Original Article

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Protective Effects of Galbanum Essential Oil on Histomorphometric Changes in Placenta of Cyclophosphamide Treated Rat

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ABSTRACT

BACKGROUND AND OBJECTIVE: Cyclophosphamide is a drug widely used to treat cancer, skin diseases and immune system disorders. Since cyclophosphamide passes placenta during pregnancy and causes disorders in fetus, this study was conducted to assess the protective effects of galbanum essential oil against cyclophosphamide toxicity in rat placenta tissue.

METHODS: In this experimental study, 19 pregnant rats were divided into 3 groups. On the 13th day of pregnancy, control group (n=7), cyclophosphamide group (n=7) and cyclophosphamide and galbanum group (n=5) received intraperitoneally a dose of normal saline and cyclophosphamide (15 mg/kg), cyclophosphamide (15 mg/kg) and galbanum essential oil (200 mg/kg), respectively. All rats were euthanized on 20th day of pregnancy. Placentas were separated and fixed in 10% buffered formaldehyde after their morphology and morphometry was studied. Tissue sections were prepared using the routine techniques of tissue sections preparation and their histology and histometry were studied by light microscopy.

FINDINGS: Cyclophosphamide decreased 0.4 g of placental weight compared with control group (0.5 g) and decreased the thickness and length of large and small diameter of placenta from 3.62, 11.2 and 14.15 mm in control group to 2.81, 9.25 and 11.37 mm, respectively ($p \leq 0.05$). Histologically, it decreased the thickness of the labyrinth and basal layers to 385.73 and 72.80 µm and decreased the number of giant cells to 2.45 compared with control group ($p \leq 0.05$). Co-administration of galbanum essential oil and cyclophosphamide increased the length of large diameter, thickness of the labyrinth and basal layers and number of giant cells to 12.77 mm, 467.64 and 91.1 µm and 7.60, respectively ($p \leq 0.05$).

CONCLUSION: Results of the study revealed that galbanum essential oil can protect placenta tissue against toxic effects of cyclophosphamide.

KEY WORDS: *Galbanum, Placenta, Cyclophosphamide, Rat.*

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Introduction

Cyclophosphamide is a drug widely used for cancer treatment and is an effective immunosuppressive drug to prevent the rejection of transplanted organs and tissues (1). It is also used to treat skin diseases and immune system disorders (2). On the other hand, cyclophosphamide has mutagenic properties that interfere in antioxidant defense system of tissues and produces a considerable amount of free radicals. Cyclophosphamide passes placenta during pregnancy and causes disorders in fetus (3). A great deal of teratogenic effects of the drug is due to the mechanism of oxidative stress (4).

Najafzadeh et al. have shown that cyclophosphamide administration causes various skeletal malformations in rat fetus, including cleft palate, limb defects and exencephaly (5); Delucia et al. have indicated in their study that cyclophosphamide administration during pregnancy delays fetal growth, decreases placental weight and shortens the length of umbilical cord (6); Finally, Padmanabhan et al. have reported histopathological changes in placenta after cyclophosphamide administration (7).

There is much evidence to prove that some herbal compounds such as flavonoids, polyphenols, carotenoids and phytosterols can act as mutagenic inhibitors (8).

Galbanum (Ferula gummosa Boiss) contains phenolic and flavonoid compounds (9) and they have great potential to attach to oxygen free radicals (10). This plant is used in traditional medicine for epilepsy and convulsion treatment, memory improvement (11), wound healing and treating skin infections and rheumatism (12).

It is also well-known for its antimicrobial activities (13, 14). Rashidi et al. have reported in their study that galbanum essential oil can decrease cleft palate induced by caffeine in rat fetuses (15).

Placenta plays a key role in fetal growth and since cyclophosphamide harms the placenta by inducing oxidative stress and as a result causes fetal malformations, galbanum seems to have the ability to prevent the histological changes in rat placenta due to its antioxidant compounds.

Since the effect of galbanum essential oil on cyclophosphamide-induced histological changes in placenta has not been studied yet, the present study was conducted to investigate the protective effect of galbanum essential oil on cyclophosphamide-induced histological changes in rat placenta.

Methods

19 adult female Wistar rats were used in this experimental study. 10-12 weeks old male and female rats with a mean weight of 200±20 g were kept separately in wire cages under light conditions (12 hours of light and 12 hours of darkness), in room temperature (23±2ºC) and constant humidity (50±10 %) for two weeks before the experiment. They were fed regularly with drinking water and compressed food provided from Pars animal feed company in Tehran. Every step was taken according to basic principles of research ethics approved by Ethics Committee of Chamran University.

Every three female rats were kept with one male rat in one cage for 12 hours for mating and vaginal plug of female rats was checked the next day to confirm their pregnancy. The day of detection of the vaginal plug was designated as day 0 of pregnancy. The pregnant rats were randomly divided into 3 groups. On the 13th day of pregnancy, the first group (control, n=7) was administered with normal saline in equal amount of cyclophosphamide intraperitoneally; the second group (cyclophosphamide, n=7) was administered with cyclophosphamide (provided from Baxter International Inc., Germany) at a dose of 15 mg/kg body weight intraperitoneally (16) and the third group (n=5) like the second group was administered with cyclophosphamide along with galbanum essential oil (prepared from galbanum essential oil company, Kashan, Iran) at a dose of 200 mg/kg body weight intraperitoneally (15).

All rats were euthanized on 20th day of pregnancy according to ethical considerations. After opening abdominal cavity and cutting uterine horns, placentas and fetuses were removed. After examining the appearance, they were weighed on a scale and the placental diameter and thickness was measured with a caliper. 5 µm tissue sections were prepared using the routine techniques of tissue sections preparation.

After staining with Haematoxylin and Eosin (H&E) and Periodic acid-Schiff (PAS), 5 samples from each group, 5 slides from each sample and 5 microscopic fields of view from each slide were studied randomly with a light microscope (Olympus BX51). After taking photographs with a digital camera (Olympus DP71) attached to the microscope, thickness of basal, labyrinth and decidua zones were measured with ImageJ software. Trophoblast giant cells in each section were also enumerated and changes in the size and shape of giant and glycogen-containing cells were

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studied. The obtained data was analyzed using SPSS 16.0, the one-way analysis of variance (ANOVA) and Least Significant Difference (LSD) test and p≤0.05 was considered significant.

Results

The morphological and morphometric results: Mean weight of the group that received cyclophosphamide (0.3994±0.01 g) was significantly less than control group (0.5024±0.01 g), but it was not significant compared with the group that received cyclophosphamide plus galbanum (0.4486±0.02 g). Mean thickness of placenta in cyclophosphamide group (2.81±0.12 mm) was significantly less than control group (3.62±0.22 mm) (p≤0.05), while the mean thickness of placenta in the group that received cyclophosphamide plus galbanum (3.26±013 mm) was more than cyclophosphamide group, though not statistically significant (p=0.09) and it was less than control group (p≤0.05) (table 1). Mean length of small diameter in cyclophosphamide group (9.25±0.27 mm) and the group that received cyclophosphamide plus galbanum (9.85±0.21 mm) was less than control group (11.02±0.23 mm) (p≤0.05) and there was a significant difference between the two treatment groups (p=0.11). Mean length of large diameter in cyclophosphamide group (11.37±0.32 mm) and the group that received cyclophosphamide plus galbanum (12.77±0.30 mm) was less than control group (14.15±0.26 mm) (p≤0.05) and no significant difference were observed between the two treatment groups (p≤0.05) (table 1).

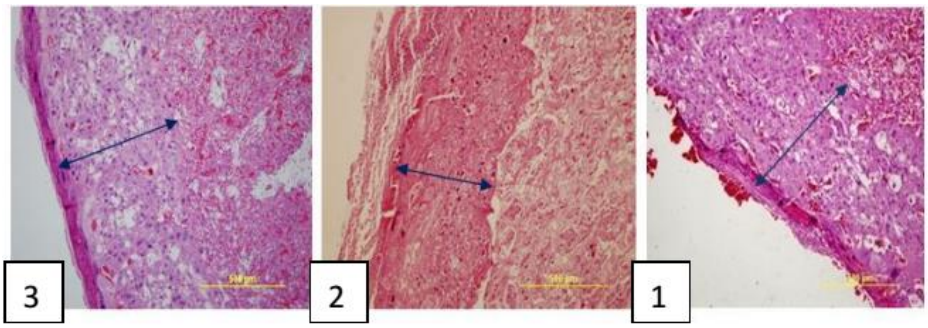
Morphologically, placentas in the control group were in light red and revealed no sign of transformation or hyperemia. Placentas in cyclophosphamide group were small and revealed hyperemic spots while placentas in the group that received cyclophosphamide plus galbanum were morphologically identical to the control group.

Histological and histometrical results: Mean thickness of labyrinth zone in cyclophosphamide group (385.73±39.81 µm) and the group that received cyclophosphamide plus galbanum (467.64±17.18 µm) was less than control group (456.14±15.63 µm) (p≤0.05) and no significant difference were observed between the two treatment groups. Mean thickness of basal zone in cyclophosphamide group (72.8±3.29 µm) and the group that received cyclophosphamide plus galbanum (91.1±9.45 µm) was less than control group (94.04±3.10 µm) (p≤0.05) and no significant difference were observed between the two treatment groups (Fig 1).

Table 1. A comparison of mean weight, small diameter, large diameter and thickness of placenta in witness group, cyclophosphamide group and cyclophosphamide plus galbanum group

Groups Parameters	Witness Mean±SD	Cyclophosphamide Mean±SD	Cyclophosphamide plus galbanum Mean±SD
Placenta weight (g)	0.50 ± 0.01^{b}	0.40 ± 0.01^{a}	0.44 ± 0.02^{ab}
Small diameter (mm)	11.02 ± 0.23^{b}	9.25 ± 0.27^{a}	9.85 ± 0.21^{a}
Large diameter (mm)	14.15 ± 0.26^{c}	11.37 ± 0.32^{a}	12.77 ± 0.30^{b}
Thickness (mm)	3.62 ± 0.22^{b}	2.81 ± 0.12^{a}	3.26 ± 0.13^{ab}

Dissimilar letters in each horizontal row represent a meaningful difference between groups at p≤0.05 level.



Picture 1. Microscopic structure of placenta in groups under study. Decrease in thickness of basal layer (blue arrow) in cyclophosphamide group (2) compared with witness group (1) and cyclophosphamide plus galbanum group (3) is clearly visible in this picture (H&E, 10×).

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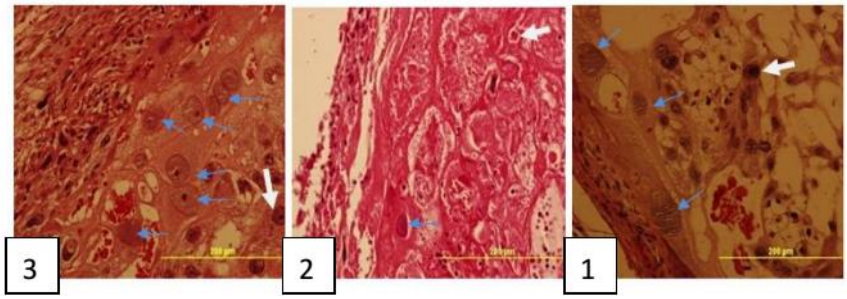
Mean thickness of decidua zone in cyclophosphamide group (43.12±4.82 µm) was more than control group (28.8±2.51 µm) ($p\leq0.05$) but did not show a significant difference with the group that received cyclophosphamide plus galbanum (27.69±0.97 µm). Average number of giant cells in cyclophosphamide group (2.45±0.17) was less than control group (5.6±0.54) ($p\leq0.05$) but administration of cyclophosphamide plus galbanum increased the number of these cells significantly (7.60±0.65) ($p\leq0.05$) (table 2). Studying the histology revealed that cyclophosphamide causes shrinkage in the nucleus of giant cells, severe necrosis of spongiotrophoblast cells along with hyperemia in labyrinth layer, shrinkage of trophoblast cells in labyrinth zone and necrosis of decidua cells; but administration of cyclophosphamide plus galbanum did not reveal any change in the nucleus of giant cells or necrosis of spongiotrophoblast cells and decreased hyperemia in basal zone. Furthermore, trophoblast cells maintained their natural structure in the labyrinth zone like the control group (Fig 2). Overall, galbanum essential oil increased the thickness of basal layer, thickness of labyrinth layer and the number of giant cells and decreased the thickness of decidua layer significantly compared with the cyclophosphamide group ($p\leq0.05$).

Table 1. A comparison of mean thickness of labyrinth, basal and decidua layers and average number of giant cells in witness group, cyclophosphamide group and cyclophosphamide plus galbanum group

Groups / Parameters	Witness Mean±SD	Cyclophosphamide Mean±SD	Cyclophosphamide plus galbanum Mean±SD
Thickness of labyrinth (mµ)	456.14±15.63^{b}	385.73±39.81^{a}	467.64±17.18^{b}
Thickness of basal (mµ)	94.04±3.10^{b}	72.8±3.29^{a}	91.1±9.45^{b}
Thickness of decidua (mµ)	28.8±2.51^{a}	43.12±4.82^{b}	27.69±0.97^{a}
Number of giant cells	5.60±0.54^{b}	2.45±0.17^{a}	7.60±0.65^{c}

Dissimilar letters in each horizontal row represent a meaningful difference between groups at $p\leq0.05$ level.



Picture 2. Microscopic structure of placenta in groups under study. Increase in number of giant cell (blue arrow) in cyclophosphamide plus galbanum group (3) compared with cyclophosphamide group (2) and also shrinkage and necrosis of spongiotrophoblast cells (white arrow) and hyperemia in labyrinth zone in cyclophosphamide group compared with the other two groups is clearly visible in this picture (H&E, 40×).

Discussion

Administering cyclophosphamide decreased placental weight, diameter and thickness in this study. Delucia et al. have reported that intraperitoneal administration of cyclophosphamide decreased the size of rat placenta on the 10th day of pregnancy (6). Park et al. also have reported weight loss in rat placenta after administration of cyclophosphamide (4).

Najafzadeh et al. have reported that cyclophosphamide administration causes various skeletal malformations in rat fetus (5). In the present study, treatment with cyclophosphamide caused significant histological and histometrical changes such as decreasing the number of giant cells and increasing the thickness of decidua zone compared with control group which was accompanied by severe hyperemia and cellular necrosis and this was in accordance with findings of Padmanabhan et al. (7). Acrolein, as one the metabolites of cyclophosphamide, combines with other molecules by interfering in antioxidant defense system and generating a great deal of oxygen free radicals and causes direct oxidation and inhibits their normal performance and launches apoptosis (17). During the process of cell division, acrolein combines with DNA and breaks it down into single-stranded DNA

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molecules and ultimately leads to formation of micronucleus and death of cell (18). Ghaffarie et al. have well illustrated the relationship between genotoxicity and oxidative stress in many experimental animal models (19).

Thus, according to multiple studies it seems that administration of cyclophosphamide induces oxidative stress and reactive oxygen species in placenta tissue which leads to cell death. Co-administration of cyclophosphamide and galbanum increased the thickness of large diameter, the thickness of labyrinth and basal layers and the number of giant cells and decreased hyperemia and necrosis compared with cyclophosphamide group.

Medicinal plants, particularly the ones rich in polyphenolic compounds and flavonoids act as protective agents against chemical drugs due to their antioxidant characteristics (8). Galbanum has antioxidant characteristics which are related to its phenolic compounds and flavonoids (20). Phenolic compounds reveal protective characteristics against harmful effects of genotoxic carcinogens by inhibiting reactive oxygen species (ROS) and reinforcing antioxidant defense system of the host (21). Galbanum attracts hydrogen peroxide and this characteristic is due to presence of phenols in this extract (22). Concerning the antioxidant characteristics of galbanum, Dehpour et al. have indicated in their study that phenolic compounds of galbanum act as good donors of electron and hydrogen atom and thus should be able to prevent free radical chain reactions and conversion of reactive oxygen species (ROS) to stable products (23). In the present study, administration of cyclophosphamide probably increased the level of hydrogen peroxide in placenta tissue and therefore generated oxidative stress and toxicity in this tissue and antioxidant compounds of galbanum inhibited them to a large extent. In a study, Kim et al. have assessed the protective effect of diallyl disulfide against cyclophosphamide-induced toxicity in the process of rat fetus development and indicated that diallyl disulfide can inhibit the toxic effects of cyclophosphamide on rat fetus development by maintaining the enzymatic activities of antioxidants due to its antioxidant compounds (24).

Kim et al. have reported in another study that administration of cyclophosphamide causes decrease in fetal and placental weight, increase in fetal resorption and fetal disorders and co-administration of cyclophosphamide and pine bark extract (pycnogenol) inhibits the toxic effects of cyclophosphamide due to its potent antioxidant activities (25).

Results of the present study reveal that co-administration of cyclophosphamide (15 mg/kg body weight) and galbanum essential oil (200 mg/kg body weight) can protect rat placenta against cyclophosphamide-induced histomorphometric damages.

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