Histopathological impact of cisplatin on the brain and lung of pregnant albino mouse (*Mus musculus*)

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**Abstract**

Cisplatin, also known as (SP-4-2)-diamminedichloridoplatinum (II), is a potential approach, and it is generally used as prescription medicine for the healing of a diverse range of man malignancies. Cisplatin's biological action was identified by chance more than 30 years ago. The present study examined the impact of cisplatin on the brain and lungs of pregnant white mice *Mus musculus*. In the current investigation, 15 pregnant mice were used. All of the animals were separated into three groups. Each group contained five pregnant mice. Two concentrations of the drug were used in the current study, 3.5 and 6.5 mg/kg of body weight. The medication was administered intraperitoneally (IP) on embryonic development's 8th, 13th, and 16th. The results showed thickening of alveolar septa by inflammatory exudative cells, thickening of the bronchiolar wall with inflammatory cells infiltration, necrosis of bronchial epithelial cells, and pulmonary blood vessel congestion in the Cisplatin 3.5 mg treated group (group). The severity of the histopathological lesions of the lung become more detectable in the Cisplatin 6.5 mg/kg of b.w (group 3). The brain tissue from group 2 displayed perivascular oedema, vacuolization, satellitosis, and neuronal necrosis. Previous lesions became more severe in the brain of the Cisplatin 6.5 mg/ kg of b.w. Treated group (group 3). The thickening of the alveolar septa and layers of the cerebral cortex was statistically studied. In conclusion, the pathological lesions mentioned above demonstrate that the medication has negative consequences during pregnancy. The drug must be provided under medical supervision, considering the patient's health conditions.

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**Introduction**

Worldwide, there were 18.1 thousand young cases of malignancy diagnosed in 2018 and 10 million cancer-related deaths. In addition to 2040, it is predicted that there will be 29.5 million new cases of cancer every year and 16.4 million deaths from carcinoma (1). In the treatment of solid tumors and hematological malignancies, like cancers of the reproductive organs, uterus, head, neck, throat, gut, and lungs, as well as leukemia and osteosarcoma, Cisplatin (diamminedichloroplatinum II) is a typically used as an antitumor medication (2). The significant adverse reactions of a cisplatin overdose involve morning sickness, nephropathy, electrolyte imbalance, bone marrow suppression, ototoxicity, nerve damage, hepatotoxicity, and eye problems (3). The chemotherapeutic advantages of cisplatin are well understood after 40 years of usage and intensive research. Both prevalence and toxicity rise with consistently higher doses and extended exposure periods to cisplatin. Typically, neuropathic pain appears following a maximum dose of 250-350 mg/m² (4). These consequences of cisplatin on malignant cells are wanted via the drug-activated cell death and programmed cell death pathways, and the same process in healthy tissue creates various levels of intoxication (5). The medicine also can cause histological abnormalities in the white mice *Mus musculus* liver and
The medication is used in pregnant and non-pregnant women to fight ovarian cancer. The medication can pass through the placenta. The risk of poisoning in the mother embryo may increase as mothers consume more rugs (7). The implications of such DNA damage may have clinical significance since pregnant women diagnosed with ovarian cancer during gestation are often treated with cisplatin mitochondrial toxicity brought on by cisplatin in the embryonic and maternal rat kidneys (8). In adults, cisplatin treatment is frequently associated with renal interstitial fibrosis, which causes long-term kidney harm. Once given to pregnant women, this medication can potentially damage the embryo. Preterm birth, intrauterine growth restriction, and oligohydramnios have all been linked to pregnant women using this medicine (6). In a twin pregnancy, it has been demonstrated that the amniotic fluid contains significant cisplatin (10% of the maternal concentration) (9). Several medical research studies have found cisplatin transplacental transfer to be meaningful. Cisplatin, for example, was found in the umbilical blood of babies exposed to the drug during embryonic development (10). The medication impacted the expectant mothers and the animals' respiratory systems (11). More than 75% of those who receive chemotherapy for malignancies just outside the brain and nerve suffer from cognitive issues, such as difficulties focusing, thinking, processing information quickly, and speaking clearly (12). It has additionally resulted in a rise in neurologic and auditory abnormalities. According to dos Santos et al., cisplatin causes ototoxicity, peripheral (the most common), and central (rare) neurotoxicity. The influence of cisplatin-induced lung injury on cilia is unclear. One of the leading causes of cisplatin's adverse effects has been identified as oxidative stress (11).

The study aims to examine the results of the medication cisplatin at dosages of 3.5 and 6.5 mg/kg on the histological structure and a few histomorphometric parameters of the lung and brain of the adult pregnant mouse on days 8, 13, and 16 of pregnancy.

Materials and methods

Ethical approve

The University of Mosul, College of Veterinary Medicine, Animal Care and Use Committee reviewed and approved every step of the investigation's processes. The approval date is 3/9/2022 and the Ref: UM.VET.2022.066.

Animal housing

The current research includes 15 pregnant adult mice. The animals weighed 31+29g each. They were 58 days old. The animals were collected from the veterinary medical college's animal house at Mosul University in Mosul, Iraq. The animals were exposed to equal hours of light and dark (9). Model animal home maintenance, feeding, and breeding procedures have been applied to animals (6). Fully developed males were housed in the early evening for 48 hours with females at a ratio of one male to two females per cage for mating (16). The presence of a genital barrier was considered the first-day conception (day = 0) (17). They were left alone until the seventh day of their pregnancy, at which point the females were removed from the mating cages and separated into groups (13). The animals were cared for and handled using laboratory animal guidelines, and it is used in the College of Veterinary Medicine, University of Mosul. Kang Pharmacy, Haryana, India, produced the cisplatin medication solution (50 mg/100 ml) utilized in this investigation (14).

Experimental design

The pregnant adult mice (n=15) were placed into three groups: Group 1 (consisting of five mice) was the control group, receiving distilled water (0.3ml) through intraperitoneal injection (IP). The cisplatin IP (3.5 mg/kg b.w.) was given to Group 2 (consisting of five mice). Group 3 (composed of five mice) received the cisplatin IP (6.5 mg/kg b.w.). All groups received distilled water and medicine at both doses on the 8th, 13th, and 16th days of pregnancy. The amounts were estimated using Platinum's LD50 for mice, which is 7.6mg/kg body weight (15).

Histopathological examination

The animals were euthanized with chloroform at the end of the research. The pregnant animals were autopsied. Their lungs and brain were dissected. Both organs were fixed with a10% formaldehyde solution (formalin) for two days before being rinsed for 2 hours with filtered (distilled) water to prepare them for the next step. The routine histopathological process of paraffin-embedded tissue processed the samples. Sections were passed with graded concentrations of ethyl alcohol. The samples were passed with a mixture of xylene and melted wax at a ratio of 1:1 for two times for 15 minutes each time. Then, it is transferred to the melted wax for two hours for two times. Then, the wax molds were prepared. The wax had a melting point of 55-56 C. Wax molds were cut using a rotary microtome with a thickness of 5 μm. The tissue sections were dewaxed using warm xylene for 20 minutes, and the process was repeated twice. Delafield's hematoxylin and eosin were employed to color the sections. The sections of both organs were stained for 5-10 min in the hematoxylin and for 1 min in the alcoholic eosin. Sections were mounted with D.P.X. and examined using a light microscope (16). For Photography, the software of microscope camera Omax Toup View was used.

Statistics analyses

The thickness of the alveolar septa and brain layers were expressed as mean ± standard deviation (SD). One-way Analysis was used to examine the findings scientifically. The means of test groups 2 and 3 were compared to the mean of healthy controls in Dunnetts's sub-test to look for differences (group 1). The present outcomes were statistically calculated.
using the statistical package Graph Pad Prism 5.0 (San Diego, USA). The level of significance was chosen at *P < 0.05, which is meaningful; ** P < 0.01, which is strongly meaningful; and *** P < 0.001, which is very strongly meaningful (6).

Results

Mother’s lungs

The histological examination of the control lung (group 1) showed normal architectures of alveoli, bronchioles, respiratory bronchiole, and blood vessels (Figure 1). Alveoli septa thickness was also measured in the control section to compare them with the others in the experimental groups (Figure 2). Lung sections of group 2 (3.5 mg /Kg) revealed some lesions represented with a thickness of the alveolar septa by inflammatory exudative cells, thickening of the wall of the bronchiole with inflammatory cells infiltration, necrosis of bronchial epithelial cells, congestion and hyperaemia of the blood vessels (Figures 3 and 4). Lung sections of group 3 (6.5mg /kg of b.w.) showed several histopathological changes represented by thickening of the alveolar septa by inflammatory exudative cells, focal inflammatory cells infiltration surrounding bronchioles, necrosis of the epithelial cells lining bronchioles as well as serofibrinous, exudate, congestion and hyperaemia of the blood vessels (Figure 5). The thickness of the alveolar septa was affected by the treatment with both doses of cisplatin (Figure 6). Alveolar septa thickness is affected by the two doses of cisplatin, 3.5 and 6.5 mg/kg b.w., that was administrated by IP injection to pregnant mice on the 8th, 13th and 16th days of gestation and showed a substantial improvement in group 2 individuals and a dramatic improvement in group relative to group 1 (Figure 7).

Figure 1: Photomicrograph Group 1 (control group) lung demonstrating normal structures of alveoli (A), Bronchus (B), respiratory bronchiole (C), and blood vessels (D). 100X H&E stain.

Figure 2: Photomicrograph of group 1 (control group) lung demonstrating normal structures of alveoli (A) and bronchioles (B) with alveolar septa measurements in micrometer (measurements/field 60.08 m2/400X) using microscope camera software Omax ToupView. H&E stain, 400X.

Figure 3: Photomicrograph of group 2 (Cisplatin 3.5 mg/kg) lung. The drug was injected (IP) on the 8th, 13th, and 16th days of gestation, demonstrating thickening of alveolar septa by inflammatory exudative cells (A), thickening of the bronchiolar wall with inflammatory cells infiltration (B), necrosis of bronchial epithelial cells (C) and excessive accumulation of blood within a vessel (congestion) and blood vessels hyperemia (D). H&E stain, 100X.
Figure 4: Photomicrograph of group 2 (Cisplatin 3.5 mg /kg) lung. The drug was injected (IP) on the 8th, 13th, and 16th days of gestation, showing thickening of alveolar septa by inflammatory exudative cells (A), thickening of the bronchiolar wall with inflammatory cells infiltration (B), necrosis of bronchial epithelial cells (C), and congestion of the blood vessels (D). The measurements of the alveolar septa in micrometer (measurements/field 60.08 µm2/400X) using the software of microscope camera Omax ToupView. H&E stain, 400X.

Figure 5: Photomicrograph of group 3 (Cisplatin 6.5 mg /kg) lung. The drug was injected (IP) on the 8th, 13th, and 16th days of gestation, showing thickening of alveolar septa by inflammatory cells (A) and serofibrinous exudate (B), severe necrosis of epithelial cells lining bronchioles (C), serofibrinous exudate (D), excessive accumulation of blood within a vessel (congestion) and blood vessels hyperemia (E). H&E stain, 100X.

Figure 6: Photomicrograph of group 3 (Cisplatin 6.5 mg /kg) lung. The drug was injected (IP) on the 8th, 13th, and 16th days of gestation showing thickening of alveolar septa by inflammatory cells (A) and serofibrinous exudate (B), severe necrosis of epithelial cells lining bronchioles (C) with the measurements of the alveolar septa in micrometer (measurements/field 60.08 µm2/400X) using the software of microscope camera Omax ToupView. H&E stain, 400X.

Figure 7: Exhibits the impact of cisplatin on the alveolar septa thickness. The findings were presented as (SD). All values were regarded as meaningful at *P> 0.05. and very strongly meaningful at *** P < 0.001.

Microscopic notes of the mother’s brain

The microscopic assessment of the control group indicated normal cerebral mantle layers. The sections also demonstrated the intact neurons, glial cells, and blood arteries of the neocortex (Figure 8). The brain sections also showed variable lesions, including singular necrosis of neurons, vascularization, satellitosis, and perivascular
oedema at 3.5 mg /kg (Figure 9). Lesions become more observable at the dose of 6.5mg/kg, represented by singular necrosis of neurons, gliosis satellitosis, neuronophagia, and perivascular edema (Figure 10). The thickness of the leptomeninges increased exponentially in both experimental groups 2 and 3, as shown in the current data. Although group 3 exterior granular layer of the brain revealed marked improvement, group 2 demonstrated a substantial considerable rise in its thickness. In groups 2 and 3, the thickness of the exterior molecular layer rose somewhat but considerably. Additionally, groups 2 and 3 had a non-meaningful improvement in the thickness of the exterior molecular pyramidal layer (Figure 11).

**Figure 8**: Photomicrograph group 1 brain of the control group showing cortex mantle with intact nervous cells (A), neuroglia (B), and blood vessels (B). H&E stain, 400X.

**Figure 9**: Photomicrograph of group 2 (Cisplatin 3.5 mg/ kg) brain. The drug was injected (IP) on the 8th, 13th, and 16th days of gestation, showing singular necrosis of neurons (A), vacuolization (B), satellitosis (C), and perivascular edema (D). H&E stain, 400X.

**Figure 10**: Photomicrograph of group 2 (Cisplatin 6.5 mg/kg) brain. The drug was injected IP on the 8th, 13th, and 16th days of gestation, showing singular necrosis of neurons (A), gliosis (B), satellitosis (C), neuronophagia (D), and perivascular edema (E). H&E stain, 400X.

**Discussion**

Cisplatin is one of the drugs commonly used in cancer treatment, and the current results have shown adverse effects on the brain and lungs in pregnant mice when used on specific days of pregnancy. The findings indicated that both tested groups 2 and 3 had numerous lung lesions like degeneration, necrosis, infiltration of the inflammatory cells, and congestion. The severity of lesions depended on the
dose concentration. The findings revealed thickening of the alveolar septa, which exhibited a meaningful rise in the lung of group 2. The alveolar septa improved significantly in their thickness. The results of this study were comparable to those of Han et al. (11) and Atwa et al. (17). The findings were similar to those of Chen et al. (18), who described peroxidation as one of the disadvantages of cisplatin that can impact the lungs and other tissues and organs. The results were also somewhat consistent with those of Unver et al. (19), who found that administering 50 mg/kg of cisplatin to rats can result in significant alveolar disruption, exhibiting edema and significant alveolar septal fibrosis. Necrosis in this study may be due to the cisplatin increasing the production of superoxide anion and peroxidation by lowering the expression of MnSOD IDH; and catalyzing H2O2. The inflammatory response is the animal’s protective reaction to illness and tissue injury, and it can either prevent pathogen spread or enhance tissue repair. Infiltration of the inflammatory cells in this study might be brought on by the drug's increased production of free radicals and its ability to cross-link DNA, which prevents DNA replication and increases the free radical species (20). By increasing oxidative and pro-inflammatory marker levels and decreasing antioxidant levels, cisplatin led to pulmonary oxidative stress that suggests that the damaged area produces a combination of chemical signals, such as proinflammatory cytokines, eicosanoids, and active T-cells, which attract neutrophils and monocytes to the area of injury (19). DNA damage and oxidative stress are carried on by the medicinal use of cisplatin in non-malignant organs, such as the kidney, liver, testicles, and lungs. Reducing defence mechanisms and tissue damage may be part of cisplatin-induced reactive oxygen species formation pathophysiology. The induction of these molecules’ formation is associated with mitochondrial dysfunction (Mitochondria are unable to generate sufficient energy for the body's tissues) that causes ROS accumulation, which is the main reason for cell degeneration (21).

Observable histological alterations in the maternal brain were leptomeninges, vascularization, perivascular edema, and gliosis. Leptomeninges’ thickness rose dramatically, indicating a considerable increase. The thickness of the layers of the cerebral cortex developed and showed significant changes. The results obtained by Abdel-Mohsen et al. (22) and the recent findings were comparable. Gliosis in our study may be due to oxidative stress and increased reactive oxygen species. The results of both Mao-Ying et al. (23) and Starobova and Vetter (24) were similar to our findings. The recent findings consisted somewhat of those of Lomeli et al. (25) who discovered that acute cisplatin therapy caused mitochondrial damage, vacuolization, and cristae loss in CA3 neuronal cells. Gliosis may also be due to the release of neurotoxic chemicals by that astrocyte, such as free radicals and inflammatory cytokines, which actively assault protein molecules within neurons and cause neuronal damage and inflammation. The generation of reactive oxygen species (ROS) can result in the peroxidation of membrane lipids, which disrupts membrane permeability and leakage and can cause harm to cell biomolecules, which may be the reason for vascularization occurrence (17).

Conclusions

Although cisplatin is one of the most comprehensive treatments for many forms of cancer, it has negative impacts, notably on the maternal brain and lungs, which are significant parts of the body. This is mainly if it is used within the pregnancy period so the mother’s organs are damaged and develop a variety of pathological abnormalities. The dosage and length of the therapy period affect how severe these lesions are. Because of this, it must be given under a doctor's supervision and only with approved dosages, especially for pregnant women.

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Conflict of interest

There is no conflict of interest.

References


