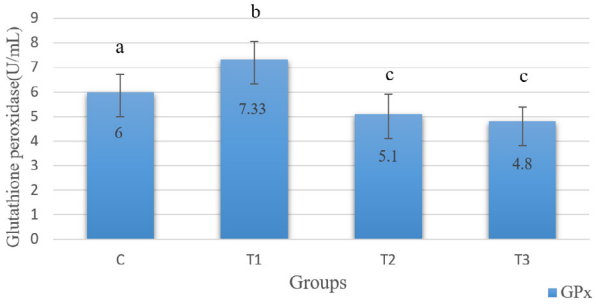

Figure 5 Results of GPx assessment in maternal rats across the experimental groups. All data are presented as Mean ± SD. Columns marked with different letters (a, b, c) indicate statistically significant differences ($p < 0.05$)

Microscopic Evaluation of Fetal Kidney Tissue

Histopathological examination of fetal kidney tissue across the experimental groups (H&E staining) showed that increasing the drug dose led to mild disruption of tubular architecture and dilation of renal tubules. These alterations, including tissue disorganization and tubular dilation, were more prominent in experimental group 3 (Figure 6).

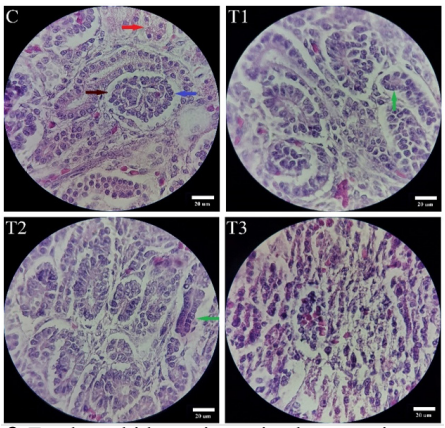

Figure 6 Fetal rat kidney tissue in the experimental groups (H&E, 1000×).
Red arrow: proximal convoluted tubule; green arrow: distal convoluted tubule; brown arrow: urinary space; blue arrow: renal corpuscle

Histopathological assessments using PAS staining revealed the presence of abundant glycoproteins in the brush border of proximal convoluted tubule cells. Based on this staining pattern, brush-border staining intensity increased in experimental groups 1 and 2, whereas a reduction in staining intensity was observed in experimental group 3 (Figure 7).

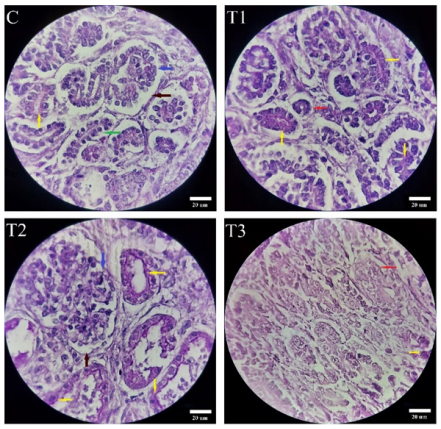

Figure 7 Fetal rat kidney tissue in the experimental groups (PAS, 1000×). Red arrow: proximal convoluted tubule; green arrow: distal convoluted tubule; brown arrow: urinary space; blue arrow: renal corpuscle; yellow arrow: glycoproteins

The results of fetal kidney tissue evaluation using Masson's trichrome staining (in which blue staining indicates collagen deposition and tissue fibrosis) showed no evidence of tissue fibrosis in any of the experimental groups (Figure 8).

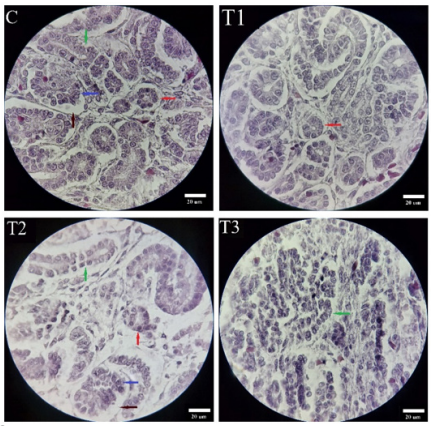

Figure 8 Fetal rat kidney tissue in the experimental groups (Masson's Trichrome, 1000×).
Red arrow: proximal convoluted tubule; green arrow: distal convoluted tubule; brown arrow: urinary space; blue arrow: renal corpuscle

4 Discussion

Herpesviruses are currently recognized as among the most prevalent infections, with a high risk of vertical transmission to the fetus, potentially leading to mild

diseases, congenital disabilities, structural anomalies, or even fetal death. Therefore, preventive measures, including antiviral therapy and cesarean delivery in specific cases, are considered necessary.[13,14] Valacyclovir is a suitable antiviral regimen against herpesviruses. It is an oral L-valyl ester prodrug that rapidly converts to acyclovir in the body, enhancing its efficacy compared to oral acyclovir. Antiviral drugs can cross the placental barrier and exert significant effects on fetal development, which vary depending on the drug type and usage context.[15] Valacyclovir is a nucleoside analogue antiviral. Another drug in this class, ribavirin, was studied by Magdy et al. for its embryotoxic effects in Wistar rats during different stages of pregnancy. They reported that ribavirin administration negatively affected fetal development, causing intrauterine growth retardation, reduced fetal weight, and morphological abnormalities.[16]

Paluch et al. found that high-dose acyclovir injections could induce renal failure and nephrotoxicity due to drug crystallization in renal tubules. Elevated plasma concentrations and central nervous system (CNS) effects may also lead to serious neuropsychiatric side effects, including confusion, delirium, or other psychiatric symptoms.[17] The findings of the present study align with Paluch et al.'s results, as increased drug doses were associated with restlessness and anorexia.

A study by Aydemir investigated the anti-inflammatory and immunomodulatory effects of valacyclovir on mammalian macrophages. It showed that valacyclovir reduced the production of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), in LPS-activated macrophages, indicating the drug's potential as an anti-inflammatory agent. Aydemir concluded that valacyclovir could serve as a clinically relevant antiviral drug with anti-inflammatory properties, contributing to the reduction of inflammation under various conditions.[18] In studies by Hu et al. on diabetic mice, animals treated with low doses of acyclovir exhibited reduced oxidative stress.[19] Considering the decrease in MDA at 100 mg/kg and its increase at 300 mg/kg, along with the rise in GPx at 100 mg/kg and its reduction at 300 mg/kg, and the histological findings, it can be concluded that the present study's low-dose results are consistent with the findings of Aydemir and Hu, indicating that valacyclovir exerts dose-dependent negative effects. Adikwu and Kemelayefa investigated the protective effects of curcumin against acyclovir-induced nephrotoxicity. In their study, one group received only acyclovir, showing signs of renal toxicity, including elevated serum Cr and BUN levels, along with increased inflammatory responses and oxidative stress.[20] Similarly, Badawi's evaluation of vitamin D on acyclovir-induced kidney injury in adult male albino mice showed histopathological kidney damage and cellular necrosis caused by acyclovir. Badawi also reported increased serum Cr and BUN, indicating reduced renal function, as well as elevated oxidative stress in groups not receiving vitamin D, reflected by increased MDA and decreased antioxidant levels, such as superoxide dismutase and GPx.[21] These findings align with the results of the present study.

In another study, Lu et al. examined acyclovir-induced nephrotoxicity in mice. Histological analyses of the kidneys revealed tubular necrosis and glomerular structural damage following acyclovir administration, with significant increases in serum Cr and BUN levels, indicating impaired renal function.[22] The present study observed similar kidney tissue damage. It demonstrated that high-dose valacyclovir administration caused renal tissue injury, potentially linked to increased oxidative stress, elevated serum Cr, and BUN levels. Histopathological findings confirmed these destructive effects. Moreover, this study showed that increasing the administered drug dose significantly exacerbated the observed renal damage.

5 Conclusion

Based on the morphological, histopathological, and biochemical analyses conducted in this study, it can be concluded that although valacyclovir administration during pregnancy does not induce specific morphological abnormalities in the fetus, increasing the drug dose can significantly reduce fetal weight and cranio-caudal length. Furthermore, at higher doses, the drug contributed to oxidative stress by generating reactive oxygen species, leading to a decrease in GPx enzyme activity and an increase in MDA levels. The significant elevation of renal function indices (Cr and BUN) in the high-dose groups also indicated maternal kidney damage, representing another adverse effect of the drug. Histopathological evaluation of fetal kidneys revealed tissue injury and dilation of renal tubules. Therefore, although further studies are needed to assess the long-term effects, the findings of this study suggest that valacyclovir administration during pregnancy should be approached with caution.

Declarations

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Artificial Intelligence Disclosure
The authors declare that this manuscript was prepared without the use of AI tools.

Authors' Contributions
The authors contributed to the conception, study design, data collection, and drafting of the manuscript. All authors have reviewed and approved the final version and have no disagreements regarding any sections of the paper.

Availability of Data and Materials
The datasets generated and/or analyzed during the current study are not publicly available due to privacy and confidentiality agreements with the participants. Still, they are available from the corresponding author upon reasonable request.

Conflict of Interest
The authors declare that this work was conducted independently and that there are no conflicts of interest with any organizations or individuals.

Consent for Publication
Not applicable.

Ethical Considerations
This study was conducted in accordance with ethical principles and approved by the University Ethics Committee under the Code of Ethics IR.ARDAKAN.REC.1403.044.

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