ORGINAL RESEARCH ARTICLE

Effect of Paxlovid on Liver Morphogenesis in Animal Model Embryo (Rat)

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Abstract

Background The antiviral drug Paxlovid has been approved for the treatment of COVID-19 in adult patients. This study aimed to investigate the potential effects of Paxlovid administration on liver morphogenesis in rat embryos.

Methods Pregnant rats were randomly assigned to four groups (n = 10 per group): a control group (vehicle), experimental group one (Paxlovid 60 and 100 mg/kg), experimental group two (Paxlovid 200 and 300 mg/kg), and experimental group three (Paxlovid 1000 mg/kg). The Animals were treated daily via oral gavage. Maternal body weight was recorded on gestational days seven, 14, and 21. On gestational day 17, blood samples were collected from five animals in each group at five different time points. Paxlovid concentrations in the serum samples were quantified using high-performance liquid chromatography.

Results The results showed no structural changes in liver tissue. However, the maternal body weight on day 21 of pregnancy was significantly lower in experimental group one compared with the control group ($p < 0.05$). Fetal weight in all three experimental groups was significantly reduced compared with the control group. In addition, crown–rump length and abdominal circumference in experimental groups one and two were significantly decreased compared with the control group ($p < 0.05$). Measurement of systemic drug concentration indicated that experimental group two had the highest level of drug absorption, while experimental group three showed the lowest absorption.

Conclusion Overall, our findings indicate that although Paxlovid, at the investigated doses, may affect maternal weight and fetal growth parameters, it does not induce morphological or histological abnormalities in the fetal rat liver.

Keywords Liver, Morphogenesis, Nirmatrelvir/ Ritonavir, Paxlovid, Rats, SARS-CoV-2

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1 Introduction

The liver is a vital center for many physiological processes. It is composed of several cell types with distinct embryonic origins, including hepatocytes, biliary epithelial cells, Kupffer cells, and liver sinusoidal endothelial cells. Each of these cells has unique functions that collectively regulate liver activity at different levels.[1,2] Hepatocytes are the main functional cells of the liver, responsible for diverse physiological activities. In mammals, the fetal liver also serves as a hematopoietic site.[3] Early liver development begins as an endodermal bud derived from the anterior foregut, which invades the surrounding mesenchyme. This mesenchyme induces the endoderm, ultimately giving rise to the hepatic epithelium. The portion of the hepatic bud located near the foregut develops into the hepatic outflow tract, and branches derived from this duct form the gallbladder.[4] During these developmental processes, specific hepatic genes and signaling pathways—including Hhex, alpha-fetoprotein, bone morphogenetic protein, and hepatocyte nuclear factors—are activated in these cells and play essential roles.[5–7]

Since the onset of the COVID-19 pandemic, several antiviral drugs have been introduced and developed to combat severe acute respiratory syndrome coronavirus (SARS-CoV-2). The use of Paxlovid, a combination of nirmatrelvir and ritonavir, has been approved by the U.S. Food and Drug Administration for the treatment of COVID-19 patients.[8] Paxlovid consists of nirmatrelvir, a second-generation protease inhibitor, and ritonavir, a pharmacological enhancer, and is used to treat SARS-CoV-2 infection.[9] As an oral therapeutic option, Paxlovid is effective for patients with mild to moderate COVID-19, reducing disease severity in those at high risk. Nirmatrelvir is a potent and selective inhibitor of the main protease (Mpro), which plays a critical role in viral replication. By preventing the cleavage of two viral polyproteins, it inhibits viral propagation.[10,11]

Furthermore, nirmatrelvir blocks the key enzyme required by SARS-CoV-2 to assemble functional viral particles. As a result, after treatment, released viral particles are no longer able to infect healthy cells. Ritonavir, initially developed for HIV treatment, is included in Paxlovid not for direct antiviral activity against SARS-CoV-2 but to enhance nirmatrelvir's effect through inhibition of CYP3A4.[12] In fact, while nirmatrelvir is a peptidomimetic inhibitor of the SARS-CoV-2 main protease, ritonavir is a protease inhibitor of HIV-1 and a CYP3A4 inhibitor that is prescribed to improve the pharmacokinetics of nirmatrelvir without any direct activity against SARS-CoV-2 Mpro.[7,13]

Pregnant women are among the most vulnerable groups to COVID-19 infection, and evaluating potential fetal risks associated with Paxlovid use is of particular importance. With the increasing prescription of Paxlovid in pregnant patients with COVID-19, concerns have been raised about its possible effects on the development and growth of vital fetal organs, especially the liver. Despite regulatory approval, evidence regarding its safety during pregnancy and its impact on intrauterine development remains limited. Therefore, the present study was designed to investigate the effects of Paxlovid on liver morphogenesis in a fetal animal model.

2 Methods

A total of 40 pregnant Wistar rats, 11 weeks of age and weighing 190–250 g, were obtained from the Animal Breeding and Research Center of the Reproductive Sciences Institute. Pregnancy was confirmed by vaginal plug observation, and animals were considered at gestational day one upon confirmation. Rats were randomly housed in standard polyethylene cages (10 animals per cage) with free access to fresh water and a standard rodent diet, under controlled laboratory conditions (relative humidity 40–60%, temperature 22 ± 2°C, and a 12:12 h light–dark cycle).

The pregnant rats were divided into four groups: one control group and three experimental groups. Drug administration was performed orally by gavage in two distinct time intervals. From gestational days 0–6, the control group received 1 mL of distilled water (drug vehicle) daily. In contrast, the experimental groups T1, T2, and T3 received Paxlovid at doses of 60, 200, and 1000 mg/kg, respectively, in the same volume. From gestational days 7–17, the doses were increased: group T1 received 100 mg/kg, group T2 received 300 mg/kg, and group T3 continued at 1000 mg/kg.

On gestational day 17, five rats from each experimental group were randomly selected for tail-snip blood collection. Sampling was performed at baseline (30 minutes before drug administration) and at 30, 60, 120, and 240 minutes post-administration. In the control group, two rats were sampled once to obtain control serum. All animals continued receiving treatment until the end of the study. On gestational day 21, all 40 rats were euthanized (chloroform anesthesia).[11]

Blood samples were centrifuged, and sera were stored in microtubes at –60°C until analysis. Systemic drug concentrations were determined using a high-performance liquid chromatography (HPLC) system (AZURA, KNAUER, Germany). This chromatographic method achieved complete separation of nirmatrelvir (retention time 4.9 min) and ritonavir (retention time 6.8 min), allowing quantification in the range of 1.0–20.0 µg/mL in both pure and pharmaceutical forms.[14] On day 21, liver samples were fixed in buffered formalin and processed for histological staining using hematoxylin–eosin (H&E), periodic acid–Schiff (PAS), and Masson's

trichrome.

Maternal weight, fetal weight, crown–rump length, and abdominal circumference were analyzed using SPSS version 22. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied for group comparisons. Data were expressed as mean ± standard deviation (SD), and statistical significance was set at $p < 0.05$.

3 Results

Maternal Body Weight

Pregnant rats showed the most significant weight gain on gestational day 21 compared with day seven. Although no clinical symptoms of illness were observed, weight reduction on day 14 of pregnancy was noted in all three experimental groups, which may indicate systemic drug effects. Maternal weight in experimental group one was significantly lower than in the control group on day 21 ($p < 0.05$). In experimental groups two and three, a reduction was also observed on day 21, though it did not reach statistical significance (Figure 1).

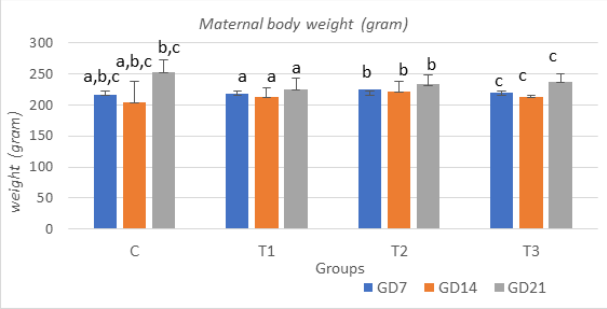

Figure 1 Maternal body weight on gestational days seven, 14, and 21

Fetal Weight

The mean fetal weight was significantly reduced in all three experimental groups compared with the control group ($p < 0.05$). However, there was no significant difference in mean fetal weight between experimental groups one and two ($p < 0.05$) (Figure 2).

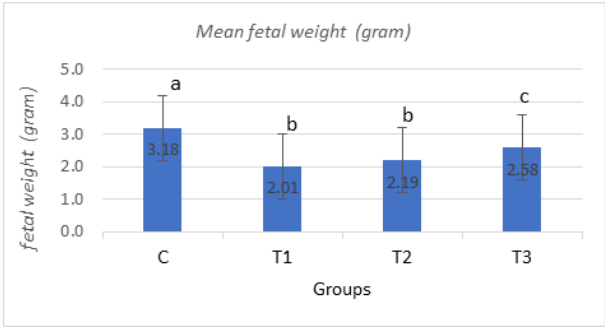

Figure 2 Mean fetal weight in different groups

Fetal Abdominal Circumference

According to the results, the mean abdominal circumference was significantly reduced in experimental groups one and two compared with the control group ($p < 0.05$). No significant difference was found in group three relative to the control (Figure 3).

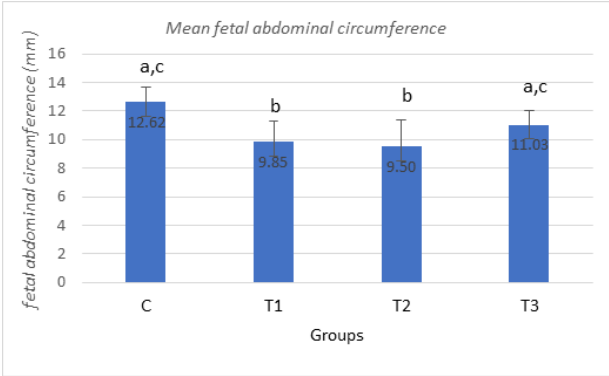

Figure 3 Mean fetal abdominal circumference

Fetal Crown–Rump Length

The mean crown–rump length in group 3 did not differ significantly from the control group. However, significant reductions were observed in experimental groups one and two compared with the control group ($p < 0.05$) (Figure 4).

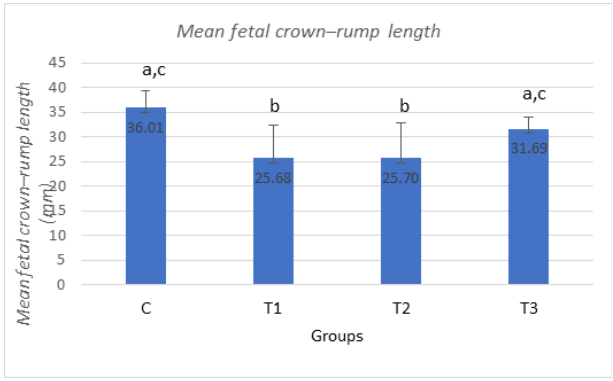

Figure 4 Mean fetal crown–rump length

Systemic Drug Concentration (HPLC Analysis)

Systemic drug concentration analysis showed that experimental group two exhibited the highest absorption, while group three had the lowest. The retention times were 1.81 minutes for nirmatrelvir and ritonavir (Figures 5 and Figure 6).

Histopathological Findings

- Hematoxylin and Eosin (H&E) Staining:

Microscopic examination of fetal liver tissue in the control group revealed a regular arrangement of hepatocytes with distinct nuclei. Blood cells and multinucleated megakaryocytes with abundant cytoplasm were usually observed. No significant differences were detected in the

experimental groups compared with the control. The liver tissue appeared entirely normal and free of pathological lesions in all groups (Figure 7).

• Periodic Acid–Schiff (PAS) Staining:

In the control group, hepatocyte arrangement was regular and orderly. PAS staining revealed hepatocytes with pink cytoplasm, and glycogen deposits were clearly visible in pink to magenta in both cytoplasmic and perinuclear regions. The amount of glycogen was comparable across all groups (Figure 8).

• Masson's Trichrome (MT) Staining:

All groups showed regularly arranged cells. The absence of collagen fibers in all four groups confirmed the lack of pathological lesions such as fibrosis (Figure 9).

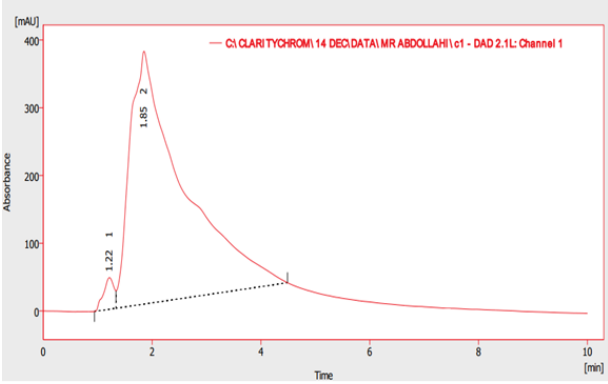

Figure 5 Systemic drug concentration curve

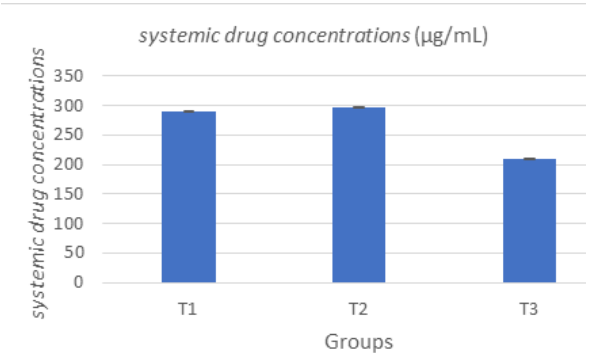

Figure 6 Comparison of systemic drug concentrations across groups

Liver Morphogenesis in Experimental Groups

Macroscopic evaluation of fetal liver in all three experimental groups compared with the control revealed no pathological lesions, including shrinkage, swelling, or hemorrhage (Figure 10).

4 Discussion

The present study demonstrated that Paxlovid had no significant impact on fetal liver morphogenesis in the rat model, and liver tissue remained completely normal in both morphological and histological assessments.

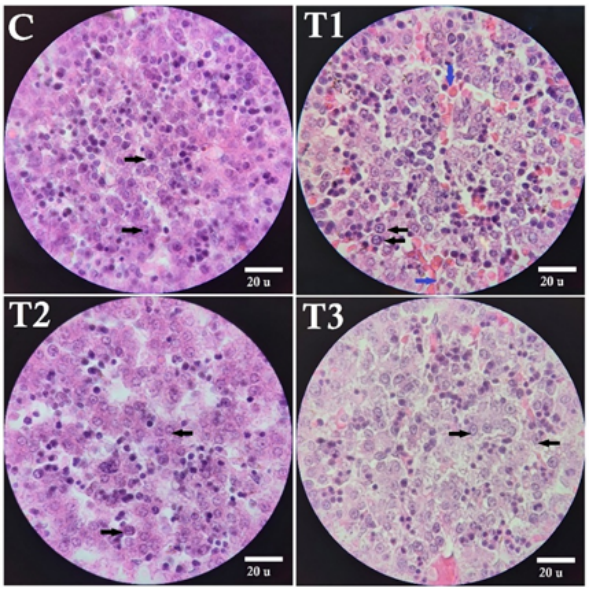

Figure 7 Microscopic view of fetal rat liver tissue in all groups (H&E, ×1000). Blue arrows: red blood cells and hematopoietic lineage cells; black arrows: hepatocytes.

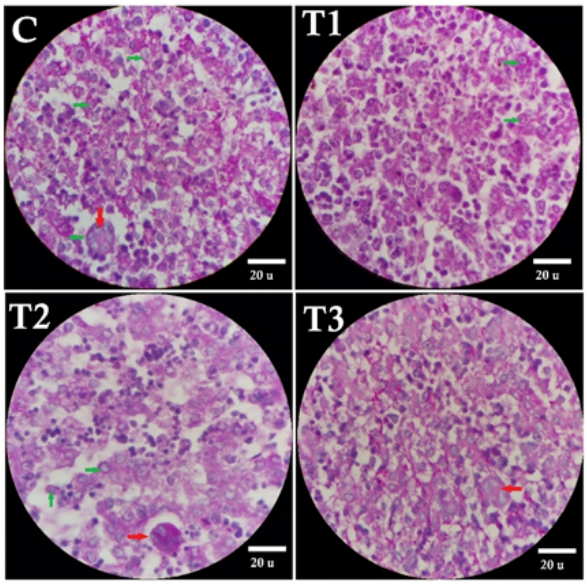

Figure 8 Microscopic view of fetal rat liver in all groups (PAS, ×1000). Green arrows: glycogen accumulation in the cytoplasm; red arrows: megakaryocytes.

Morphological evaluation showed no changes in texture, color, or size of the liver in the experimental groups compared with the control. These findings were consistent with histological staining results, including hematoxylin–eosin, PAS, and Masson's trichrome. H&E staining revealed that all cellular components—including hepatocytes, central veins, and megakaryocytes—were arranged normally. PAS staining confirmed the presence of glycogen without evidence of cellular damage. Similarly, Masson's trichrome staining showed no signs

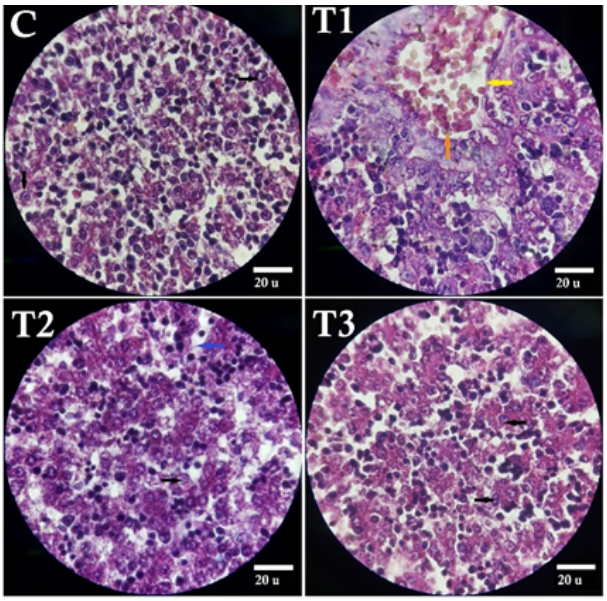

Figure 9 Microscopic view of fetal rat liver in all groups (MT, ×1000). Black arrows: hepatocytes; blue arrows: sinusoids and hematopoietic cells; yellow arrows: central vein with epithelial lining cells; orange arrows: blood cells.

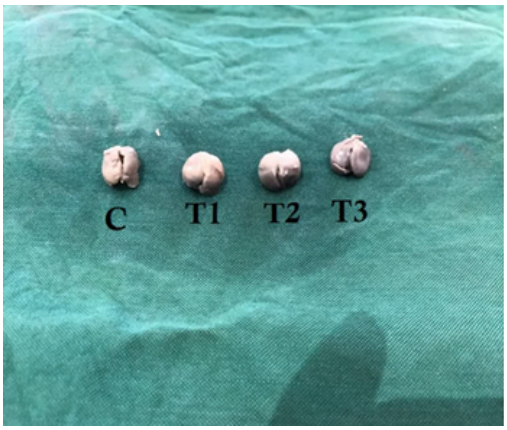

Figure 10 Gross morphology of fetal liver in experimental groups

of fibrosis or collagen deposition. Taken together, these results indicate that Paxlovid administration during gestation does not induce pathological alterations in fetal liver morphogenesis.

In addition, crown–rump length analysis showed no significant reduction in experimental group three (1000 mg/kg) compared with the control, whereas significant decreases were observed in groups one and two. Maternal weight assessment revealed that, on gestational day 21, experimental group one showed a significant reduction compared with the control. In contrast, reductions observed in groups two and three were not statistically significant.

HPLC analysis provided further insight into systemic drug absorption. Interestingly, groups receiving intermediate doses (200 and 300 mg/kg) exhibited higher systemic concentrations compared with those receiving the highest dose (1000 mg/kg). This finding may reflect saturation of the absorption pathway or enhanced metabolism at higher doses, highlighting the importance of optimized dosing to achieve maximum efficacy with minimal adverse effects.

Comparable observations have been reported with other protease inhibitors. Lopinavir/ritonavir, a protease inhibitor boosted with ritonavir, is commonly used in combination antiretroviral therapy for pregnant women with HIV to prevent mother-to-child transmission. A systematic review evaluating maternal and neonatal safety outcomes reported no concerns regarding safety or efficacy when standard doses of lopinavir/ritonavir were used in pregnant women.[15] Similarly, Vangelotti et al. investigated the effects of lamivudine and ritonavir, administered at varying doses, on fetal liver development in a murine model. Their findings showed no impact on normal hepatic morphology, with liver histology in all experimental groups comparable to controls.[16] These results are consistent with the present study, as no evidence of significant hepatic damage was observed across experimental groups.

Future research should aim to elucidate the mechanisms of absorption and metabolism of Paxlovid at higher doses, as well as to investigate its long-term effects on liver growth and function.

5 Conclusion

In this study, microscopic sections of fetal livers were examined after dissection. The results showed that all extracted livers were within normal limits in terms of their appearance, including softness of the tissue, color, and size, and no abnormal changes were observed. These results indicate that the normal state of the fetal liver was maintained under the conditions studied and suggest that the factors that may affect the morphology of this organ were not active in these samples. Systemic analysis of drug concentration in blood serum showed that the highest drug level was observed in the second experimental group and the lowest in the third experimental group. The findings demonstrated that Paxlovid administration at the studied doses did not induce histological alterations in fetal liver tissue; however, it did influence fetal growth indices.

Declarations

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Authors' Contributions
All authors contributed to the initial conception, study design, data collection, and manuscript drafting. All authors have reviewed and approved the final version of the manuscript and declare no disagreements regarding its content.

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
All authors have read and approved the final manuscript and have provided their consent for publication.

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Ethical Considerations
The Ethics Committee of Ardakan University approved this study under the Code of Ethics IR.ARDAKAN.REC.1403.045.

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

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