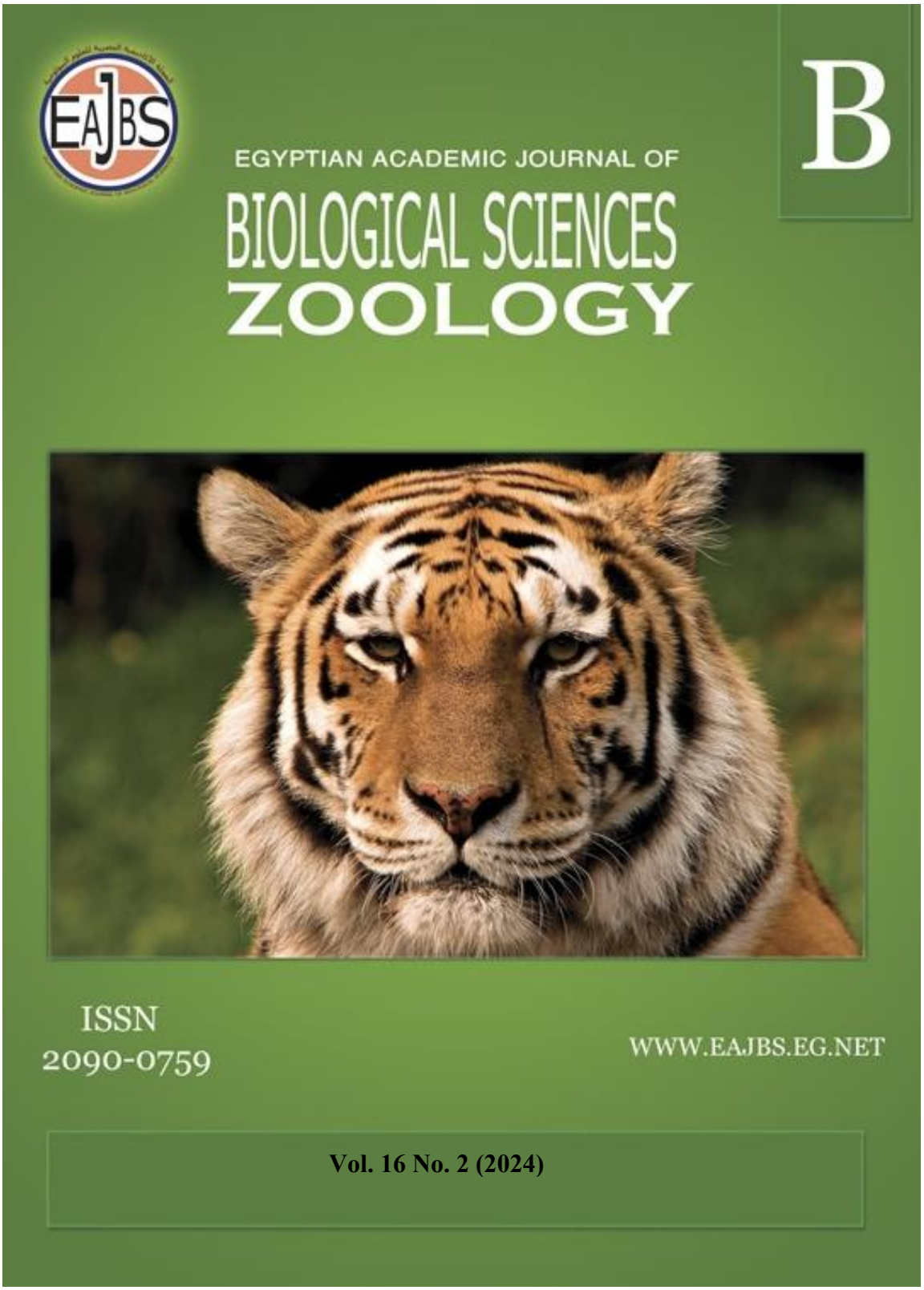

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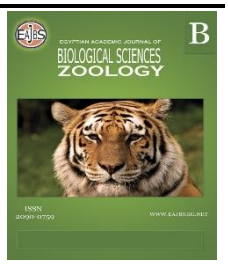

Developmental Toxicity Induced by Prenatal Exposure to Remdesivir in Pregnant Female Rats and Their Offspring

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Abstract

Remdesivir is one of the nucleoside prodrugs with a broad spectrum of activity against zoonotic and human corona-viruses. Remdesivir was the first drug to be approved for the treatment of COVID-19, and studies on hospitalized patients needing oxygen therapy have shown that it can speed up their recovery. Our study aimed to assess the development and effects of remdesivir on pregnant rats and their fetuses during the organogenesis phase of pregnancy. Thirty pregnant female rats were randomly assigned into 3 groups (n=10). These groups were control, remdesivir (10 mg/kg/rat), and remdesivir (20 mg/kg/rat). The developmental toxicity caused by remdesivir exposure was evidenced by increased resorption sites and fetuses suffering from growth retardation, and skeletal deformities. The histological Examination of the maternal placental tissues in remdesivir-treated groups revealed numerous histopathologic changes. Considering the results of our study, it appears that the risk of remdesivir to the maternal tissues and their fetuses is present but in faint type. This study is critical because there has to be a broad examination of the possible adverse effects of remdesivir due to its increasing usage in medical treatments and the related health consequences. Therefore, it is essential to investigate innovative approaches.

INTRODUCTION

In recent years, much knowledge has been acquired on the impact of COVID-19 on pregnancy and perinatal outcomes. The results are alarming; pregnant individuals in both high-income nations and low and middle-income countries have been seen to have an increased incidence of serious illness and problems. Furthermore, there is a correlation between severe and catastrophic cases of COVID-19 during pregnancy and increased incidence of preterm delivery and health issues in newborns (Villar *et al.*, 2021; Norman *et al.*, 2021).The treatment of pregnant individuals with COVID-19 has distinctive clinical and ethical difficulties, which are further intensified by the limited availability of thorough, high-caliber evidence about the safety and effectiveness of therapies for this group (Malhame *et al.*, 2020; Taylor *et al.*, 2021). Pregnant women are consistently excluded from biomedical research, despite the evident necessity, hindering the ability to offer treatment based on scientific data (Taylor *et al.*, 2021).

Remdesivir is a type of drug that is converted into an active form in the body and has the ability to fight a wide range of coronaviruses that affect both humans and animals (Jorgensen et al., 2020). The medicine in question was the initial one to receive official

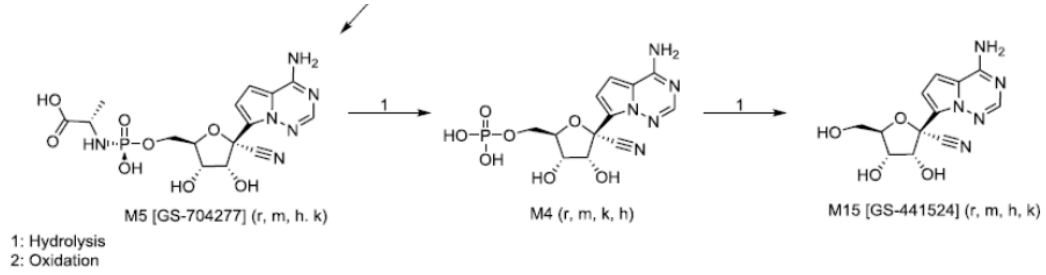
approval for treating COVID-19. it has been shown to reduce the duration of recovery in hospitalized patients who need oxygen therapy (Beigel *et al.,* 2020). The effect of this medication on mortality is not clear, as indicated by the variation in consensus treatment guidelines (Wang *et al.,* 2020; WHO 2021). Some guidelines advocate its use in severe COVID-19 cases among hospitalized patients, while others advise against it. (Bhimraj *et al.,* 2021). Pregnant and nursing individuals were deliberately not included in any scientific trials assessing the effectiveness of remdesivir for COVID-19 (Wang *et al.,* 2020; WHO 2021). Therefore, there is no direct proof of its efficacy in this particular group. During the Phase III trial assessing the effectiveness of remdesivir for Ebola virus infection, pregnant individuals were considered for participation. Out of all the participants who were randomly assigned to receive remdesivir, six of them were pregnant (accounting for 3.4% of the remdesivir group). It is worth noting that no significant negative effects on the mother, fetus, or newborn were observed in this subgroup (Mulangu *et al.,* 2019). Twenty-one Data on the safety of Remdesivir in pregnant individuals with COVID-19 are being collected via post-marketing registries, compassionate use programs, and case series/reports since it has been administered outside of clinical trials (Anderson *et al.,* 2020; Burwick *et al.,* 2021).

Remdesivir (VekluryÒ, GS-5734) is an antiviral medicine that targets the RNA-dependent RNA polymerase. It was the initial medication authorized by the U.S. Food and Medicine Administration (FDA) for the treatment of coronavirus disease 2019 (COVID-19). This medication is a prodrug of nucleotide and has the ability to enter cell membranes. Once inside, it is transformed into the primary metabolite GS441524 monophosphate (Gordon *et al.,* 2020), monophosphate undergoes biotransformation to create its active triphosphate metabolites. These metabolites have the ability to hinder the replication of RNA polymerase in many viruses, including COVID-19 (Sheahan *et al.,* 2017). Therefore, GS-441524 possesses the potential to be further developed as an orally administered antiviral medication (Wei *et al.,* 2021). Pregnant women, constituting around 9% of COVID-19 cases (Zambrano *et al.,* 2021), are impacted by the pandemic along with the rest of the population. Pregnant women who have COVID-19 have a significantly higher risk, up to five times, of being hospitalized to critical care and needing invasive mechanical ventilation (Collin *et al.,* 2020). Additionally, it presents a greater likelihood of premature delivery, that is 75% (Gurol-Urganci *et al.,* 2021), and complications in newborns (Jorgensen *et al.,* 2021). However, because of concerns regarding the safety of remdesivir in pregnant women, especially its ability to pass through the placenta (Louchet *et al.,* 2020), more than 80% of clinical trials have excluded pregnant women as participants (Taylor *et al.,* 2021). As a result, there is a scarcity of data about the pharmacokinetics of remdesivir in pregnant individuals (Lampejo, 2021).

This study is critical because there has to be a broad examination of the possible adverse effects of remdesivir due to its increasing usage in medical treatments and the related health consequences. Therefore, it is essential to investigate innovative approaches.

MATERIALS AND METHODS

1. Chemical:



2. Experimental Animals:

The Wister albino rats (Rattus norvegicus) used in this study were all healthy and had been examined by a veterinarian for abnormal symptoms, injuries, or infections. We procured male and female virgin specimens weighing between 140 and 180 grammes from VACSERA, a biologics and vaccines company based in Helwan, Egypt. The zoology department of the Faculty of Science at Al-Azhar University housed the animals in the animal house. Before the experiment began, the animals were given a week to acclimatise to a laboratory environment with unlimited access to food and water. There was a constant temperature of 22 ± 1 °C and a relative humidity of 55 ± 5 % as well as a light-dark photoperiod of 12:12 hours.

3. Treatments and Crosses:

One male and two females were kept in a cage overnight so that they could mate. According to **(**Burdan *et al.,* 2011), females were examined daily for the presence of a white plug covering most of the vaginal cervical junction with a substance used to produce a vaginal smear indicating the onset of pregnancy. Pregnant females were divided into three cohorts of ten animals each and housed separately in polyacrylic cages measuring 38 cm × 23 cm × 10 cm. The control and treated groups received the appropriate treatment of distilled water and Remdesivir orally once daily during organogenesis, i.e. from day 6 to day 15 of gestation

Control group: Pregnant rats were administered an equal amount of (distilled water) by oral gavage.

Low dose of remdesivir group: Pregnant rats were orally administered remdesivir at 10 mg/kg, via oral gavage.

High dose of remdesivir group: Pregnant rats were orally administered remdesivir at 20 mg/kg via oral gavage.

Daily observations were performed for all dams in the control and treated groups for indications of general toxicity, bleeding, and/or mortality.

4. Assessment of Fetal Skeleton Development:

According to the procedure outlined by **(**Sadeghi *et al.,* 2015), fetuses were fixed in 95% ethyl alcohol, skinned, eviscerated, and then stained with double staining of the skeleton. Cartilage was stained for 48 hours using a solution of alcian blue dye. Two hours were spent in 95% ethyl alcohol on each of the specimens, followed by two hours in descending grades of ethyl alcohol (75%, 40%, and 15%) for each concentration. After rinsing the specimens in multiple changes of distilled water for two to three hours, they were placed in 1% potassium hydroxide (KOH) until the skeleton was visible. To stain the bony portions of the specimens, they were rinsed in 0.001% aqueous alizarin red for three days. After that, they were submerged in 1% KOH three times for several hours each time. For each step, the samples were treated for 24 hours with an increasing series of glycerol in 1% KOH. To stop mold from growing, they were put in pure glycerin for long-term storage along with a thymol crystal. Under a stereo microscope, the blue embryonic cartilage and purplish-red bone were examined. Every stained skeleton underwent a thorough examination to look for any skeletal anomalies, such as missing or atypically shaped or sized bones.

5. Transmission Electron Microscopic Technique (TEM):

Small placental fragments (1 mm) were preserved in phosphate buffer (4F1G), which is a 4:1 mixture of formaldehyde and glutaraldehyde. Ultramicrotomy was used to prepare 1 μm semi-thin sections of the placenta from resin capsules. The slices were stained with toluidine blue, dried at room temperature, and then examined using a light microscope to identify and isolate the regions of interest. These regions were further analyzed using a transmission electron microscope (TEM). The ultra-thin placenta sections (60–70 nm) from specific regions were prepared at the Electron Microscope Unit of the Regional Center for

Mycology and Biotechnology, Al Azhar University, Egypt. These sections were then double-stained with uranyl acetate and lead citrate (Bancroft *et al.*, 2013**)** and examined using a Joel 100 CX transmission electron microscope.

6. Histopathological Examination:

A biopsy of placental tissue was immediately taken from the mothers and fetuses of all three groups, and the samples were fixed in 10% neutral buffered formalin for 24 hours. Hematoxylin and eosin staining and preparation of kerosene sections were performed according to the previously published protocol of **(**CDER 2020**).** A BX53M light microscope (Olympus, Japan) was used to examine each section and images were captured using an Olympus DP74 camera.

7. Statistical Analysis:

The statistical analysis was performed using SPSS version 22. The impact of both treatment modalities on the parameters being investigated was assessed using a one-way analysis of variance, and the findings were found to be statistically significant (P <0.05).

RESULTS

The present study "Effects of different doses of compound remdesivir on maternal weight, embryonic development, and pregnancy outcomes in rats" presents data from a study that investigated the impact of two doses of remdesivir (T1: 10 mg/rat and T2: 20 mg/rat) on various parameters related to maternal health and embryonic development in rats. The data is compared to a control group (Table 1& Figs.1 -4).

Table 1: Effects of different doses of remdesivir on maternal body weight, and pregnancy outcomes in rats.

Parameter	Control	Ramedsvir10 mg/kg	Ramedsvir 20 mg/kg
Body weight	176.0 ± 5.9[a]	185.7 ±7.5[a]	182 ± 7.8[a]
Placenta	2.0 ±.00[a]	1.7 ± 0.18[a]	1.3 ± 0.2[b]
Embryo	2.0 ± .00[a]	1.9 ± 0.14[a]	1.67 ± 0.21[a]
Corpus lutem	8.8 ± 0.5[a]	7.4 ± 0.64[a]	7.3 ± 0.2[a]
N. OF embryos	8.8 ± 0.47[a]	7.4 ± 0.6[a]	7.3 ± 0.2[a]
Live embryos	8.8 ± 0.4[a]	6.4 ± 0.8[a]	6.8 ± 0.3[a]
Dead embryos	00 ± 00[a]	0.43 ± 0.20[b]	0.5 ± 0.22[b]
Resorbed	00 ± 00[a]	0.57 ± 0.4[b]	0.17 ± 0.1[c]

Mean± SE, significant P<0.05

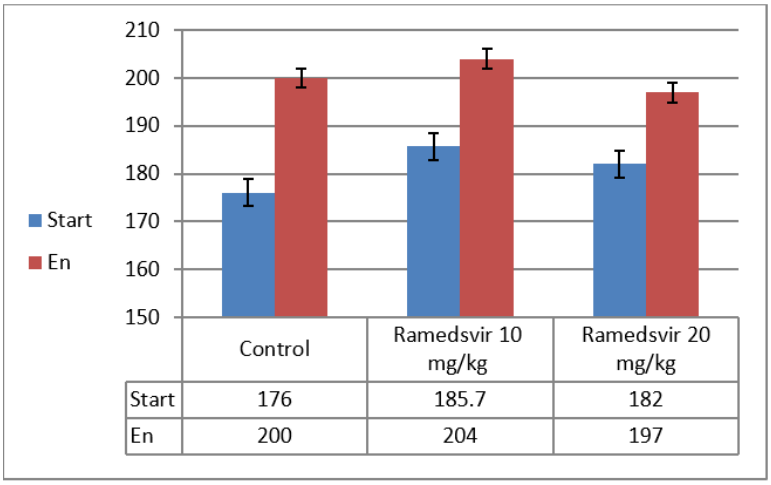
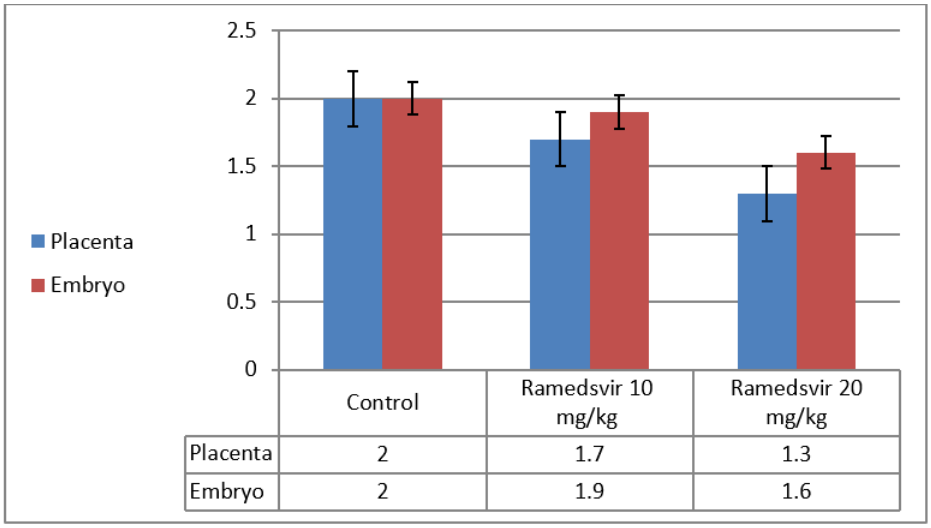
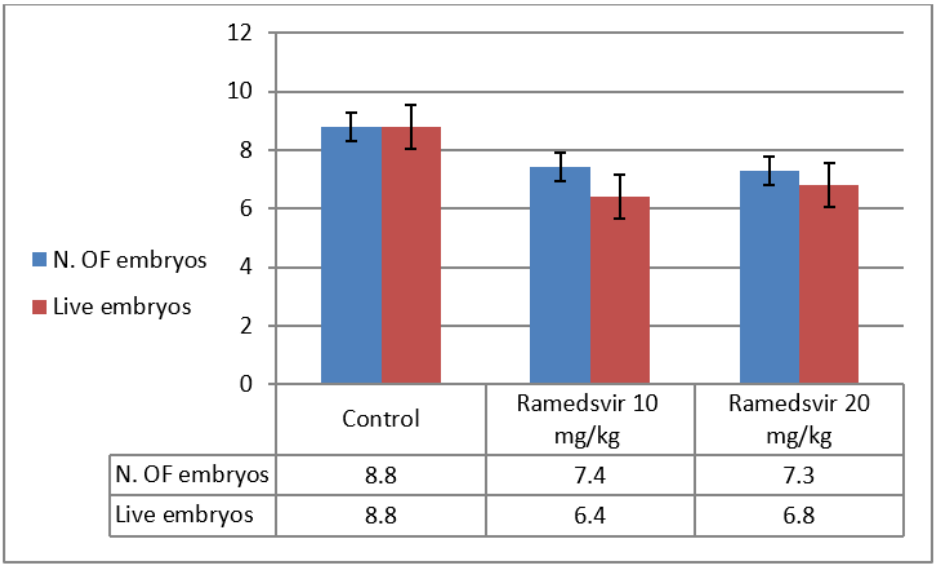


Fig.1: Effects of different doses of remdesivir on maternal body start and end weight.



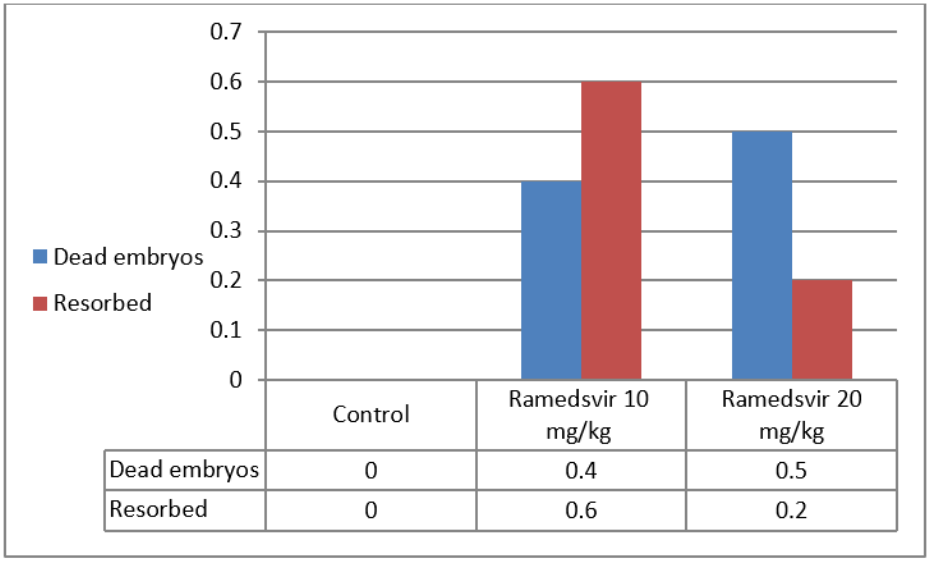
	Control	Ramedsvir 10 mg/kg	Ramedsvir 20 mg/kg
Placenta	2	1.7	1.3
Embryo	2	1.9	1.6

Fig. 2: Effects of different doses of remdesivir on placental & embryo weight.



	Control	Ramedsvir 10 mg/kg	Ramedsvir 20 mg/kg
N. OF embryos	8.8	7.4	7.3
Live embryos	8.8	6.4	6.8

Fig. 3: Effects of different doses of remdesivir on number & live embryo.



	Control	Ramedsvir 10 mg/kg	Ramedsvir 20 mg/kg
Dead embryos	0	0.4	0.5
Resorbed	0	0.6	0.2

Fig. 4: Effects of different doses of remdesivir on resorbed & dead embryo.

1. Effect On the Gravid Uteri Morphology:

As shown in Plate 1, the isolated uteri from control animals that received only distilled water and from treated pregnant rats (10 and 20 mg/kg Remdesivir) during gestation can be seen. Plate 1 (A) clearly shows the symmetrical distribution of embryos in both uterine horns, with no signs of resorption or other abnormalities. The uteri isolated from (B) and (C) show an uneven distribution of embryos, the right horn is shorter than the left, and one shows an abnormal reduction of the implantation sites (R.I.), reflecting the signs of dysmorphology. The symmetrical distribution of embryos in the uterine horns of the treated groups (D) is shown in **Plate (1).**

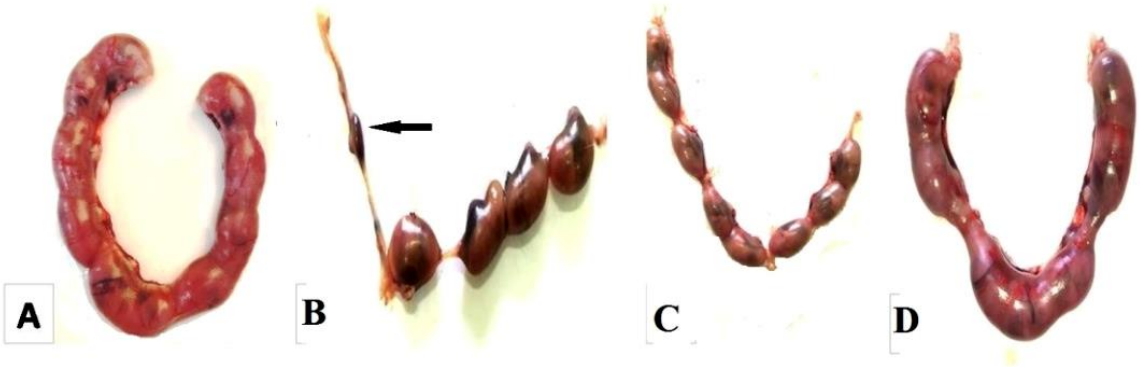


Plate (1): Observations of the isolated uterus from female rats during gestation. A: Control group, (B&C), Low dose of remdesivir group and (D), the high dose of remdesivir group.

2. Total Number of Corpus Lutem:

The mean numbers of the rat corpus lutem and rat embryos (implantation sites) in gravid uteri in the control group were (8.8±0.5), and remdesivir groups were (7.4±0.6 & 7.3±0.2), in 10 mg and 20 mg respectively. The findings showed a decline in the number of embryos in the experimental groups and no significant variation in corpus luteum in any of the treatment groups ($P<0.05$), as presented in Table 1.

The average number of live embryos retrieved from the control group was 8.8±0.4, whereas the remdesivir group had 6.4±0.8 and 6.8±0.3, respectively. The observed alterations exhibited a statistically insignificant reduction as compared to the control group ($P<0.05$), as indicated in Table (1) &Plate 2**.** The mean values of dad embryos in the control and remdesivir groups were (0.0±0.0), (0.4±0.2 & 0.5±0.2) respectively. The observed alterations exhibited a statistically significant reduction as compared to the control group ($P<0.05$), as indicated in Table (1) & Figures (3;4). The control group and the remdesivir group had resorbed embryos with average values of (0.0±0.0) and (0.57±0.4 & 0.17±0.1) respectively. The changes observed in this study were found to be statistically significant, indicating a considerable increase when compared to the control group ($P < 0.05$), as seen in Table 1.

Placenta; the average placenta weight in the control group was 2.0±0.0, whereas in the remdesivir group, it was 1.7±0.18 and 1.3±0.2, respectively. The study revealed a statistically significant drop in the high dosage of remdesivir compared to the control group, as indicated in Table (1). However, there was an insignificant decrease in the low dose of remdesivir compared to the control group ($P <0.05$).

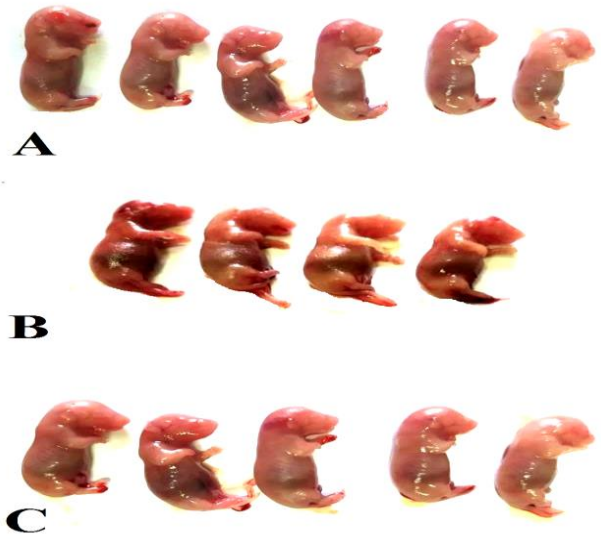


Plate (2): Numbers of the rat embryos from female rats. A: Control group, (B), Low dose of remdesivir group and (C), the high dose of remdesivir group.

3. Skeletal Study:

The control fetuses have well-developed cartilage and bone in many regions of the skeleton. The processes of chondrification and ossification have clearly been finished in the fetuses from the control group (Plate 3A). In contrast, fetuses of rats treated with T1 during gestation have exhibited partial ossification of the skull, fore and hind limbs (Plate 3B) as compared to control. On the other hand, fetuses maternally treated with T2 showed faintly stained cartilaginous and bony parts of the skull, fore and hind limbs (Plate 3C).

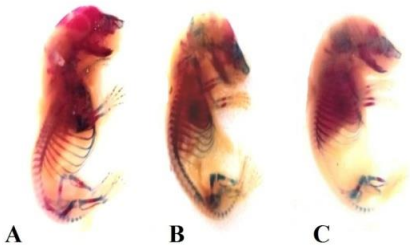


Plate (3): Skeletal study of the rat embryos from female rats. A: Control group, (B), Low dose of remdesivir group and (C), the high dose of remdesivir group.

4. Maternal Placenta Tissue:

The placental tissues obtained from the untreated group show the average stroma, average trophospongium, and average labyrinth zone (A). (B) This high magnification image displays a layer of large cells that is of typical size, with an average number of glycogen cells. (C) shows the mean number of trophoblastic cells and the mean number of blood sinusoids. (D) illustrates glycogen cells with average characteristics, a labyrinth zone with average maternal sinusoids (MS), average fetal blood arteries containing nucleated red cells, and average trophoblastic septa as shown in Figures 5 A, B, and C.

Group 10: the rat placenta (A) shows stroma with minor regions of bleeding, trophospongium with dilated blood vessels, and significant cytolysis of glycogen cells, as well as an average labyrinth. (B) The image displays stroma with hemorrhages, a thin layer of large cells, and trophospongium demonstrating significant destruction of glycogen cells. (C) The image displays a thin layer of large cells, with the trophospongium revealing significant destruction of glycogen cells and highly congested blood sinusoids. (D)

Depicting a trophospongium with trophoblastic cells of average size, a labyrinth with maternal sinusoids of typical size, fetal blood arteries with nucleated red cells of average size, and trophoblastic septa of average size (Fig. 6 A, B & C).

Group 20: the rat placenta (A) has decidualized stroma with significantly dilated congested blood vessels and regions of bleeding. The trophospongium displays areas of cytolysis and an enlarged labyrinth zone. The stroma has significant regions of bleeding, whereas the trophospongium displays pronounced destruction of glycogen cells. (C) This view displays the stroma with significantly enlarged and congested blood vessels, an average layer of giant cells, and trophospongium with significant destruction of glycogen cells. The trophospongium has a small number of glycogen cells, the trophoblastic septa are swollen and contain dying trophoblastic cells, and the fetal blood arteries have nucleated red cells on average (Fig. 7 A, B, and C).

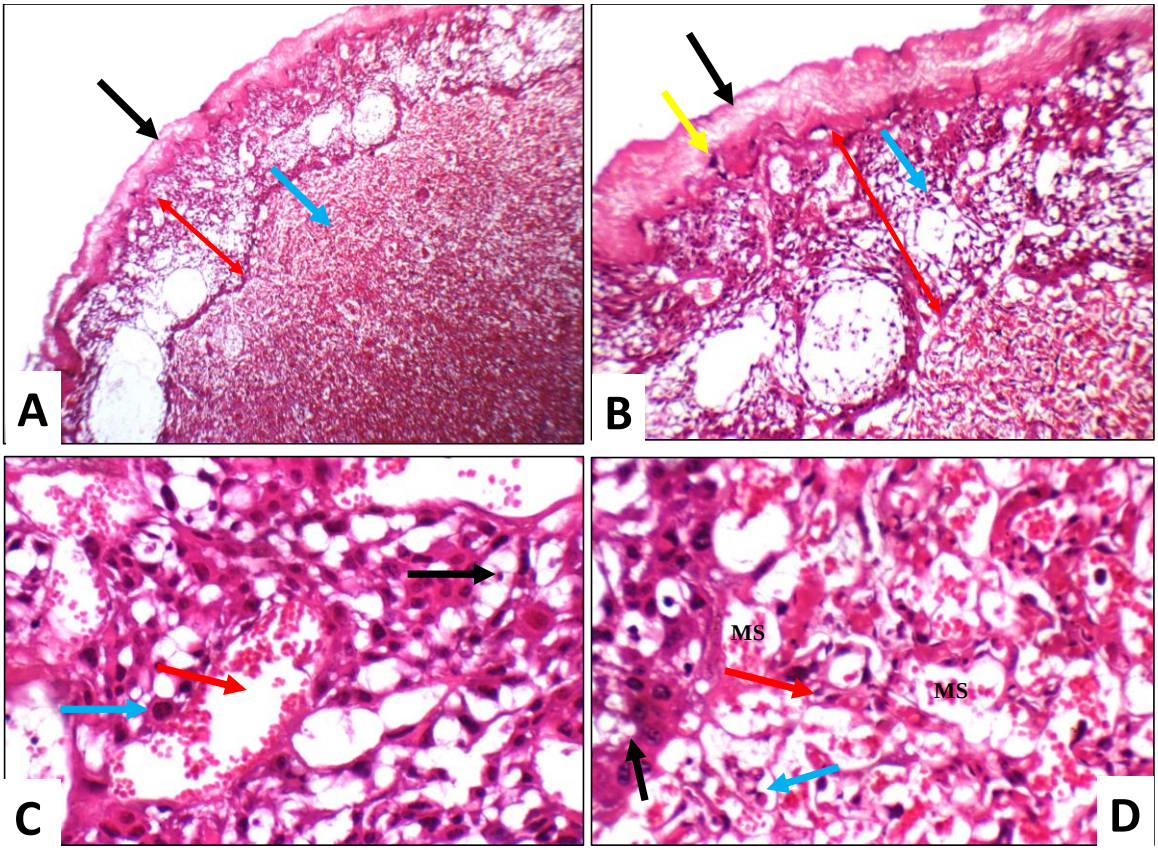


Fig 5:(Control): This image displays a rat placenta (A) with the average stroma indicated by a black arrow, the average trophospongium indicated by a red arrow, and the average labyrinth zone indicated by a blue arrow. The image was taken using H&E staining at a magnification of 100x. (B) This image displays a magnified view of the stroma (indicated by the black arrow), the large cell layer (indicated by the yellow arrow), the trophospongium (indicated by the red arrow), and the glycogen cells (indicated by the blue arrow). The magnification level is 200 times and the staining used is H&E.(C) Image of trophospongium with glycogen cells (shown by black arrow), trophoblastic cells (indicated by blue arrow), and blood sinusoids (indicated by red arrow). The image displays a trophospongium with glycogen cells (shown by the black arrow), a labyrinth zone with maternal sinusoids (MS), fetal blood arteries with nucleated red cells (indicated by the blue arrow), and trophoblastic septa (indicated by the red arrow). The magnification used is H&E X 400.

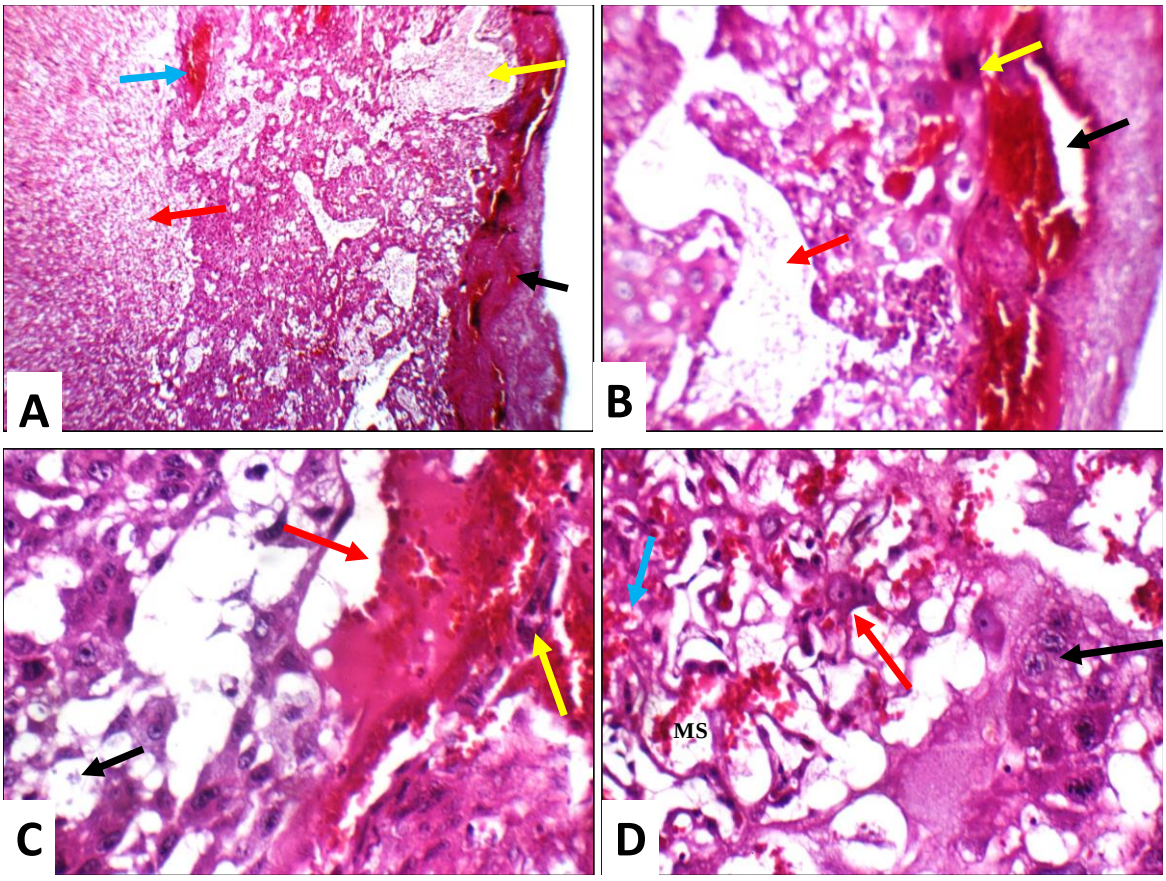


Fig.6:(Group 10): The picture depicts a rat placenta (A) with stroma that exhibits small areas of bleeding (shown by the black arrow), trophospongium that shows increased blood vessels (indicated by the blue arrow), and substantial damage to glycogen cells (indicated by the yellow arrow). In addition, the typical labyrinth may be visible, as indicated by the red arrow, with a magnification of 100X using the H&E staining technique. (B) The picture shows stroma with small areas of bleeding (shown by black arrows), a thin layer of big cells (indicated by a yellow arrow), and trophospongium with noticeable damage to glycogen cells (indicated by a red arrow) (H&E X 400). (C) The picture depicts a narrow layer of enlarged cells (shown by the yellow arrow), trophospongium showing substantial degradation of glycogen cells (indicated by the black arrow), and densely packed blood sinusoids (indicated by the red arrow). The illustration depicts a trophospongium with characteristic trophoblastic cells (shown by the black arrow), a labyrinth including typical maternal sinusoids (MS), fetal blood arteries with nucleated red cells (indicated by the blue arrow), and trophoblastic septa (indicated by the red arrow). The picture was enlarged by a factor of 400 and treated with hematoxylin and eosin (H&E) stain.

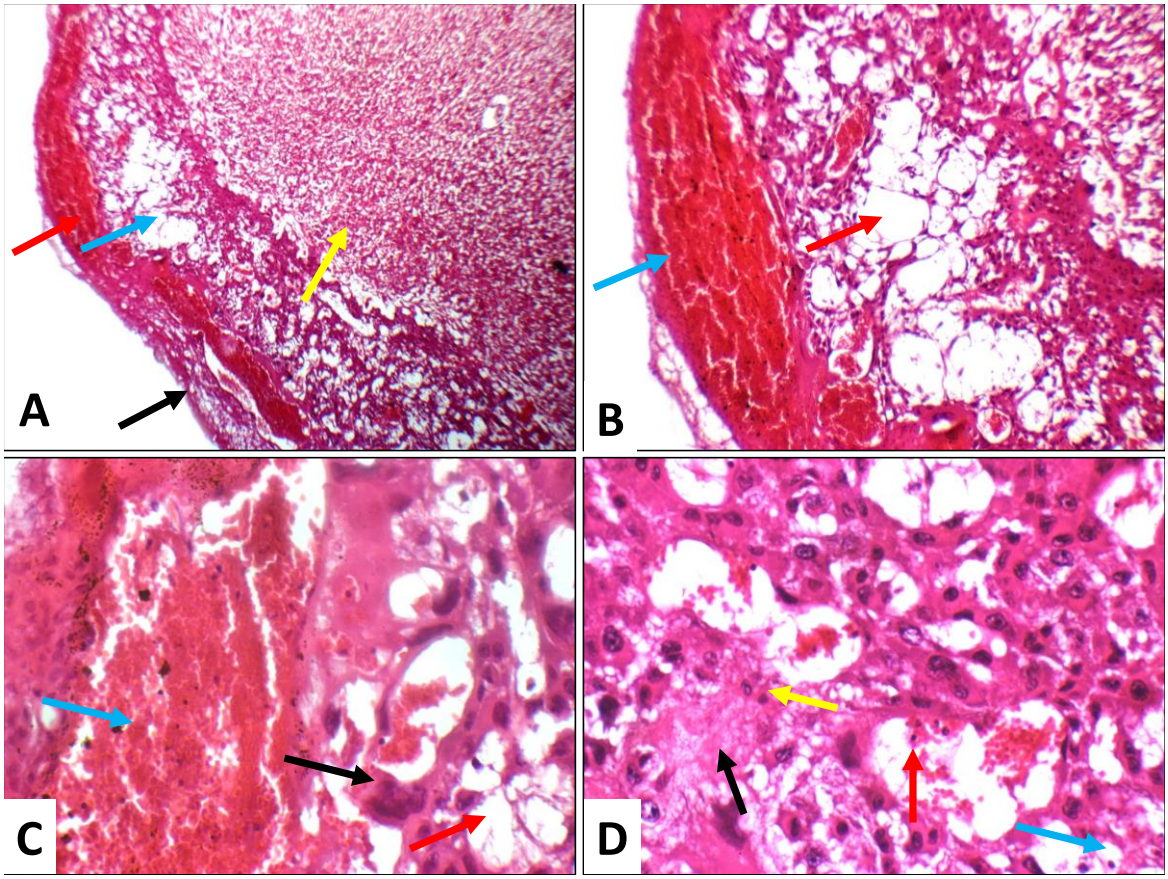


Fig 7: (Group 20): The image displays a rat placenta (A) with decidualized stroma (shown by a black arrow), which exhibits significantly dilated congested blood vessels and regions of bleeding (indicated by a red arrow). The trophospongium also shows areas of cytolysis (indicated by a blue arrow) and an enlarged labyrinth zone (indicated by a yellow arrow). The magnification used for this image is H&E X 100. The stroma exhibits significant regions of bleeding (shown by the blue arrow), while the trophospongium shows pronounced destruction of glycogen cells (indicated by the red arrow) (H&E X 200). (C) The stroma is observed with significantly enlarged and congested blood vessels (blue arrow), an average layer of gigantic cells (black arrow), and trophospongium revealing significant destruction of glycogen cells (red arrow), as seen under a microscope at a magnification of 400 times using the H&E staining technique. The trophospongium has a small number of glycogen cells (blue arrow), the trophoblastic septa are swollen (black arrow) and contain dying trophoblastic cells (yellow arrow), and the fetal blood arteries include nucleated red cells (red arrow). The image was magnified 400 times and stained with hematoxylin and eosin (H&E).

5. Transmission Electron Microscopic Technique:

A. Control rat placenta showing average blood sinusoids (BS) with average endothelial cells (EC), cytotrophoblasts with nuclei showing prominent nucleoli, dispersed chromatin, average ovoid mitochondria, and a few scattered electron-dense granules. **B.** Control rat placenta showing average syncytiotrophoblast (S) with nuclei (N) showing dispersed chromatin and indented nuclear membrane (blue arrow), well-formed endoplasmic reticulum, and few scattered electron-dense granules. **C.** rat placenta (10 mg), showing syncytiotrophoblast (S) with large nuclei (N) showing dispersed chromatin and regular nuclear membrane, small mitochondria and small cytoplasmic vacuoles, cytotrophoblasts (C) with small nuclei (N) showing faint nucleoli, average endothelial cell

(EC), and few electrons dense granules. **D**.10 mg rat placenta showing large cytoplasmic vacuoles, cytotrophoblasts (C) with nuclei (N) showing clumped chromatin and prominent nucleoli, and electron-dense elongated bands. E&F. 20 mg rat placenta showing syncytiotrophoblast (S) with nuclei (N) showing clumped chromatin, irregular nuclear membrane, large cytoplasmic vacuoles, swollen mitochondria, and excess electron-dense bodies (Fig. 8 A, B& C).

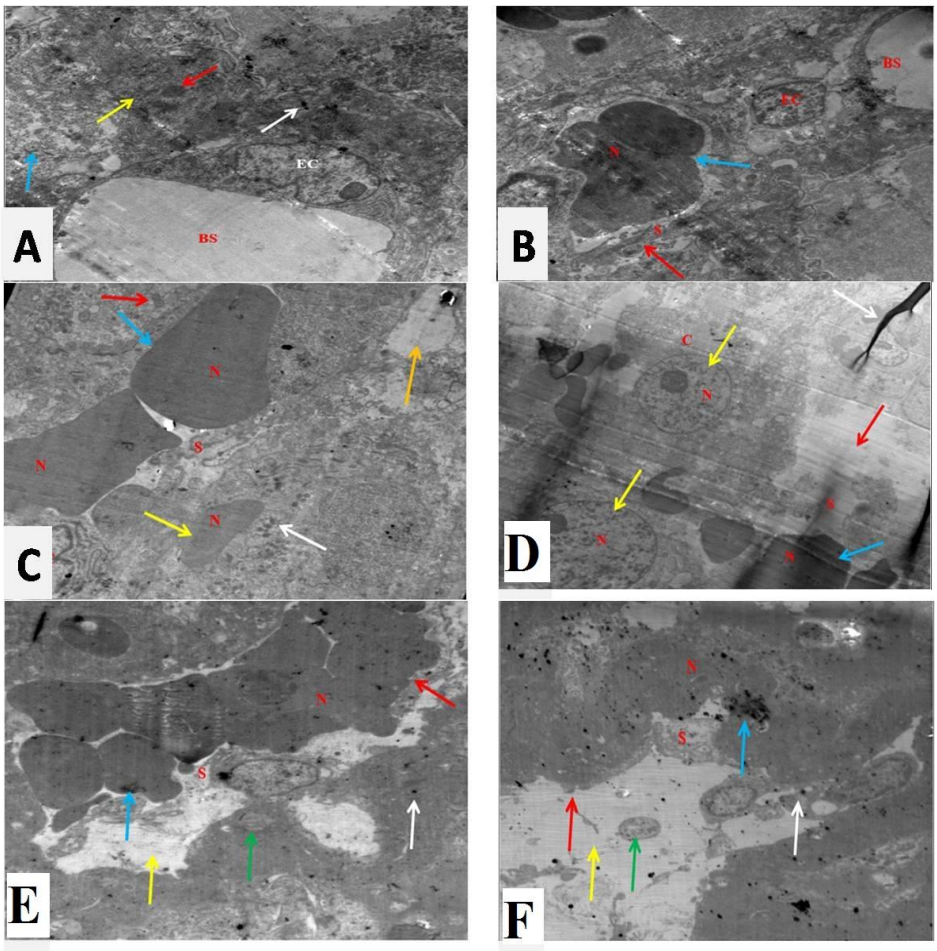


Fig 8: A. Control rat placenta showing average blood sinusoids (BS) with average endothelial cells (EC), cytotrophoblasts with nuclei showing prominent nucleoli (red arrow), dispersed chromatin (yellow arrow), average ovoid mitochondria (blue arrow), and few scattered electron-dense granules (white arrow). B. **Control** rat placenta showing average syncytiotrophoblast (S) with nuclei (N) showing dispersed chromatin and indented nuclear membrane (blue arrow), well-formed endoplasmic reticulum (red arrow), and few scattered electron-dense granules (white arrow). C. rat placenta (10 mg), showing syncytiotrophoblast (S) with large nuclei (N) showing dispersed chromatin and regular nuclear membrane (blue arrow), small mitochondria (red arrow) and small cytoplasmic vacuoles (orange arrow), cytotrophoblasts (C) with small nuclei (N) showing faint nucleoli (yellow arrow), average endothelial cell (EC), and few electrons dense granules (white arrow). D. **10**mg**:** rat placenta showing syncytiotrophoblast (S) with nuclei (N) showing dispersed chromatin (blue arrow) and large cytoplasmic vacuoles (red arrow), cytotrophoblasts (C) with nuclei (N) showing clumped chromatin and prominent nucleoli (yellow arrow), and electron-dense elongated bands (white arrow). E&F. 20mg: rat placenta showing syncytiotrophoblast (S) with nuclei (N) showing clumped chromatin (blue arrow), irregular nuclear membrane (red arrow), large cytoplasmic vacuoles (yellow arrow), swollen mitochondria (green arrow), and excess electron-dense bodies (white arrow).

DISCUSSION

Remdesivir has been evaluated in animal studies, specifically examining its effects on embryo-foetal development in rats and rabbits, fertility in rats, and prenatal and postnatal studies (Jorgensen *et al.,* 2020). The results of the in vitro and in vivo studies have shown that Remdesivir is not genotoxic. In the current study, the effects of two different remdesivir doses (T1: 10 mg/rat and T2: 20 mg/rat) on maternal weight, embryonic development and pregnancy outcome in rats were investigated. The results showed a reduction in the number of corpora lutea, embryo number and viable embryos in the experimental groups compared to a control group".

Female rats exposed to a dose of 10 mg/kg/day of remdesivir during a prenatal study (equivalent to 1-fold exposure to the standard human dose) showed a decrease in the mean number of corpora lutea, implantation sites and viable embryos. This was exacerbated by a reduced average weight of the ovary and reproductive organs, including the uterus, cervix and fallopian tubes (Lin et al., 2022). The "no observed adverse effect level" (NOAEL) for female reproductive and embryonic toxicity was reported to be 3 mg/kg/day, which is approximately 0.34 times the typical human exposure. The results were related to the use of Remdesivir. In embryonic and fetal development experiments, pregnant rats and rabbits exposed to doses up to four times higher than those recommended for humans showed no adverse effects on the growth, survival or physical structure of their offspring **(Gonzalez *et al.,* 2013).**

While conclusive investigations on the transplacental transfer of remdesivir are still ongoing, its chemical composition and pharmacokinetics provide a basis for making fair predictions. Remdesivir is a compound that is converted into a nucleoside monophosphate analogue by esterases inside cells after being given intravenously. The intracellular nucleotide triphosphate analogue is generated by a sequence of supplementary metabolic processes (Bohnert and Gan, 2013). Based on the available research, it is improbable that remdesivir will pass through the placenta in substantial quantities with therapeutic relevance. Nevertheless, the characteristics of the primary metabolites in circulation, including their extended half-lives, small molecular sizes, and significant unbound proportions, suggest a potential for greater transfer across the placenta.

In the present work by (Watanabe et al., 2018), an established UHPLC-MS/MS analysis technique was used in combination with in vivo microdialysis. Using the number 14, transplacental transfer of remdesivir and GS-441524, the main by-product of the drug, in the placenta, amniotic fluid and maternal blood was investigated. The approach used in this study conformed to the 3Rs concept (replacement, reduction and refinement) by reducing the number of animals required for the longitudinal study and quantifying the pharmacologically important unbound form of the drug in the extracellular compartment. The efficacy (Tempestilli *et al.,* 2020) and movement within the body (Sun, 2022) of a drug are strongly influenced by the amount of the drug that is not bound to proteins in the blood.

The protein unbound fraction is the only portion that may freely go from the plasma to the tissues. In the tissues, it interacts with target proteins, such as enzymes, receptors, and ion channels, which play a role in pharmacology (Eastman *et al.,* 2020). Moreover, employing this technique enables the concurrent identification of medicines in an animal at many sample locations.

After administration of remdesivir, the remdesivir level in the mother's blood decreased rapidly, as shown by the pharmacokinetic profiles. This indicates a significant degree of metabolic activity and biotransformation (Eastman *et al.,* 2020). The major metabolite GS-441524 is formed by the rapid hydrolysis of remdesivir, consistent with the properties of the drug. The elimination half-life of remdesivir is shortened due to its rapid metabolic conversion (Sukeishi *et al.,* 2021). Carboxylesterase hydrolyzes remdesivir to a

monophosphate nucleoside core, which is more water-soluble and tends to remain in the tissue phase (Xie and Wang, 2021). This conclusion is supported by the substantial volume of distribution of GS-441524, which is 6.36 liters per kilogram. In this study, transplacental transmission of remdesivir was not observed due to its rapid biotransformation. However, only GS-441524 successfully penetrated the placenta and reached the fetus. The final results for C max and AUC for GS-441524 were observed in the following order: maternal blood, amniotic fluid, fetus and placenta. The AUC values for the placenta (8.39 ± 2.13 min mg/ml) and fetus (10.85 ± 3.46 min mg/ml) were approximately twice as high as the values for amniotic fluid (16.70 ± 4.48 min mg/ml).

A plausible hypothesis is that prominent members of the CYP family, such as 1A1, 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, or 3A5, encounter challenges in metabolizing GS-441524. These results suggest that GS-441524 is not metabolized in the liver but remains as GS 441524 and is directly eliminated in the fetus's urine (Griffiths and Campbell, 2015).

Drug transfer across the placenta can occur by four main mechanisms: active transport, assisted diffusion, simple diffusion and pinocytosis (Dehelean *et al*., 2020). Of these four mechanisms, passive diffusion, which is subject to Fick's diffusion rule, is the primary mechanism by which the drug crosses the placenta

Efficient placental transfer is facilitated by low molecular weight (<500 Da) and strong lipophilicity (high LogP value). Remdesivir and GS-441524 have molecular weights of 603 Da and 291 Da, with LogP values of 2.01 and -1.9, respectively (Smith *et al.*, 2015; Deb *et al.*, 2021). The tissue penetration rate of Remdesivir is generally considered to be poor (Humeniuk *et al.*, 2021). Remdesivir was unable to penetrate the blood-brain barrier and was not detected in the lungs or spleen of rats or monkeys, which aligns with this argument (Deb *et al.*, 2021). Furthermore, in a collective investigation, remdesivir exhibited a small volume of distribution, with a dosage of 10-225 mg per person, ranging from 0.75 to 0.12 L/kg [(Humeniuk *et al.*, 2021). Consequently, we theorized that the higher lipophilicity of remdesivir compared to GS-441524 might improve its ability to enter the placenta. However, the ability of the unbound drug to cross the placenta is counteracted by the rate of protein binding and ionization class (Humeniuk *et al.*, 2021). As a result, the transfer ratio from mother to fetus for GS-441524 can reach up to 51%. The reports from these sources corroborate our findings about the classification of remdesivir as a prodrug with limited diffusion in tissues. The substance rapidly decomposes into its main metabolite, GS-441524, which easily penetrates the embryo.

Conclusion

From the previous results, it can be concluded that the risk of remdesivir for maternal tissues and their fetuses is present and observed but in faint type, thus this study is critical because there has to be a broad examination of the possible adverse effects of remdesivir due to its increasing usage in medical treatments and the related health consequences. Finally, it is essential to investigate innovative approaches.

Declarations:

Ethical Approval: Not applicable.

Competing interests: The authors declare no conflict of interest.

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