

Protective role of melatonin on cisplatin-induced changes in developing cerebellum of rat fetusSajjad Hejazi¹, Sina Yaghoubi(Corresponding author)^{2*}, Mohamadreza Delghandi²

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Abstract: Cisplatin is one of the most efficient anticancer drugs which are used in the recent years, there has been an increasing concern on the occurrence of abnormal generation in human beings. As such, the purpose of the present study is to investigate the protective effect of melatonin on the histological changes in the cerebellum of newborn rats which are treated by cisplatin before their birth (during pregnancy). In this experimental study 24 pregnant rats, with NIH race, were distributed to three equal groups of control, interfere I(1), and interfere II(2). The Cerebellums of 24 newborn rats were studied on the basis of the changes occurred histologically. In histological observations of both groups: interfere 1 and interfere 2 the layers of cerebellar cortex had irregular tissues (forms). The cerebellum had no foliated shape form and with a perfectly primary shape, it showed some deficiency in its trophic tissues. In interfere group 1. The followings were observed: In many tissue and pia matter of soft tissue, pletosis and edematous were seen and in the cover tissue of choroid plexus of forth ventricle and in neuroglia tissue, pletosis and apoptosis were seen dispersely. However, in interfere group 2, there was little pletosis and edematous in pia matter of soft tissue and choroid plexus of forth ventricle were observab and the occurrence of apoptosis was not seen. In present study, new events, in line with the proof of infusion mechanism, retardation in the formation of cerebellum in newborn rats which were treated by cisplatin during in-uterine lives were presented. Also, Apoptosis infusion and the distortion of cell structure in cerebellum caused by cisplatin and the anti-apoptosis effects of melatonin were presented.

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1-Introduction

In many studies, the mutagenic and teratogenic effects of cytotoxic have been investigated. This study aims to investigate the destructive effects of cisplatin on the nervous system of cerebellum followed by the prescription of melatonin as an anti-oxidant synchronously. The histological changes and the destructive effects of cisplatin and the protective effect of melatonin during the pregnancy period from mother to fetus have been investigated.

The effects of teratogenic cisplatin on fetus, and the occurrence of malformation have been proven (36,25). However, sufficient information doesn't include the cerebellum.

Cisplatin is one of the most efficient anti-cancer drugs that are used in the treatment of some kinds of tumors like: lung, neck and head (cancers), in particular ovarian cancers (5,6). Cisplatin is used in the treatment of adrenal Carcinoma, breast, uterine, gastrointestinal, lung, prostate head and neck, germ cell tumors, neuroblastoma, and sarcoma (49). The effective mechanism of cisplatin is the same as other anticancer drugs: it creates links between DNA and RNA strands, then it interferes with their performance. Its side effects include: secondary anemia, kidney toxicity, blood uric acid increase or nephropathy

accompanied with blood uric acid increase and otic toxicity (49).

As such, the purpose of this paper is to evaluate the probable protective effect of newborn rats which are treated by cisplatin.

The toxic effects of this drug is mainly posed on the cells of nervous tissues, in the studies which have been done in this field so far, and the teratogenic effects of the drug on fetus and its malformation have been proven. The cells of cerebellar tissue are examples of those cells which are sensitive to this drug. With respect to the cases mentioned above, the necessity of a study which investigates the destructive effects of melatonin during pregnancy from mother to fetus is felt.

2-Method and materials:

The conditions as how to keep and how to feed the samples, were all the same for all groups: They were kept under the daily light for 12 hours and under the darkness for 12 hours alternatively, with $21 \pm 2^\circ\text{C}$ temperature, in special cages on the bedding-matter (straw). Water and food was freely available for the animals. After one week, the rats were put to mate.

The day on which vaginal plaque were formed at the opening of their vagina, that day was considered as the first day of pregnancy (8, 33, 46). The rats, regarding to their pregnancy day which was the same for all them, were assigned to three groups of control each with 8 members, and 2 groups of interfere with 3 subgroups randomly. on the basis of a planned schedule, the pregnant rats. from interfere group 1 were injected 75mg/kg dose(43) cisplatin during their organogenesis(19) period on the 6th and 12th days of pregnancy, however, interfere group 2 were injected synthetically. In other words, they were injected 75 mg/kg dose of cisplatin on the sixth 6th and twelfth 12th days of pregnancy and for 5 days, daily from the 6th to 12th days of pregnancy, melatonin with 200mg/kg dose (orally) were fed(40). In control group, pregnant rats were injected two times, by pure water on the 6th and 12th days of pregnancy. After the end of pregnancy period (18-21 days), the newborn fetus were made unconscious. They were fixed and after weighing and measuring the length and width of their cerebellum, rats were cut into two parts longitudinally and their heads were separated.

From the fixed samples which were done by using autotechnic and color – H&E methods, some lams were provided to be observed histologically. Then, they were observed under the light-microscope.

3-Results

The gathered samples, after being fixed, were evaluated. In this study, which was done from morphological point of view externally, the parameters of newborn fetus weight, the length of newborn fetus, the width and length of cerebellum in three groups of control 1, interfere 1, and interfere 2 were recorded (table 2-4). The results were presented as mean \pm SEM. The difference among different groups were analyzed through the use of T-test, ANOVA and software SPSS statistically, and the meaningful level of α was determined as $p < 0.05$.

The morphological observations from the newborn fetus in interfere group 1 and 2 showed meaningful decrease in weight, size of cerebellum and growth of newborn fetus compared with those in control group ($p < 0.05$) (Table 1).

Table 1: shows the average of parameter observed morphologically after the injection of 2 dose cisplatin IP, on the 6th and 12th days of pregnancy in interfere group 1, melatonin from the 6th and 12th days of pregnancy accompanied with the injection of 2 dose cisplatin on the 6th and 12th days of pregnancy in interfere group 2 and control groups. Each parameter has been presented as mean \pm SEM(n=24).

Group3 (interfere2)	Group2 (interfere1)	Group1 (control)	parameter
0.08 \pm *1.19	0.05 \pm *1.16	0.13 \pm 1.71	Birth weight (gr)
1.59 \pm *21.37	1.66 \pm *21.25	0.91 \pm 27.62	length of the baby (mm)
0.51 \pm *7.37	0.51 \pm *7.37	0.83 \pm 7.87	Skull width (mm)
0.53 \pm *9.50	0.53 \pm *9.50	0.88 \pm 11.25	length of the baby (mm)

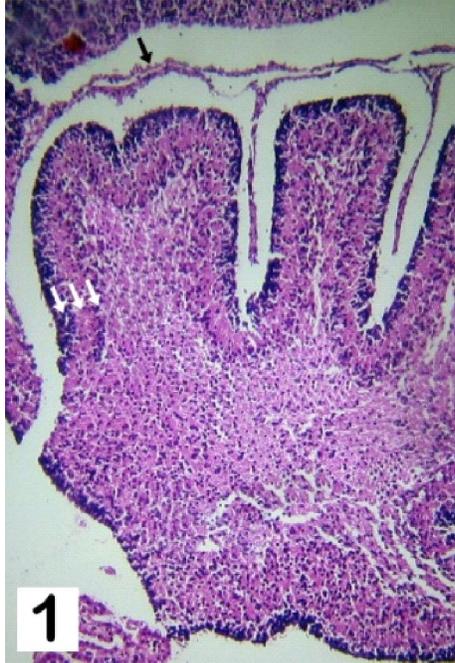
(*) shows a meaningful difference between interfere groups 1&2 and control group ($p < 0.05$)

According to the microscopic observations from the initial growth of cerebellum in control group, three-layers of cerebellum were completely recognizable and also distinguishable from each other.

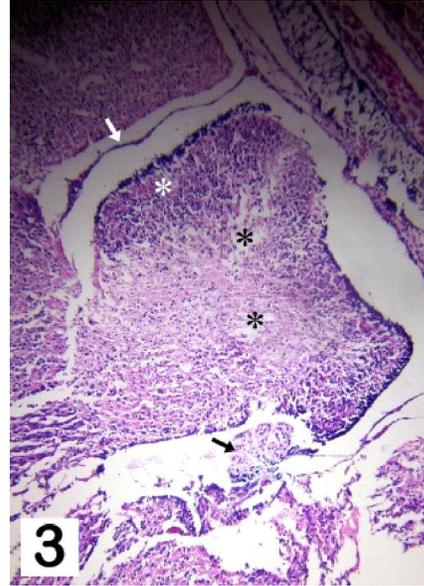
The cerebellum had a foliated shape, and the development of soft tissue of pia matter was observable around the cerebellum. In interfere group 1, the layers of molecular cells, purkinje cells and granular cell had no regular tissue and seemed distorted compared with those of control group so that the cerebellum had no foliated shape, it had no initial chin and it had a completely primary shape (image 1, 2). In interfere group 2, the same as interfere group 1, cerebellum had no foliated shape and no initial fold and it had a completely primary shape, as well as three layers of cells from the cerebellar cortex with no regularity and it seemed distorted (image 3-4). The presence of purkinje cells related to cerebellar cortex in interfere group 1 compared with the density of purkinje cells in control

groups were rarely observable (image 4,5). When the white matter of cerebellum in interfere group 1 was observed, Compared with the normal condition of control group, deficiency was observed in tropil tissue which was accompanied with a decrease in color-receiving and an increase in interstitial space and decrease in the density of neuroglia cells. Also, deficient in dismyelination of nervous fibers in the white matter of cerebellum in interfere group 1 was seen (image 4, 5). In comparing the meninge around the cerebellum in interfere group 1 with the normal condition of Meningeal tissue in control group, soft tissue of pia matter was pletosis and edematosis (image 4, 5). The results obtained from interfere group 1 was the same as those obtained from interfere group 2 ; however, in comparing these two groups, there was less edematous, and pletosis in the soft tissue of pia matter in interfere group 2 (image 6).

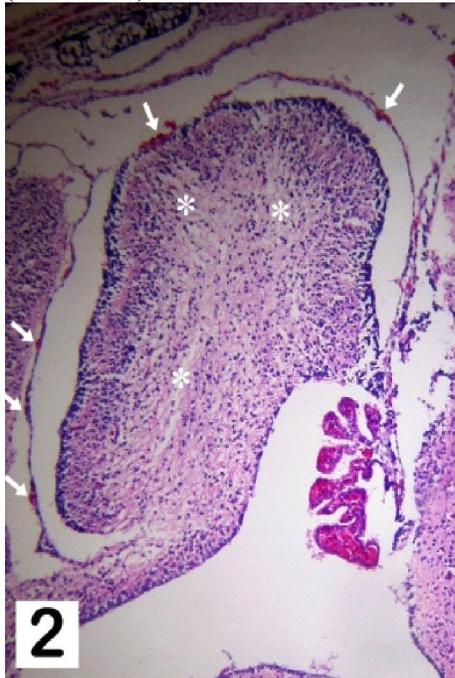
Image 1: shows a microscopic view from the cerebellar tissue with three-layers of cerebellar cortex (white flash), Meningeal membranes (black flash), and control group H&E * 200).



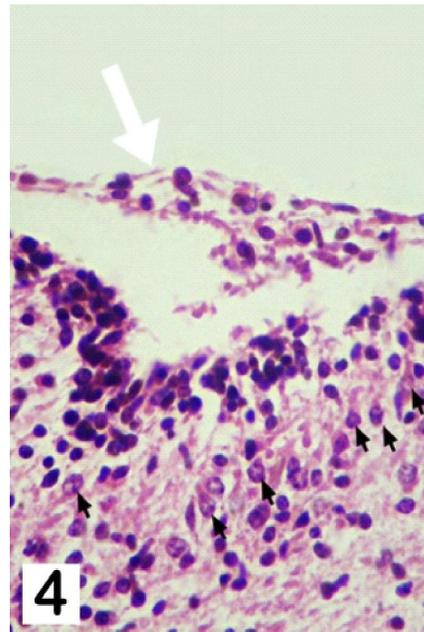
-Image 3: shows a microscopic view from the cerebellar tissue, meninge (white flash), interstitial space of white matter of cerebellum (*), choroid plexus of forth ventricle (block flash), interfere group 2, H&E*200)



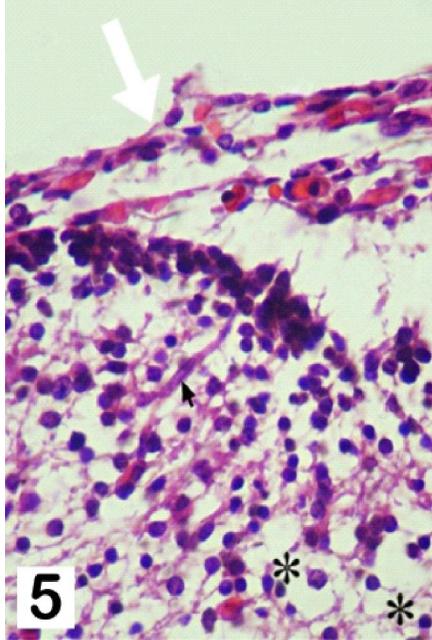
- Image 2: Shows a microscopic view from cerebellar tissue,meninge (White flash), interstitial space of white matter of cerebellum (*), interfere group 1 (H&E *200.)



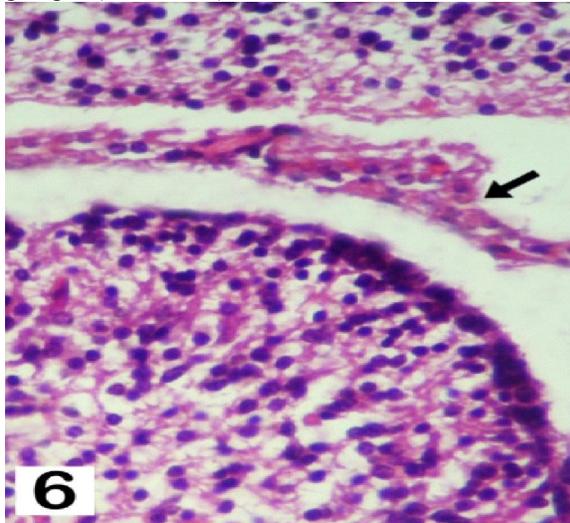
-Image 4: Shows a microscopic view from the cerebellar tissue accompanied with the dense cells of purkenje (black flash), meninge (White flash), control groupe 1 (H&E*360).



-Image 5: Shows a microscopic view from the cerebellar tissue, cells of purkenje (black flash), meninge (White flash), interstitial space of cerebellar white matter (*), interfere groupe 1 (H&E*360).



_Image 6: shows a microscopic view from the cerebellar tissue, meninge (black flash), interfere group 2 (H&E*360).

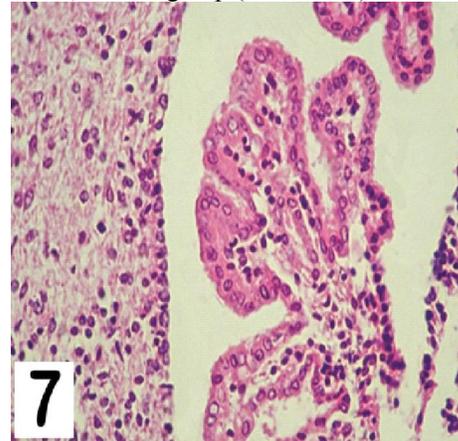


On the basis of the observations made from the forth ventricle structure around the cerebellum in control group, the choroid tissue was completely developed and the cover cells of coroid: had a normal condition. In interfere group 1, the structure of coroidi forth ventride was pletosis and the cover cells of choroid, due to the necrotic appearance had lost their uniformity and the coroidal tissue was distorted. Necrotic cast accompanied with fibrin

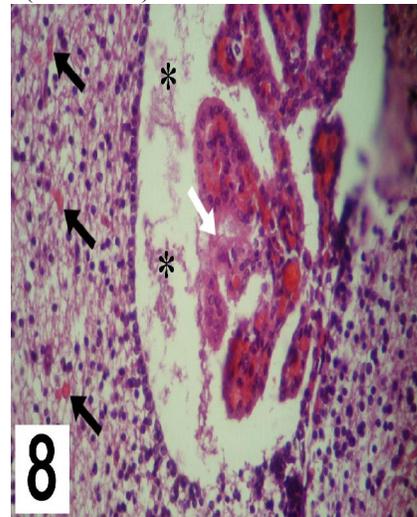
leakage, following damage to the capillaries of the choroidal plexus in the forth ventricle were rarely observable (4-7, 4-8).

On the basis of observations made from interfere group 2, showed that the condition of choroidal tissue and the cover cells of choroid, were similar to those of control group while the amount of pletosis was less than that of control group (image 3-4).

-Image 4-7: shows a microscopic view from the choroidal forth ventricle in the vicinity of cerebellar tissue, control group (H&E*320)



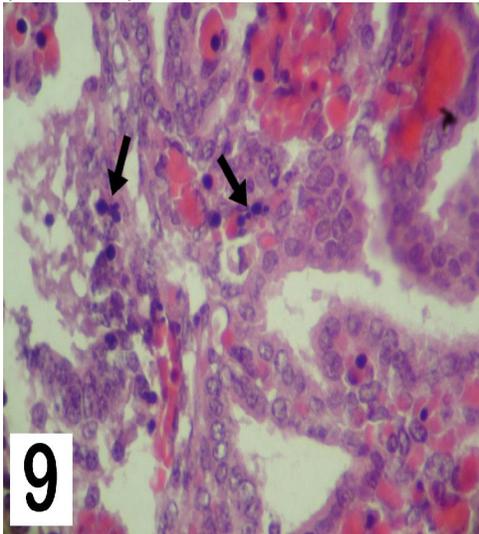
-Image 4-8: shows a microscopic view from the choroidal forth ventricle in the vicinity of cerebellar tissue accompanied with pletosis and necrotic appearance (white flash), fibrin leakage in ventricle space (*) and the development of blood veins in cerebellar tissue (black flash), interfere group 1(H&E*320).



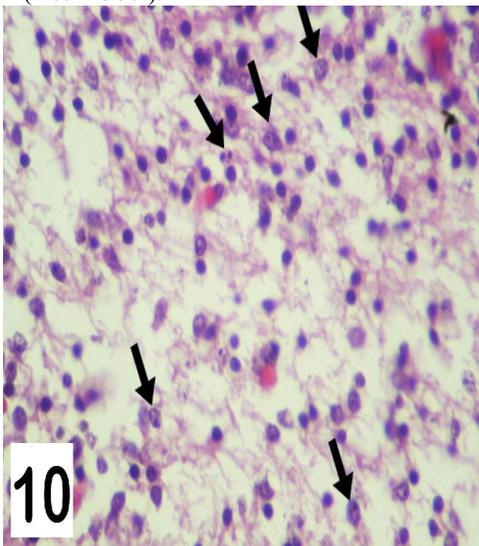
The occurrence of apoptosis in cover tissue of ventricle was one of the other observations made in interfere group 1 compared with that of the control group Apoptosis was observable with an increase in cytoplasm, condensation and segmentation of

chromatin nuclear, and finally formation of apoptosis mass (image 4-9). The occurrence of apoptosis, also, was observable dispersal in the neuroglial cells of cerebellar white matter (4-10). In changes referring to the appearance of apoptosis in the nervous cells of cerebellar Cortex was rarely observable. However, in investigating interfere group 2, the occurrence of apoptosis in the cover cells of choroidal plexus and neuroglial cells of cerebellar white matter has not been reported.

-Image 4-9: shows a microscopic view from the cover cells of coroidal forth ventricle, segmentation of chromatin nuclear, formation of apoptosis mass in the cover cells, (black flash) interfere group 1 (H&E*360).



-Image 4-10: shows a microscopic view from the apoptosis occurrence in neuroglial cells of cerebellar white matter, (black flash) interfere group 1 (H&E*360).



-Discussion and conclusion

More often the damages to cerebellar structure investigated in the experimental models of studies done on: pregnancy period, fetus edem, brain scheme, and pre-birth survys. In the present study, on the basis of observations from samples, descriptions made it clear that cerebellar structure, from the organogenesis period to the end of pregnancy period, is pertain to the cerebellar damages which are meaningful, so that such injuries may cause problems during in-uterine period, or after birth they may cause problems spiritually or cause problems during the maturity period. In a study by Hutton L.C and et al (2007) which was done by the interference of androtoxins on the development of cerebellum in sheep fetus, they pointed to the meaningfulness of injuries on cerebellar structure during the end period of pragnency.

In an investigation done by Necchi D.,and Scherini, E.(2002) which was on the occurrence of malformation in the process of development in cerebellar tissue, it has been asserted that the interference of anti-mitotic ctivity drugs or X ray in newborn rats causes the initial damages to the pia matter of meninge (32). Also, in the present study, with respect to the observations made on the damages, due to the effect of cisplatin on the newborn rats in interfere group1, pletosis and edem in pia matter around the cerebellum were reported. In studies done by Jaworek and et al (2002, 2003), Gulben and et al (2010), and also M.E srefoglu and et al (2006), it was indicated that in rats which were treated by meliatonin before infusion of acute pancreatic, the morphological signs of edem, locosite extraction, cell vaculation decreased significantly (22,27,23,31).

In the present study, also, it has been observed that there is a decrease in pletosis and edem in the cover tissue of forth ventricle and Meningeal membranes in cerebella tissue. Studies done by Bauman M., Kemper T.L.(1998) and Kern.J.K.(2003) in the field of neuropathology asserted that a high percentage of damage to cerebellar purkinje structure is accomplished with a decrease in the number and some times in the size of cerebellar purkenje cells (25,20). In addition, Amal T. Abou Elghalit and et al (2010) reported that cisplatin is well known for harmonizing DNA. And those harmonies not only prevents from proliferation and transcription from DNA but they also end to the planned death of cells (Apoptosis). Also, in their studies, cerebellar Cortex of animals which were under the treatment by cisplatin showed structurally distinguishable damages to their layer of purkenje cells (9). In present study, also, the dense conditionof purkenje cells in interfere group1 and interfere group2 was reported which was

an evidence for the destructive effect of cisplatin on the purkensis's mitotic activities. Parkash and etal(2007) in an study on the 10th and 12th of pregnancy (orgonozens period) which was done on the rats, showed that cytotoxic cyclophosphamide had a halting effect on the cover cells of choroidal girid significantly and it also caused apoptosis infusion in the brain cells (34). Also, QING and et al (2011) in their studies, reported that the resistance of cisplatin is multifaceted, such as ; it causes, a change in the development of DNA mechanism, and a decrease in the accumulation of cisplatin into the cells, finally, an increase in the deficiency of apoptosis (30). Also, in present study the effect of cisplatin on the cover cells of choroidal tissue in interfere group 1 was demonstrated by segmentation of chromatin nuclear (apoptosis) and the effect of cisplatin on the cells of pia matter with the occurrence of apoptosis were reported. In the study by Cagonli and et al (1995) it was indicated that melatin protects against apoptosis in nervous cells of rat under in-vitro condition (11). Munoz-Casares and et al (2006) in their investigation showed that melatonin is able to decrease the process of apoptosis and necrosis in pancratin(14). Moreover, Fengz and et al (2005, 2006) and Leony and et al (2006) reported that melatonin and its metabolites act as anti-oxid (17-18), are free radical absorbents, and have anti-apoptosis roles and prevent from unnatural increase of Nitric Oxide (No) in cerebellar Cortex(28). Abdel-Rahim and et al(2001) in their studies reported that melatonin is useful in cases which aflatoxic poisoning happen, in such a way that it halts the liver's apoptosis by creating a balance in the level of oxidant-anti-oxidon system (7). In other studies which were done in this field, by Maurizi (1997), Deigner and et al (200), persengiev (2001), it was indicated that apoptosis cell death, as a result of not getting energy from melatonin has been failed, and its role as an anti-apupetosis in the treatment of degenerative nervous is reported (30, 14, 35). Also, in this study, the protective role of melatonin on the decrease of apoptosis and anti-apoptosis activities has been emphasis on the basis of results obtained from the present study and review of the studies which were done previously, it can be concluded that the effects of anti-mitotic-activities (cytotoxic) can include:

- Halting role of drug on the segregation and proliferation of cells in cerebellar cortex.
- Anti-oxide of melatonin can have anti-apoptosis role in the cerebellar tissue.

Also, regarding the findings obtained from this study and those of previously done, with respect to the anti-oxide effects of melatonin, it can be concluded that:

- Melatonin can be effective in decreasing pletosis and edematous in cerebellar cortex.
- Anti-oxide of melatonin can have an anti-apoptosis activity in cerebellar cortex.

In sum, in present study, new events were presented so as to prove the influsion mechanism retardation in the formation of cerebellum in newborn rats which were treated by the chemotherapy drug of cisplatin during their intr uterin lives. Also, the apoptosis infusion and distortion of cell structure in cerebellar cortex created by cisplatin and anti apoptosis effects of cisplatin were presented.

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