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Protective Effect of Dendrobium Officinale Polysaccharide on Ovarian and Embryonic Development of Female Mice with Reproductive Damage

Chunmeng Li[1], Mingzhu Huang[1], Qijun Kong[1], Chunbin Lu[1] and Biao Liu[2]

[1]School of Jinan University, Guangzhou 510632, China;

[2]Nanjing Institute of Environmental Sciences, Nanjing 210042, China.

Abstract

To determine the protective effect of Dendrobium officinale polysaccharide (DOP) on ovarian and embryonic development of female mice with reproductive damage induced by cyclophosphamide (CP), 150 female Kunming mice were randomly separated into the following 6 groups (n=25): control; DOP(200mg/kg); CP; CP+DOP_L(100mg/kg); CP+DOP_M (200 mg/kg); and CP +DOP_H(400 mg/kg). On day 0, each group received an intraperitoneal injection of CP (75mg/kg), and the control and DOP groups received an intraperitoneal injection with sterile physiologic saline. The DOP and CP+$DOP_{L/M/H}$ groups received intragastric doses of DOP, and the CP and control groups received intragastric dosed of physiologic saline in the same amount for 7 d. At the conclusion of intragastric dosing on d 7, ten mice were randomly sacrificed in each group. The ovarian indices were calculated. The ovarian tissues stained with hematoxylin and eosin and the pathologic changes were noted. The number of follicles at different stages were counted with the aid of a microscope. The malondialdehyde (MDA) and superoxide dismutase (SOD) content in ovarian tissues was detected using a chemical method. The remaining 15 mice in each group were mated with male mice after superovulation. Four of the mice were sacrificed 2.5 d after the appearance of a vaginal plug to assess embryonic development. Four of the mice were sacrificed on d 6.5 and the number of embryo implantation sites was measured with the aid of trypan blue staining. The remaining four mice were sacrificed on d 12.5 to calculate embryonic development at different stages and the non-viable embryo rate. CP significantly reduced the number of follicles at different stages and increased the number of atretic follicles in the ovaries of female mice. Also, CP affected embryo development and significantly increased the embryo death rate. CP treatment also significantly increased the MDA content in ovarian tissues and significantly reduced SOD activity. When CP and different doses of DOP were co-treated, the number of follicles at different stages in the ovary was increased and the number of follicles was reduced ($P<0.05$). Superovulation and in vivo fertilization experiments showed that DOP increased the percentage of embryos at different stages, especially the percentage of 8-cell embryos ($P<0.05$), and significantly reduced the percentage of broken embryos ($P<0.05$). DOP treatment also increased the number of embryo implantation sites ($P<0.05$) and reduced the rate of dead embryos ($P<0.05$). In addition, medium and high doses of DOP reduced the MDA content ($P<0.001$) and increased the SOD activity ($P<0.001$) in ovarian tissues. CP caused ovarian follicle loss in mice and severely affected embryonic development. DOP effectively alleviated reproductive injury in female mice caused by CP. At a DOP dose ⩾200 mg/kg, the effect was more obvious. The therapeutic effects of reproductive damage was related to the antioxidant effects.

Keywords

Dendrobium Officinale Polysaccharide; CP; Ovary; Embryo; Anti-oxidize Effect.

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1. Introduction

CP, also known as Andoran, is a commonly used alkylating antitumor drug. CP alone or in combination with other anti-tumor drugs can be used for the treatment of Hodgkin's disease, Burkitt's lymphoma, and breast cancer [1-2]. CP is used for reproductive system cancers, including testicular [3] and ovarian cancer [4]. CP also has a good curative effect and is currently the first-line medication for cancer treatment.

Although CP is widely used, there are many reports detailing the side effects [5-7]. The reproductive damage to females caused by CP has attracted increasing attention from researchers. Studies have shown that women treated with CP have interrupted menstrual cycles, a reduced number of follicles, premature ovarian failure, and infertility [8]. The ovarian pathology revealed cortical fibrosis and a decrease in the number of follicles, especially the number of primordial follicles.

In addition, the oxidative stress caused by CP is one of the side effects that occurs during tumor treatment. Nese [10] reported that CP causes a significant increase in malondialdehyde (MDA) and superoxide dismutase (SOD), while glutathione peroxidase (GPx) activity is significantly reduced. CP induces lipid peroxidation, which leads to oxidative damage to the rat ovary and promotes cell apoptosis. Petrillo [11] conducted a study on the toxicologic effects of CP on oocytes and found that low concentrations of CP induced DNA damage in oocytes, resulting in a significant reduction in follicles, and DNA damage in oocytes increased the risk of infertility. The observed DNA double-strand breaks also responded to CP-induced ovarian oxidative stress.

In recent years the adjuvant effect of polysaccharides on cancer treatment has attracted increasing attention [12-13], and the ameliorating effect of polysaccharides on ovarian injury has also begun to be studied. In 2015, Zhang et al. [14] studied the effects of sepia ink polysaccharides (SIPS) on CP-mediated intervention of ovarian oxidative stress injury in mice and found that it can significantly reduced ovarian MDA content, increased SOD activity, increased the ovarian index, and enhanced mouse ovarian antioxidant capacity. DOP is the main medicinal ingredient of *Dendrobium officinale*, which is a member of the orchid family. Recent studies have showed that the polysaccharide had strong antioxidant, anti-tumor, and anti-aging activity, and enhanced immunity [15]. *Dendrobium officinale* protocorm polysaccharide has a scavenging effect on superoxide anion-free radicals (.O2-) and hydroxyl-free radicals (.OH-), and inhibits the production of MDA in mouse liver mitochondria and liver tissues, and has good *in vitro* antioxidant ability [16]. Zhao [17] found that DOP reduces the MDA level of rat embryonic cardiomyocytes (H9c2 cells), increases SOD activity, and inhibits the production of intracellular ROS. Studies have shown that DOP also reduces ovarian damage in mice caused by aging via inhibition of NF-κB and p53/Bcl-2 signaling pathways [18].

CP treatment causes female reproductive damage. DOP has an antioxidant effect and can be used for cancer rehabilitation. Whether DOP has a protective effect on female reproductive damage caused by CP has not been reported. This study intends to use 75 mg/kg of CP to establish a female reproductive damage model. After the model is established, different doses of DOP will be given by gavage for 7 d to determine the effect of CP on the ovaries. The effects of follicle development, embryonic development, implantation and survival, and the protective effect of DOP on these reproductive injuries and the possible mechanism will be investigated.

2. Materials and Methods

2.1 Drugs and reagents

Dendrobium officinale, Guangdong Academy of Agricultural Sciences Crop Research Institute. cyclophosphamide, Baxter Oncology, Germany. PMSG and HCG, Ningbo No. 2 Hormone Factory. SOD Assay Kit, Sigma, USA. KSOM, refer to Nagy version "*Mice embryo manipulation experiment manual*" [19] preparation. Eosin hematoxylin kit, Shanghai Biyuntian Biotechnology Co., Ltd. Coomassie Brilliant Blue G250, Guangzhou Haoma Biotechnology Co., Ltd. Thiobarbituric acid, Beijing Solei Bao Technology Co., Ltd

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2.2 Animals and groups

5-week-old, healthy adult male and female Kunming mice (SPF), purchased from Guangdong Experimental Animal Center, weight 25±2 g. The experimental mice were bred adaptively in accordance with the national laboratory animal breeding standards, and the experiment started after 1 week. The room temperature is 22~24 ℃, the indoor humidity is 40%~70%, 12 hours of light, 12 hours of darkness. The mice in each group eat freely. All experiments were performed under the principles drawn up by the Experimental Animal Ethics Committee. The mice were randomly divided into control group, DOP group, CP group, CP+DOP_L group, CP+DOP_M group, CP+DOP_H group, 25 mice in each group. The treatment of mice in each group is as follows:

Control: a single intraperitoneal injection of physiological saline on day 0, continuous intragastric administration of distilled water for 7 days;

DOP: single intraperitoneal injection saline on day 0, continuous intragastric administration DOP (200 mg/kg) for 7 days;

CP: single intraperitoneal injection 75 mg/kg CP on day 0, continuous intragastric administration of normal saline for 7 days;

CP+$DOP_{L/M/H}$: a single intraperitoneal injection of 75 mg/kg CP on day 0, with 100 mg/kg, 200 mg/kg and 400 mg/kg doses for 7 consecutive days of DOP.

Twenty-four hours after the last gavage, 10 mice in each group were randomly killed by cervical dislocation. The remaining mice were treated with superovulation and mated with male mice of appropriate age, 5 mice were randomly sacrificed on d 2.5, d 6.5 and d 12.5 after the embolism (pregnancy).

2.3 Methods

2.3.1 Extraction of Polysaccharides from Dendrobium officinale

According to the method of Zhu Xuan [20], the crude polysaccharide of *Dendrobium officinale* was extracted. The fresh stems of *Dendrobium officinale* are dried and crushed into powder. Weigh a certain amount of *Dendrobium officinale* powder, add distilled water to it in a volume ratio of 1:20, and stir in a water bath at 90 ℃ for 100 minutes for 30 minutes, and then filter with double gauze to obtain Filtrate: Repeat the above process three times, combine the filtrate, concentrate with a freeze dryer, add 4 times the volume of absolute ethanol, precipitate 12 h in a refrigerator at 4 ℃, centrifuge to get the precipitate, and dry at 40 ℃ to get *Dendrobium officinale* Crude polysaccharides. The total polysaccharide content of *Dendrobium officinale* crude polysaccharides was 88.60% by phenol-sulfuric acid method [21].

2.3.2 Body weight and ovarian index

After the gavage, the mice in each group were killed by cervical dislocation, weighed, and the ovaries were quickly taken out and weighed to calculate the ovarian index.

Ovarian index = ovarian weight (mg) / body weight (g)

2.3.3 Histomorphology

The ovaries on both sides of the mouse were taken, fixed in 4% paraformaldehyde for 48 h, dehydrated and embedded. The paraffin-embedded ovaries were serially sectioned with a thickness of 6 mm. After staining with hematoxylin and eosin, the tissue morphology was observed. According to the method of Johnson J [22] and others, the follicle counts at all levels were performed.

2.3.4 Embryo percentage

On the 2.5 d of pregnancy, the mice in each group were sacrificed by cervical dislocation. The oviducts on both sides were taken out, and all the embryos were flushed out from the fimbria of the oviduct with an oviduct needle. The embryos at all levels and the percentage of broken embryos were observed and calculated.

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2.3.5 Embryo implantation sites

On 6.5 days of pregnancy, the mice were intravenously injected with 0.3 ml trypan blue dye solution at a concentration of 0.5%. After 10 minutes, the mice were sacrificed by cervical dislocation. The uterus on both sides were removed to observe the embryo implantation site. The naked eye can see that there are blue bands in the uterus, and the stained bands are circular and arranged in parallel [23].

2.3.6 Live embryo and dead embryo rates

On the 12.5 d of pregnancy, the mice were sacrificed by cervical dislocation. At this stage, the absorption site of the unviable fetus was visible. Embryos that undergo absorption or death become smaller, purple-black in color, hemorrhage and necrosis, and even melt into blood masses. Observation under the dissecting microscope is blurry and there is no fetal membrane structure. The normal embryo is larger and the color is pink. Observation of the fetal membranes and fetus under the microscope has clear contours, good light transmittance, no bleeding or stasis [24].

Dead embryo rate = R/(V +R)×100%

R is the number of embryos absorbed (dead embryos), V is the number of viable embryos.

2.3.6 MDA content and SOD activity in ovarian tissue

The SOD activity of ovarian tissue was tested according to the experimental operation manual in the SOD analysis kit (Sigma, 19160-1KT-F). Detect the MDA content in tissues according to the method reported by Zhao Keran [25-32].

2.4 Analysis

Use SPSS16.0 software for data analysis. The results are uniformly expressed as mean ± standard deviation ($\bar{\chi}\pm s$). The difference between the groups was analyzed by t test, and the significant difference was $p<0.05$.

3. Results

3.1 Effect of DOP on the body weight and ovarian index of female reproductive injury mice induced by CP

Compared with the control group, the weight of the mice in the CP and CP+DOP groups decreased, but there was no statistical difference ($P>0.05$). After CP treatment, the ovarian weights in each group of mice were significantly reduced ($P< 0.001$). Compared with the CP group, the ovarian weight in the CP+DOP_L group increased, but there was no statistical difference ($P>0.05$). The ovarian weight in the CP+DOP_M group and the CP+DOP_H group increased significantly ($P<0.01$); there was no statistical difference between the CP+DOP_M and CP+DOP_H groups ($P>0.05$; Tab.1).

Tab. 1 Effect of DOP on weight, Ovary weight and Ovarian index of female mice induced by CP ($\bar{\chi}\pm s$, n=5)

Group	Body weight/g	Ovary weight/mg	Ovarian index
Control	28.62±4.111	11.74±1.791	41.06±2.900
DOP	28.16±2.602	11.16±1.272	39.63±2.600
CP	24.56±3.193	4.58±1.071***	18.32±2.128***
CP+DOP_L	24.78±3.079	5.22±1.074***	21.15±2.325***#
CP+DOP_M	26.24±2.716	6.61±0.629***##aa	25.24±1.503***###aa
CP+DOP_H	26.26±2.380	7.10±0.809***##aa	27.05±2.304***###aaa

***P <0.001: compared with the control group;#P<0.05,##P<0.01,###P<0.001: compared with the CP group; aa$P$<0.01,aaa$P$<0.001:compared to the CP+ DOP_L group.

CP treatment will cause the weight of the mice to decrease ($P>0.05$), and the weight of the ovaries significantly decrease ($P<0.001$), which will lead to a significant decrease in ovarian index ($P<0.001$). But when DOP and CP are treated at the same time, the two doses of CP+ DOP_M group and CP+

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DOP_H group have a better protective effect on the development of the ovary. Although DOP group can not restore the ovarian weight and ovarian index to the normal level, the ovarian index of mice is significantly higher than that of the CP-treated mice (P<0.01), DOP treatment can reduce the weight loss of ovaries caused by CP (Tab.1).

3.2 Effect of DOP on the follicles of female mice ovary with reproductive damage caused by CP

Compared with the control group, the number of preantral follicles (including primordial follicles, primary follicles and secondary follicles) in the CP group was significantly reduced (P<0.001); the number of preantral follicles in the DOP group was also significantly reduced in the control group (P <0.001). Compared with the CP group, the number of preantral follicles in the $CP+DOP_{L/M/H}$ groups increased to varying degrees, especially the number of preantral follicles in the $CP+DOP_M$ group and $CP+DOP_H$ group increased significantly (P <0.01) (Fig. 1A). Similarly, for the antral follicles, the CP group reduced the number of antral follicles (P<0.001), and the $CP+DOP_M$ group and $CP+DOP_H$ group could significantly increase the number of antral follicles (P<0.01). But compared to the control group, the number of antral follicles was still significantly reduced (P<0.001) (Fig. 1B).

For the atretic follicles, compared with the control, the CP group significantly increased the number of atretic follicles, but when different doses of DOP were treated with CP at the same time, the number of atretic follicles was significantly reduced (P<0.001), but it was still significantly higher than that of the control group (P<0.001).

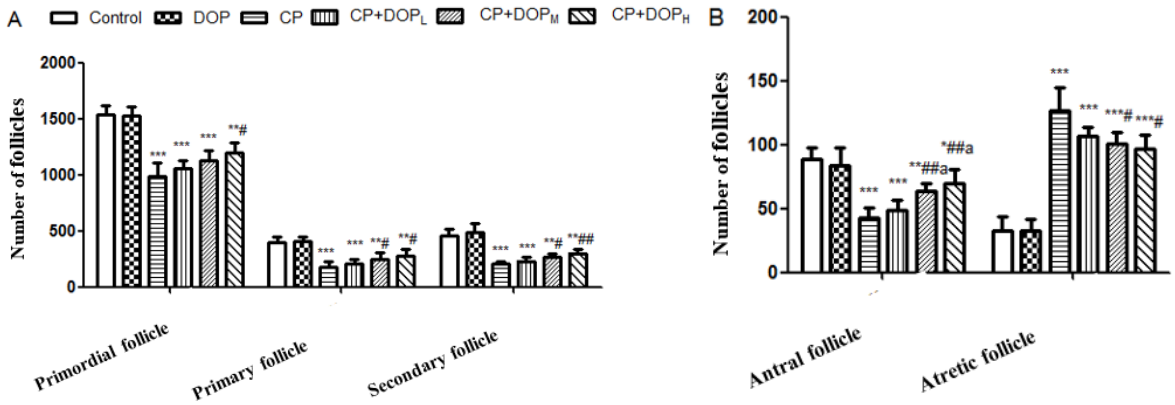

Fig. 1 Effect of DOP on the number of follicles at all levels in female mice exposed to CP. ($\bar{\chi}\pm s$, n=5)

$^{***}P$<0.001, compared with the control group; $^{\#}P$<0.05, $^{\#\#\#}P$<0.001, compared with the CP group; ^{a}P<0.05, ^{aa}P<0.01, ^{aaa}P<0.001, compared with the $CP+DOP_L$ group.

It can be seen from this that CP can significantly reduce the number of normal follicles and increase the number of atresia follicles in mice; however, when DOP and CP are treated at the same time, the $CP+DOP_H$ group and $CP+DOP_M$ group can effectively alleviate the decrease in the number of follicles at all levels and the increase in the number of atresia follicles in mice caused by CP.

3.3 Effect of DOP on the embryonic development of female mice offspring with reproductive damage caused by CP

In this experiment, the fertilization rate and the proportion of fragmented embryos were analyzed and compared when each group of mice was pregnant for 2.5 days, which are listed in Tab. 2. Compared with the control group, CP treatment resulted in a significant decrease in fertilization rate (P<0.001), and the fertilization rate in each dose group of $CP+DOP_{L/M/H}$ groups also significantly decreased (P<0.001). But compared with the CP group, DOP treatment can significantly increase the fertilization rate (P>0.05, P<0.001, P<0.001), and there is no statistical difference between the $CP+DOP_M$ group and the $CP+DOP_H$ group (P >0.05) (Tab.2).

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Compared with the control group, the proportion of 8-cell embryos in the CP group was significantly reduced by 65.77% (P<0.001), and the proportion of 8-cell embryos in the $DOP_{L/M/H}$ groups were significantly reduced by 60.99% (P<0.001), 48.81 % (P<0.001), 48.77% (P<0.001). But compared with the CP group, the 8-cell embryo ratio in the CP+DOP_L group was significantly increased by 13.94% (P>0.05), and the CP+DOP_M group and CP+DOP_H group were significantly increased by 49.54% (P<0.05), 49.64 % (P<0.05).

For the broken embryos, compared with the control group, the embryo fragmentation ratio of the CP group was significantly increased by 477.74% (P<0.001), and the embryo fragmentation ratio of the CP+$DOP_{L/M/H}$ groups were significantly increased by 366.55% (P <0.001), 250.93% (P<0.001), 205.01% (P<0.001). However, compared with CP, the embryo fragmentation ratio of each dose group of CP +$DOP_{L/M/H}$ groups was significantly reduced by 19.25% (P>0.05), 39.26% (P<0.001), 47.21% (P<0.001) (Fig.2).

Therefore, it was shown that CP treatment could reduce the fertilization rate of mice and increase the fragmentation ratio of embryos at all levels, especially the 8-cell embryo ratio at the 2.5-day pregnancy. However, when DOP and CP were treated at the same time, the CP+DOP_M group and CP+DOP_H group could significantly reduce the mice embryo fragmentation ratio, and significantly increase the 8-cell embryo ratio and the fertilization rate.

Tab. 2 Total number of embryos, broken embryo ratio and fertilization rate in each group at 2.5 days($\bar{\chi}\pm s$, n=5)

	Control (%)	DOP (%)	CP (%)	CP+DOP_L (%)	CP+DOP_M (%)	CP+DOP_H (%)
Total embryo rate	163 (100.00)	154 (100.00)	146 (100.00)	138 (100.00)	130 (100.00)	129 (100.00)
Fertilization rate	149 (91.41)	142 (92.21)	76 (50.44)***	83 (60.14)***	91 (70.00)$^{***\#\#a}$	95 (73.64)$^{***\#\#\#a}$

$^{***}P$<0.001: compared with the control group; $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001: compared with the CP group; ^{a}P<0.05: compared to the CP+ DOP_L group.

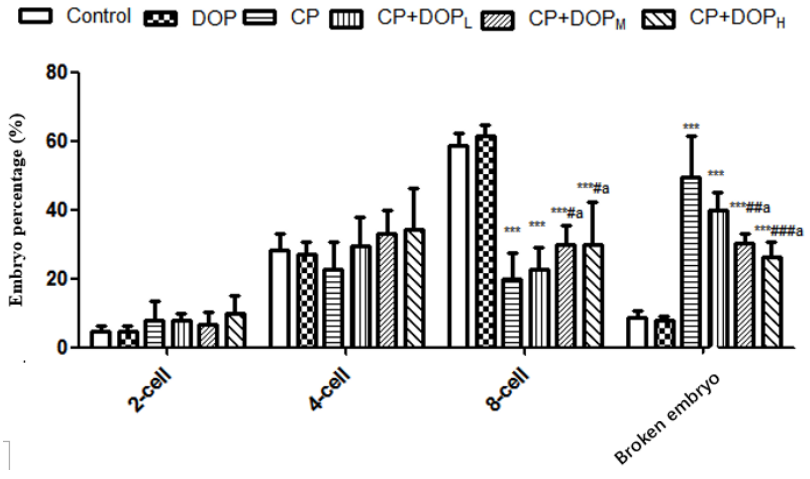

Fig. 2 Effect of DOP on embryonic development of female mice exposed to CP. ($\bar{\chi}\pm s$, n=5) $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001, compared with the control group; $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001, compared with the CP group; ^{aa}P<0.01, compared with the CP+DOP_L group.

3.4 Effect of DOP on Embryo Implantation Sites of Female Mice with Reproductive Damage Caused by CP

Compared with the control group, the number of embryo implantation sites in the CP group was significantly reduced by 39.22% (P<0.001), and the embryo implantation sites of the mice in the

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$DOP_{L/M/H}$ groups were significantly reduced by 29.41% (P<0.001). 0.001), 16.67% (P<0.05), 13.73% (P<0.05). Compared with the CP group, the number of embryo implantation sites in the CP+DOP_L group showed an increasing trend, but there was no statistical difference (P>0.05). The CP+DOP_M group mice embryo implantation sites increased significantly by 16.13% (P< 0.05), the embryo implantation site of CP+DOP_H group increased by 37.1% (P<0.01), and there was no statistical difference between CP+DOP_M group and CP+DOP_H group (P>0.05) (Figure 3).

It could be seen that CP significantly reduces the number of embryo implantation sites in mice; but when DOP and CP were treated at the same time, the CP+DOP_M group and CP+DOP_H group could significantly alleviate the decrease in the number of mice embryo implantation sites caused by CP.

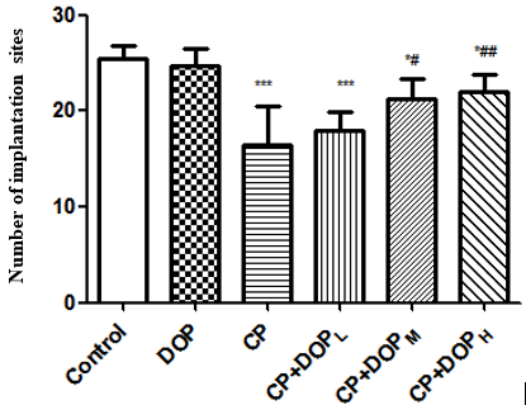

Fig.3 Effect of DOP on embryo implantation sites in female mice exposed to cyclophosphamide. ($\bar{\chi}\pm s$, n=5)

$^{*}P$<0.05, $^{***}P$<0.001, compared with the control group; $^{\#}P$<0.05, $^{\#\#}P$<0.01, compared with the CP group.

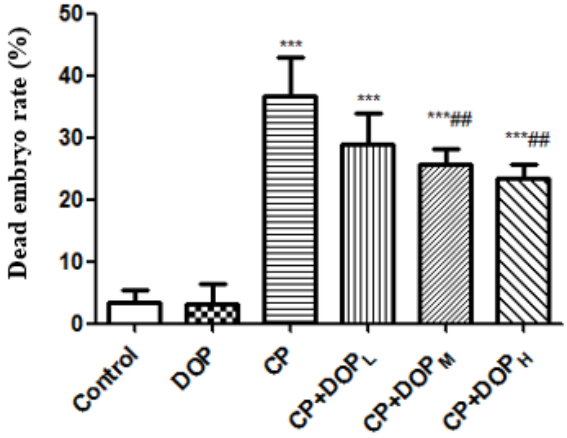

Fig. 4 Effect of DOP on the rate of dead embryos in female mice exposed to cyclophosphamide. ($\bar{\chi}\pm s$, n=5)

$^{***}P$<0.001, compared with the control group; $^{\#\#}P$<0.01, compared with the CP group.

3.5 Effect of DOP on the embryo death rate of female mice with reproductive damage caused by CP

Compared with the control group, the death embryo rate of the CP group increased by 99.26% (P<0.001), and the death embryo rate of the $DOP_{L/H/M}$ groups increased by 735.07% (P<0.001) and 640.39% (P<0.001)), 575.81% (P<0.001). Compared with the CP group, the death embryo rate of mice in each group decreased to varying degrees. The death embryo rate of the CP+DOP_L group had

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a decreasing trend, but there was no statistical difference (P>0.05). The embryo dead rate of the CP+DOP_M group was significantly reduced by 30.10% (P<0.01), the embryo dead rate of the CP+DOP_H group was significantly reduced by 36.20% (P<0.01), and there was no significant difference between the CP+DOP_M group and the CP+DOP_H group (P>0.05) (Fig. 4).

Therefore, it was shown that CP could significantly increase the embryo dead rate of mice. when DOP and CP were treated at the same time, the CP+DOP_M group and CP+DOP_H group could effectively alleviate the increase in the embryo dead rate of mice caused by CP.

3.6 Effect of DOP on the activity of MDA and SOD in the ovarian tissue of female mice with reproductive damage caused by CP

Compared with the control group, the MDA content of ovarian tissue in the CP group was significantly increased by 108.27% (P<0.001), and the $DOP_{L/H/M}$ groups were significantly increased by 88.56% (P<0.001), 71.71% (P<0.001), 55.14% (P<0.001). However, compared with the CP group, the MDA content in the ovarian tissue of the CP+DOP_L group had a decreasing trend, but there was no statistical difference (P>0.05). The CP+DOP_M group and CP+DOP_H group were significantly reduced by 17.56% (P< 0.05), 25.99% (P<0.001) (Tab. 3).

Similarly, compared with the control group, the CP group ovarian tissue SOD activity was significantly reduced by 46.5% (P<0.001), and the $DOP_{L/H/M}$ group was reduced by 40.96% (P<0.001), 32.13% (P<0.001), 30.89% (P<0.001) respectively. Compared with the CP group, the SOD activity in ovarian tissue of the CP+DOP_L group increased, but there was no statistical difference (P>0.05). The CP+DOP_M group and CP+DOP_H group significantly increased by 26.87% (P<0.001), 29.19% (P<0.001) (Tab. 3).

Therefore, it was shown that CP could significantly increase the MDA content of ovarian tissue and significantly reduce SOD activity. But the CP+DOP_M group and CP+DOP_H group could significantly reduce the MDA content and increase the SOD activity in ovarian tissue.

Tab.3 Effect of DOP on MDA content and SOD activity in ovary of mice with reproductive damage induced by CP($\bar{\chi}\pm s$, n=5)

Group	MDA content/nmol.mg^{-1} *prot*	SOD activity/U.mg^{-1} *prot*
Control	1.958±0.388	30.320±0.282
DOP	1.995±0.476	29.870±1.265
CP	4.078±0.430***	16.220±1.094***
CP+DOP_L	3.926±0.181***	17.903±0.972***
CP+DOP_M	3.362±0.401$^{***\#a}$	20.578±1.250$^{**\#\#\#aa}$
CP+DOP_H	3.018±0.225$^{***\#\#\#aa}$	20.955±1.200$^{**\#\#\#aa}$

$^{**}P$<0.01, $^{***}P$<0.001: compared with the control group; $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001: compared with the CP group; ^{a}P<0.05, ^{aa}P<0.01, ^{aaa}P<0.001, compared to the CP+ DOP_L group.

4. Conclusions

CP is a commonly used anti-cancer chemotherapeutic drug in clinical practice. CP has good therapeutic effect on a variety of cancers, including breast cancer, testicular tumors, ovarian cancer, and other reproductive system diseases, but the side effects are especially severe on the reproductive system, which limits its use. Wang et al. reported that CP causes a decrease in sperm count and vitality in mice, thereby causing damage to the male reproductive system. For females, the side effects of CP are usually associated with a high risk of infertility due to ovarian failure. Studies have shown that CP acts on oocytes and pre-granulosa cells in primordial follicles, thus leading to premature depletion of primordial follicles and affecting the development and maturity of follicles, thereby causing ovarian tissue damage. Therefore, it is very important to study the toxic effects of CP on the female reproductive system, prevention, and treatment.

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Some studies have shown that a single intraperitoneal injection of CP (75 mg/kg) in mice has no significant effect on mating or the pregnancy rate, but will cause a significant decrease in the number of follicles at all levels in the ovary and a 50% reduction in ovarian primitive follicle storage. Therefore, this method can be used to sensitively and conveniently assess damage of chemotherapeutics to fertility. In addition, exposure to CP during the follicular maturation stage has a greater impact on the reproductive performance of mice, including embryo implantation, embryo survival, and fetal malformation. Therefore, in this experiment a single intraperitoneal injection of CP (75 mg/kg) was used to establish an ovarian reserve and fertility damage model, and various experimental tests, such as ovarian, embryonic development, and fertility, were performed 7 d after CP injection. This experiment found that after a single intraperitoneal injection of CP for 7 d, compared with the control group, the ovarian index of the CP group mice was significantly reduced, the number of follicles at all levels was also significantly decreased, and the number of atretic follicles was significantly increased. The embryos of pregnant mice were significantly damaged, the number of implantation sites was significantly reduced, and the rate of dead embryos was significantly increased. This finding was consistent with existing results, indicating that CP has a significant effect on ovarian reserve and embryonic development, implantation, and embryo survival of female mice. In addition, the results of this experiment showed that DOP (200 mg/kg) significantly reduced the ratio of broken embryos in pregnant mice ($P<0.05$), significantly increased embryo implantation sites ($P<0.05$), and significantly reduced the dead embryo rate ($P<0.05$). Thus, DOP has a dose effect on the protective effect of CP-induced ovarian injury.

CP causes a significant increase in the level of oxidative stress in female ovarian tissues. In this study, compared with the control group, the MDA level of the mice was significantly increased after CP treatment, and the SOD activity was significantly reduced. DOP gavage improves the oxidative stress imbalance of ovarian tissue. In the 3 different DOP groups, at a DOP gavage dose of 200 mg/kg, the MDA level of ovarian tissue was significantly reduced ($P<0.05$) and SOD activity increased significantly ($P<0.05$). These results indicate that DOP has a good antioxidant effect, which is consistent with the results of previous studies.

In summary, this study showed that CP reduced the number of follicles at all levels of the ovary. CP affects the development of pre-implantation embryos, embryo implantation, and post-implantation embryo development in pregnant mice, which are important physiologic processes of embryonic development and reproduction. Studies have also confirmed that CP can cause oxidative stress in ovarian and endometrial tissues, and the production of excessive MDA and other oxidative free radicals is related to female reproductive damage caused by CP. The main pharmacologic component DOP in *Dendrobium candidum* significantly reduces the ovarian toxicity and the toxic damage to the follicles caused by CP, and the treatment has an important effect on implantation, a key step in reproduction and the normal development of embryos after implantation. In this experiment, DOP gavage (200 mg/kg) improved the antioxidant capacity, and at the same time had a dose-effect relationship. In addition, our research showed that DOP has a wide range of protective effects on ovarian function, which also indicated that DOP has the potential to be a drug for treating ovarian damage caused by CP.

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