

Effect of Prenatal Silymarin Administration in the Gestational Period on Fetal Growth

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ABSTRACT

Background: Silymarin is a potent inhibitor of apoptosis. The present study aimed to investigate the effect of prenatal silymarin administration during the gestational period on fetal growth.

Methods: This experimental study was conducted on 24 virgin female BALB/c mice weighing 20-30 grams. One male animal was caged with two females overnight, and they were examined for the presence of a vaginal plug in the next morning. Presence of vaginal plugs was considered to be gestational day zero (GD0). The mice were randomly divided into four groups, including three groups of pregnant mice administered with silymarin via intraperitoneal injection at doses of 50, 100, and 200 mg/kg/day (groups 1, 2, and 3, respectively) during GD6-15 (organogenesis) and a control group. Animals in the control group received normal saline via the same route in equivalent volumes. Data analysis was performed in SPSS version 18.0, and the pathological scores were compared using Kruskal-Wallis, ANOVA, and Dunn's multiple comparison tests. P-value of less than 0.05 was considered statistically significant.

Results: Administration of silymarin had no effect on the weight gain of the mothers. However, placental weight gain decreased in the second and third group compared to the other groups ($P \leq 0.001$). In addition, head circumference was observed to reduce in all the treatment groups compared to the control group ($P \leq 0.001$). Also, the findings showed significant differences in the resorption rate and weight gain in all the treatment groups compared to the control group ($P = 0.001$).

Conclusion: According to the results, silymarin administration during gestation may lower weight gain and decrease placental circumference in the fetus of mice.

Keywords: Birth weight, Growth, Head circumference, Height, Silymarin

Introduction

Silybum marianum (milk thistle) is a medicinal plant from the Asteraceae family. It is native to the Mediterranean countries and grows in southern Europe as a medicinal crop. Since ancient times, seeds of milk thistle have been used in the treatment of liver diseases and to increase breast milk (1, 2).

Silymarin and silibinin are known to provide cytoprotection and hepatoprotection. In the literature, the antioxidant properties of silibinin have been demonstrated in the rats poisoned with ethanol or paracetamol (3, 4). Moreover, silymarin and silibinin exert protective effects against lipid peroxidation (5).

Silymarin has been reported to reduce the plasma cholesterol in hyperlipidemic rat and increase protein synthesis in the injured liver as a mechanism to explain liver tissue regeneration

(6-8). In addition, it has anti-inflammatory and anti-carcinogenic properties (6, 9, 10). Silymarin has proven effective in protecting HaCaT cells against apoptosis at some levels of UVB damage (11).

The photoprotective effects of silymarin are associated with potential antitumor mechanisms, including the inhibition of DNA synthesis and induction of apoptosis, accompanied by the modulation of p53. Furthermore, the hepatoprotective properties of silymarin against ethanol abuse have been established (12, 13).

Silymarin is able to cross the placenta during pregnancy (14). Several factors can contribute to the inhibition of intrauterine growth and fetal death (15-20). Moreover, various chemical, pharmaceutical, and remedies are known to cause fetal growth restriction and fetal resorption (21).

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Although apoptosis is essential to eliminating genetic damage, it may lead to the loss of normal cells. Programmed cell death in mammalian blastocysts is one of the main stages in normal development (17). Deviation of apoptosis could impair fetal maturation and lead to embryonic death. Apoptosis plays a pivotal role in the formation of the embryo, as well as the other stages of normal fetal development (15). In this regard, Manna (16) claimed that among various cytokines, the tumor necrosis factor (TNF) is the most significant inducer of apoptosis, and silymarin is a potent inhibitor of TNF; therefore, silymarin is considered to be a potent inhibitor of apoptosis. The apoptotic effects of this herb may cause intrauterine growth retardation and fetal death.

The present study aimed to investigate the effect of prenatal silymarin administration during the gestational period on fetal growth.

Methods

Animals and Treatment

This experimental study was conducted on 24 virgin female BALB/c mice weighing 20-30 grams aged approximately two months during 2014-2015. Animals were provided by Avicenna Research Institute in Mashhad, Iran. The mice were kept in a 12-hour light-dark cycle at the temperature of $23\pm 2^{\circ}\text{C}$ and had unlimited access to food and water. All animal experiments were approved by the Animal Care and Ethics Committee of Mashhad University of Medical Sciences.

To perform the experiment, one male mouse was caged with two females overnight, and they were examined for the presence of a vaginal plug in the next morning. Presence of the vaginal plugs was considered as gestational day zero (GD0). The animals were randomly divided into four groups, including three groups of pregnant mice administered with silymarin via intraperitoneal injection at doses of 50, 100, and 200 mg/kg/day (groups 1, 2, and 3, respectively) during GD6-15 (i.e., organogenesis). Animals in the control group received normal saline with tween via the same route in equivalent volumes.

Body weight of the pregnant mice was measured

on GD1 and GD15 in all the groups. On GD18, the pregnant females were killed by cervical dislocation, and their fetuses were removed by cesarean section. Fetuses and placenta were weighed and fixed in 7% formaldehyde at 4PoPC. In addition, fetal resorption and birth rate were reported in each group. Crown-rump length and head and placental circumference of the fetuses were measured using a caliper. Head circumference was measured based on the following formula:

$$C = \pi \{3(A+B) - [(3A+B)(A+3B)]^{1/2}\}$$

Statistical Analysis

Data analysis was performed in SPSS version 18.0, and data were presented as mean and SEM. Moreover, the pathological scores were compared using Kruskal-Wallis, ANOVA, and Dunn's multiple comparison tests. In all statistical analyses, P-value of less than 0.05 was considered significant.

Results

Maternal and Fetal Body and Placental Weight Gain

Silymarin administration had no effect on the body weight gain of the mothers, and body weight gain of the fetuses was found to be lower in the second and third group compared to the control group ($P \leq 0.001$). Additionally, a significant decrease was observed in the second and third groups during silymarin administration (Table 1). Also, placental weight gain reduced in the second and third group compared to the other groups ($P \leq 0.001$) (Table 1).

Head and Placental Circumference and Crown-Rump Length of the Fetuses

According to the information in Table 1, head circumference showed a reducing trend in all the treatment groups compared to the control group ($P \leq 0.001$). In addition, placental circumference of the fetuses was lower in the mice administered with silymarin ($P \leq 0.001$) (Table 1). On the other hand, a significant reduction was observed in the crown-rump length of the treatment groups compared to the control

Table 1. Body Weight Gain, Crown-Rump Length, Head and Placental Circumference, and Placental Weight in Study Groups

	Control	Silymarin (50 mg/kg/day)	Silymarin (100 mg/kg/day)	Silymarin (200 mg/kg/day)
Body weight Gain (g)	0.03 (0.02-0.03)	0.03 (0.01-0.04)	0.03 (0.01-0.04)*	0.03 (0.009-0.04)*
Placental Weight	0.017 (0.008-0.02)	0.018 (0.009-0.02)	0.019 (0.004-0.01)*	0.019 (0.004-0.02)*
Head Circumference (cm)	0.53 (0.5-0.6)	0.51 (0.4-0.5)*	0.46 (0.4-0.5)*	0.42 (0.4-0.5)*
Placental Circumference (cm)	0.86 (0.7-0.9)	0.74 (0.6-0.9)*	0.73 (0.5-0.9)*	0.70 (0.6-0.8)*
Crown-Rump Length (cm)	1.3 (0.9-1.4)	1.12 (1-1.3)*	1.10 (0.9-1.5)*	1.10 (0.9-1.4)*

Data presented as median (min-max); * $P \leq 0.001$ compared to controls (1PstP, 2PndP, and 3PrdP groups injected with silymarin during GD6-GD15)

group ($P \leq 0.001$) (Table 1).

Fetal Resorption Rate

According to the data, rate of fetal resorption had a significant difference in the groups and increased in all the treatment groups compared to the controls ($P=0.001$). Resorption rate was zero in the control group, while it was 4.95% in first group (50 mg/kg/day), 11.2% in the second group (100 mg/kg/day), and 15.12% in the third group (200 mg/kg/day).

Discussion

According to the results of the present study, silymarin may cause intrauterine growth restriction (IUGR) and fetal resorption by affecting apoptosis. Apoptosis eliminates genetic damage and is regulated to prevent the loss of normal cells. Programmed cell death in the mammalian blastocysts occurs until the next normal developmental stage. During normal development, both parts of the blastocyst (inner cell mass and trophectoderm) undergo apoptosis; however, both parts have different sensitivities toward the factors that cause apoptosis. Deviation in normal apoptosis in the blastocysts may lead to IUGR or intrauterine fetal death (14-17, 22).

Silymarin is an anticancer agent, which inhibits the activation of the *STAT3* gene and activates caspase and apoptosis in human prostate carcinoma DU145 cells (23). In the bladder transitional-cell papilloma, the apoptosis caused by silymarin is partly mediated through the Cip1/p21 cleavage by caspase, which is reversed by Cip1/p21 siRNA (24). According to the literature, the other mechanisms of apoptosis induction by silymarin involve p53-caspase-2 activation and caspase-mediated cleavage of Cip1/p21 (21). Treatment with silymarin down-regulates the protein levels of the anti-apoptotic proteins FLIPL and FLIPS (25).

In a recent study, silymarin administration was reported to modulate multiple components in the death receptor-mediated apoptotic pathway and was responsible for its ability to recover TRAIL sensitivity in the TRAIL-resistant glioma cells (21). Furthermore, silymarin preferentially activated the DNA-PK-p53 pathway for apoptosis in response to the UVB-induced DNA damage, which might also be a predominant mechanism of silymarin efficacy against UVB-induced skin cancer (26). Another study suggested that silymarin could induce p53-mediated autophagic cell death by activating the ROS-p38 and JNK pathways and inhibiting the MEK/ERK and PI3K/Akt pathways (27).

Other findings in this regard have demonstrated that enhanced apoptosis through caspase-3 may be involved in the occurrence of IUGR without maternal symptoms (28, 29), which is consistent with the results of the present study. In our previous study, silymarin caused apoptosis in some internal organs in the fetuses of mice (unpublished data).

In a research by Wilkinson, in-utero exposure to ginger tea resulted in the increased rate of early embryo loss, while it also decreased the birth weight and crown-lump length of the surviving fetuses (30). These findings are in line with the results of the present study. In another study, it was reported that *Zingiber officinale* significantly lowered the weight gain of pregnant mice, while the uterus weight reduced noticeably. Moreover, weight and crown-lump length of the treated embryos decreased (21). Similar to the current research, the mentioned study has certain implications.

According to the literature, consumption of blue cohosh could lead to IUGR and fetal resorption (31). These studies have also suggested that using some herbal remedies might impair fetal growth and even cause early fetal resorption.

Conclusion

According to the results, silymarin administration during gestation could lower the weight gain and decrease the placental circumference in the fetuses of mice. However, several factors may require further investigation in this regard in order to verify the exact mechanisms of the detrimental effects associated with prenatal exposure to silymarin.

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