Int J Clin Exp Med 2019;12(1):212-219
www.ijcem.com /ISSN:1940-5901/IJCEM0081345

Original Article

Lycopene protects from perfluorooctanoic acid induced liver damage and uterine apoptosis in pregnant mice

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Received June 15, 2018; Accepted September 6, 2018; Epub January 15, 2019; Published January 30, 2019

Abstract: This study aimed to investigate the protective effects of lycopene on liver oxidative damage and decidual apoptosis induced by perfluorooctanoic acid (PFOA) in early pregnancy mice. Fifty pregnant mice were randomly divided into five groups of 10 each. Mice in the control group were fed with 0.1 mL of soybean oil every morning from gestation day (GD) 1-GD 7, while mice in the PFOA model group were fed with 20 mg/kg PFOA daily. Mice in the other three lycopene groups were fed with 20 mg/kg PFOA daily and different concentrations of lycopene at doses of 10 mg/kg (Low), 20 mg/kg (Medium), and 40 mg/kg (High). Liver and uterus samples were collected on GD 9 and the organ index was calculated. Contents of SOD, GSH-Px, and MDA in the liver homogenate were measured and expression of apoptotic proteins (Bax, Bcl-2, Fas, FasL, Caspase-3) in uterine cells was detected by immunohistochemistry. Weight loss in lycopene groups was alleviated, compared to the PFOA model group. Liver indexes decreased significantly in medium and high dose lycopene groups. Uterine indexes and average weights of medium and high groups were increased significantly ($P<0.01$). In addition, activities of SOD and GSH-Px were significantly elevated in the high dose group (E) and levels of MDA in medium- and high-dose lycopene groups were significantly lower than controls. Moreover, levels of Caspase-3 and Fas were significantly decreased in medium and high groups ($P<0.01$), while levels of Bcl-2 and FasL and Bcl-2/Bax ratios were significantly increased ($P<0.01$). Results of the present study revealed that lycopene ameliorated PFOA induced liver oxidative damage and uterine apoptosis in early pregnant mice.

Keywords: Lycopene, perfluorooctanoic acid, liver oxidative damage, uterine apoptosis, pregnant mice

Introduction

Due to its unique molecular structure and good chemical properties, perfluorooctanoic acid (PFOA) has been widely used in many fields closely related to human daily life, such as cosmetics, carpets, non-stick pans, and fire extinguishers. PFOA, a new type of persistent organic pollutants (POPs), is widely present in various environmental media, such as atmosphere [1], soil [2], sediments [3, 4], lakes, and rivers [5, 6]. This poses a serious threat to the ecological environment. However, in recent years, PFOA has been detected in foods, such as vegetables, meats, eggs, and milk, as well as drinking water [7, 8]. Thus, it endangers the health of human beings and animals, arousing the attention of many scholars. Numerous studies have shown that PFOA can cause severe liver damage [9, 10] and early pregnancy loss [11], as well as affecting embryonic development [11], reproductive toxicity [12], and genetic toxicity [13, 14].

Lycopene, a red-orange carotenoid present in tomatoes, has received much attention in recent years. Lycopene has a wide range of sources, being found in tomatoes, peaches, pumpkins, papaya, and other fruits and vegetables [15]. It has characteristics of high safety and low side effects. Therefore, lycopene has been commonly used in food, medicine, cosmetics, and other industries. Moreover, lycopene has been developed in animal husbandry and aquaculture as a new, highly efficient, green, and multi-functional feed additive. Plenty of studies have demonstrated that lycopene has anti-oxidant [16, 17], anti-inflammatory [18], anti-cancer [19, 20], and anti-apoptotic [21] activities. It also can promote animal growth, improve animal production performance [22], and affect animal reproduction [23].

In this study, a PFOA exposure mice model was established by intragastric administration to investigate the protective effects of lycopene on liver and uterine injuries induced by PFOA in early pregnant mice.

Materials and methods

Materials

Lycopene was purchased from Sigma (≥85% purity, Sigma-Aldrich). PFOA was purchased from Fluka (>98% purity, Sigma-Aldrich). SOD, GSH-Px, and MDA kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Bax, Bcl-2, Fas, FasL, and Caspase-3 antibodies were obtained from Boster Biological Technology Co. Ltd (Wuhan, China).

Animals

Kunming mice, at 8 weeks of age, including females and males, were obtained from SPF animals Biotech Co. Ltd., Beijing, China and housed in a room with a controlled temperature (20-24°C) and light cycle (12-hour light/12-hour dark). Animals had free access to water and were fed with a standard commercial pellet diet [24]. Female mice were mated with males overnight. The next morning was considered gestation day (GD) 0 if a vaginal plug was observed. All animal studies were conducted with approval by the Council for Animal Care in Hebei Province.

Fifty pregnant mice were randomly divided into five groups: control group, PFOA model group, PFOA+low dose lycopene group, PFOA+medium dose lycopene group, and PFOA+high dose lycopene group. Soybean oil was administrated to pregnant mice in the control group and model group every morning from GD1-GD7, while mice in the other groups were given different doses of lycopene (10, 20, 40 mg/kg) daily. Distilled water was administrated to pregnant mice in the control group every afternoon from GD1-GD7, while mice in other groups received 20 mg/kg PFOA daily from GD1-GD7 by gavage.

Body weights of pregnant mice were recorded from GD1 to GD9. Pregnant mice were sacrificed by cervical dislocation after collecting blood on GD9. Liver and uterus samples were collected and organ indexes were calculated. Contents of SOD, GSH-Px, and MDA in the liver homogenate were measured and expression of apoptotic proteins (Bax, Bcl-2, Fas, FasL, Caspase-3) in uterine cells was detected by immunohistochemistry.

Liver oxidative damage

Saline was added to 0.1~0.2 g liver tissue to dub 10% liver tissue homogenate in the ice bath environment with homogenizer, according to the quality (g): volume (mL) = 1:9, 3000 r/min centrifugation for 10 minutes. Supernatant was collected to detect the activity of SOD and GSH-Px and levels of MDA, according to manufacturer instructions.

Immunohistochemistry

Collected uterine tissues were fixed with 4% paraformaldehyde to prepare paraffin sections. These were cut into 5-μm sections. Next, expression of apoptotic proteins (Bax, Bcl-2, Fas, FasL, Caspase-3) in uterine cells was detected by immunohistochemistry. Sections were observed under light microscope.

Statistics

Data were recorded using the Excel database and statistical analyses were performed using SPSS19.0 analytic software (IBM Corporation, Armonk, NY, USA). Eight different regions per slice were selected from 5 slices per group under 400x light microscope. The optical density of every apoptotic protein was measured by Image J software. Results of Immunohistochemistry were analyzed by semi-quantitative pathological evaluations. All charts were graphed using GraphPad Prism 5.0.

Differences in parameters were analyzed by analysis of variance (ANOVA) and multiple comparisons between groups were performed using Duncan's method. Significance levels are $P<0.05$ or $P<0.01$.

Results

Effects of lycopene on body weights of pregnancy mice

Body weights (BW) of each group were about 27-28 g on the first day of pregnancy. Body weights of the control group showed a slow increasing trend from GD1-GD9. Body weights

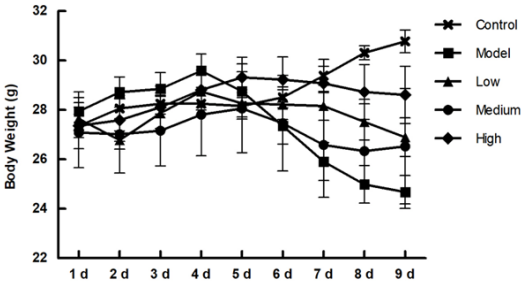

Figure 1. Effects of lycopene on body weights of pregnant mice.

of the PFOA model group started to decrease suddenly on GD 5 and continued until GD9. This decreasing trend in body weights of lycopene groups showed some improvement, compared with the PFOA model group. Low and medium dose lycopene groups started to decrease on GD 6, while the high dose lycopene group started on GD 7. Although administration of lycopene did not bring PFOA decreased body weights back to normal, lycopene significantly elevated BW values of the mice (**Figure 1**).

Effects of lycopene on the liver and uterus

Effects of lycopene on the liver and uterus were evaluated in early pregnant mice. Compared to the control group, PFOA treatment significantly increased (P<0.01) liver indexes. However, compared with the PFOA model group, liver indexes were significantly decreased in the medium dose lycopene group (P<0.05) and high dose lycopene group (P<0.01). Results suggest the protective effects of lycopene on PFOA-induced liver injuries (**Figure 2A**).

Uterine indexes and average uterine weights of the model group were significantly decreased in the PFOA model group (P<0.01), while those increased after treatment with different doses of lycopene. Compared to the PFOA model group, uterine indexes of the low dose lycopene group were significantly lifted (P<0.05). Moreover, uterine indexes and average uterine weights were very significantly increased, close to those of the control group in medium- and high-dose lycopene groups (P<0.01) (**Figure 2B**, **2C**).

Effects of lycopene on liver oxidative damage

Effects of lycopene on PFOA induced liver oxidative damage were evaluated in pregnant mice. As shown in **Figure 3**, PFOA induced a significant increase in levels of MDA and decreased levels of SOD and GSH-Px (P<0.01). Lycopene groups (low, medium, and high) not only elevated levels of SOD and GSH-Px but also reduced levels of MDA. Compared to the PFOA model group, only administration of the high dose lycopene significantly inhibited decreases of SOD (P<0.01) and GSH-Px (P<0.05) (**Figure 3B**, **3C**). In addition, medium and high dose lycopene significantly reduced levels of MDA (**Figure 3A**).

Effects of lycopene on uterine apoptosis

Bax and Bcl-2: Bax and Bcl-2 were expressed mainly in decidual cells surrounding the uterine blastocyst and some in the myometrium, as shown in **Figures 4** and **5**. They presented as brown in the cytomembrane and cytoplasm. In the nucleus, they showed strong positive expression.

There were significant differences in expression of Bax and Bcl-2 between control and model groups (P<0.01). Compared to the model group, low dose lycopene significantly inhibited the decrease of Bcl-2 (P<0.05) and increase of Bax (P<0.05) caused by PFOA. However, medium and high dose lycopene groups significantly upregulated expression of Bcl-2 and ratios of Bcl-2/Bax (P<0.01), while downregulating expression of Bax. Results showed that lycopene ameliorated PFOA-induced uterine apoptosis of early pregnant mice in a certain dose-dependent manner (**Figure 6**).

Caspase-3, Fas, and FasL: As shown in **Figures 7-9**, Fas and FasL were expressed mainly in decidual cells presenting brown surrounding the uterine blastocyst, mostly located in the cytoplasm of decidual cells. Some were expressed in the myometrium and uterine glandular epithelial cells. Moreover, brown particles of Caspase-3 presented as a hollow ring and were mainly expressed in the decidual cell layer of embryonic contact sites.

Compared to the control group, expression of Caspase-3 and Fas proteins in the PFOA model group was significantly increased (P<0.01), while FasL levels were significantly decreased (P<0.01). Compared to the PFOA model group, levels of Caspase-3 in the low dose lycopene group were significantly decreased (P<0.05), but there were no significant differences between Fas and FasL levels. However, levels of Caspase-3 and Fas in medium and high do-

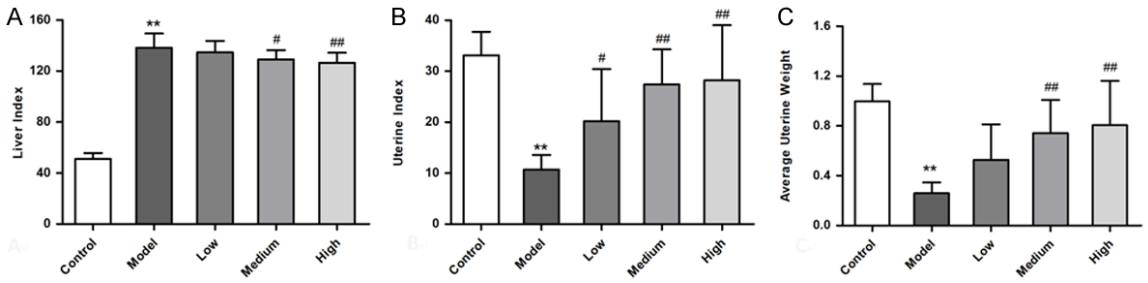

Figure 2. Effects of lycopene on the liver and uterus of early pregnant mice. Mice were given PFOA (20 mg/kg) and low, medium, or high doses of lypocene. Liver index (A), uterine index (B), and average uterine weight (C) were calculated. $^{**}P<0.01$, compared with the control group. $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with the model group.

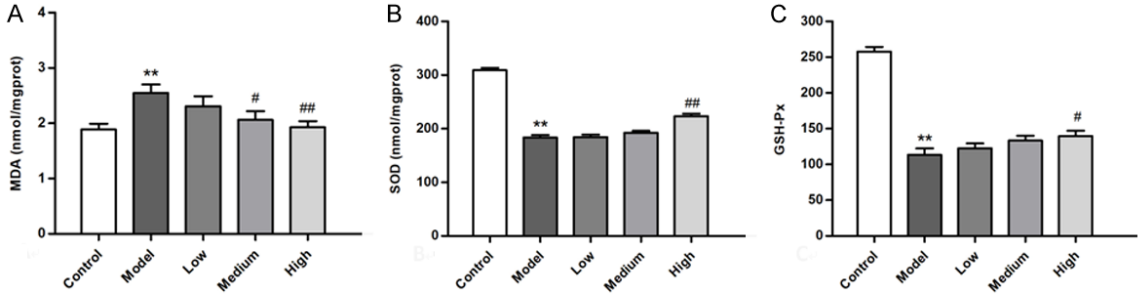

Figure 3. Effects of lycopene on liver oxidative damage of early pregnant mice. Mice were given PFOA (20 mg/kg) and low, medium, or high doses of lycopene. Levels of MDA (A), SOD (B), and GSH-Px (C) were detected. $^{**}P<0.01$, compared with the control group. $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with the model group.

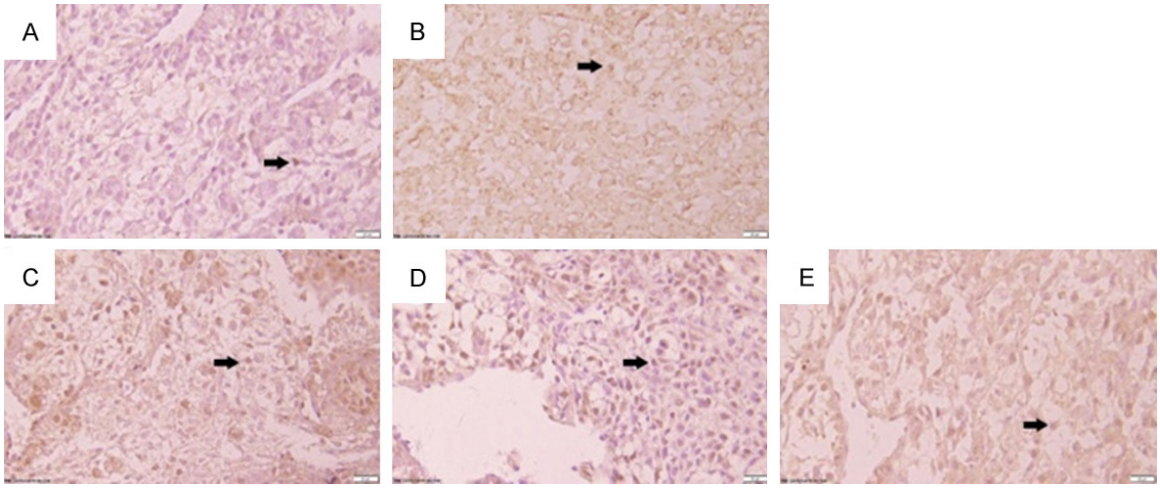

Figure 4. Expression of Bax in the uterus of early pregnant mice. A: Control group; B: Model group (20 mg/kg PFOA); C: Low group (20 mg/kg PFOA+10 mg/kg Lycopene); D: Medium group (20 mg/kg PFOA+20 mg/kg Lycopene); E: High group (20 mg/kg PFOA+40 mg/kg Lycopene). Bar = 20 μm.

se lycopene groups were significantly lower ($P<0.01$) and FasL levels were significantly increased ($P<0.01$). When lycopene doses were increased, expression of these three proteins gradually returned to control group levels (**Figure 10**).

Discussion

Results of the present study showed that lycopene ameliorated PFOA-induced liver oxidative damage and uterine apoptosis of early pregnant mice.

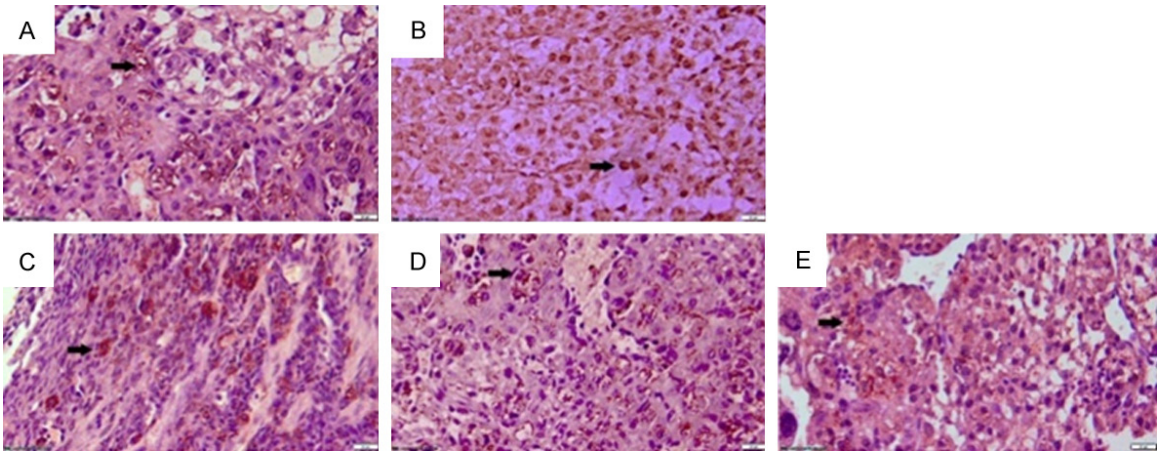

Figure 5. Expression of Bcl-2 in the uterus of early pregnant mice. A: Control group; B: Model group (20 mg/kg PFOA); C: Low group (20 mg/kg PFOA+10 mg/kg Lycopene); D: Medium group (20 mg/kg PFOA+20 mg/kg Lycopene); E: High group (20 mg/kg PFOA+40 mg/kg Lycopene). Bar = 20 μm.

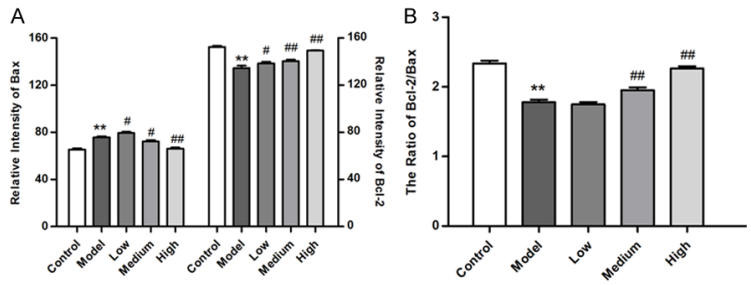

Figure 6. Effects of lycopene on expression of Bax and Bcl-2 of early pregnant mice. Mice were given PFOA (20 mg/kg) with low, medium, or high doses of lypocene. Expression of Bax and Bcl-2 (A) was measured. The value of Bcl-2/Bax (B) was calculated. $^{**}P<0.01$, compared with the control group. $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with the model group.

In this study, a PFOA exposure model was established by intragastric administration in pregnant mice. Weight loss caused by PFOA was ameliorated after giving lycopene. Decreased indexes and MDA levels of the liver were observed, as well as improved SOD and GSH-Px activities, indicating that lycopene has protective effects on liver injuries caused by PFOA exposure. Perfluorooctanoic acid (PFOA) is a member of perfluoroalkyl and polyfluoroallkyl substances designated by the acronym PFASs. PFOA is mostly distributed in the liver and plasma once entering the body. One study found a positive association between PFOA concentrations and ALT serum levels, a marker of hepatocyte damage [25]. However, results of the current study also suggest that lycopene could alleviate liver injuries. Lycopene, a carotenoid found in large quantities in tomatoes, has excellent nutritional health properties and is considered an effective antioxidant for improving bioavailability after processing and cooking. Lycopene can reduce pathological injuries, oxidative stress, and pro-inflammatory cytokines in rat liver tissues, as well as Mtx-induced AST and ALT elevation in rat serum [24]. Numerous studies have identified an inverse relationship between dietary intake of lycopene and oxidative stress and cancer risks [26]. Studies have provided evidence that lycopene can inhibit oxidative damage, regulate intracellular signaling that leads to reducing proliferation, and increase sensitivity to apoptosis and other mechanisms [27].

Abnormal expression of apoptotic proteins during pregnancy may affect placental development and function. Studies have shown that apoptosis plays a vital role in maintaining normal embryonic implantation and immune tolerance in embryos [28]. There are two possible pathways for uterine cell apoptosis, including Bax/Bcl-2 mediated mitochondrial pathways and Fas/FasL mediated death receptor pathways. Caspase family is the final common effecter of these two pathways.

Bcl-2 and Bax are the most representative anti-apoptotic and pro-apoptotic proteins, respectively. Abnormal expression of Bax and Bcl-2

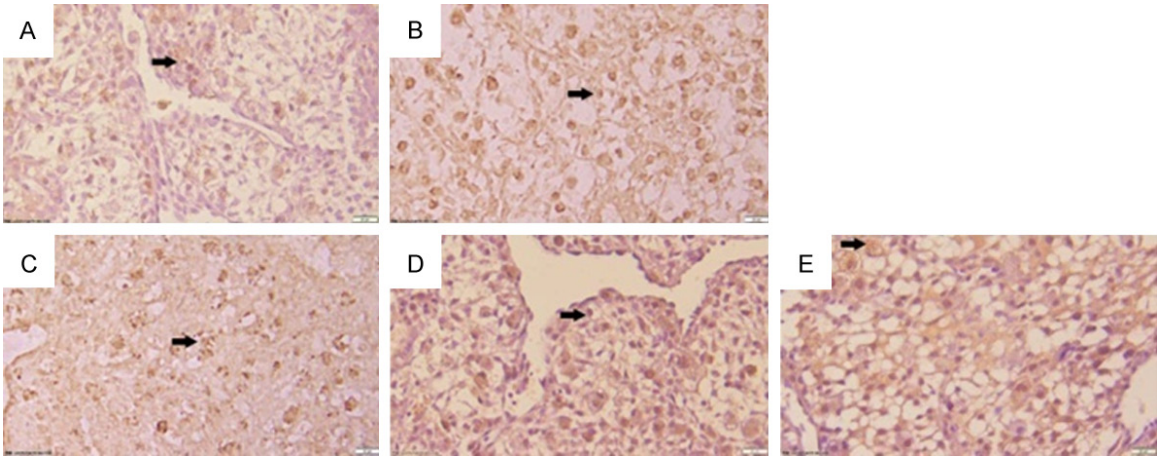

Figure 7. Expression of Caspase-3 in the uterus of early pregnant mice. A: Control group; B: Model group (20 mg/kg PFOA); C: Low group (20 mg/kg PFOA+10 mg/kg Lycopene); D: Medium group (20 mg/kg PFOA+20 mg/kg Lycopene); E: High group (20 mg/kg PFOA+40 mg/kg Lycopene). Bar = 20 μm.

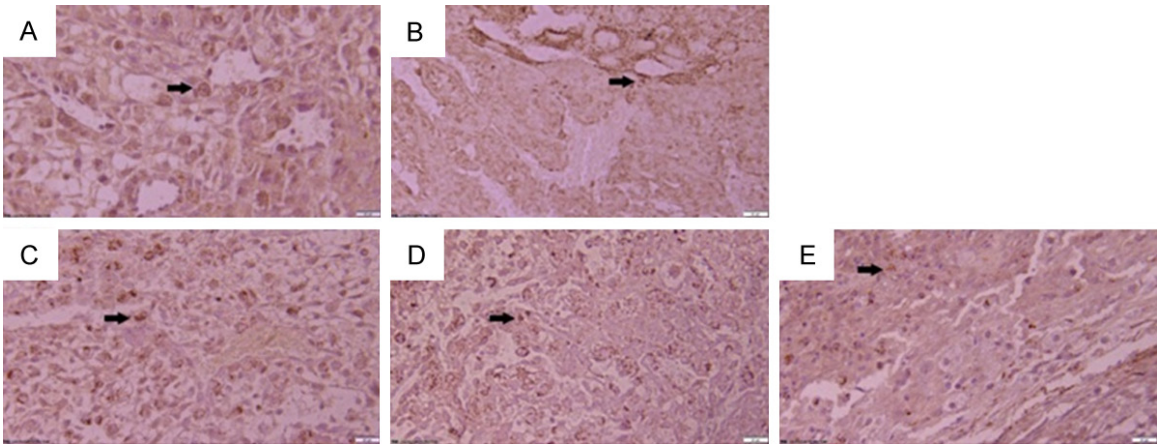

Figure 8. Expression of Fas in the uterus of early pregnant mice. A: Control group; B: Model group (20 mg/kg PFOA); C: Low group (20 mg/kg PFOA+10 mg/kg Lycopene); D: Medium group (20 mg/kg PFOA+20 mg/kg Lycopene); E: High group (20 mg/kg PFOA+40 mg/kg Lycopene). Bar = 20 μm.

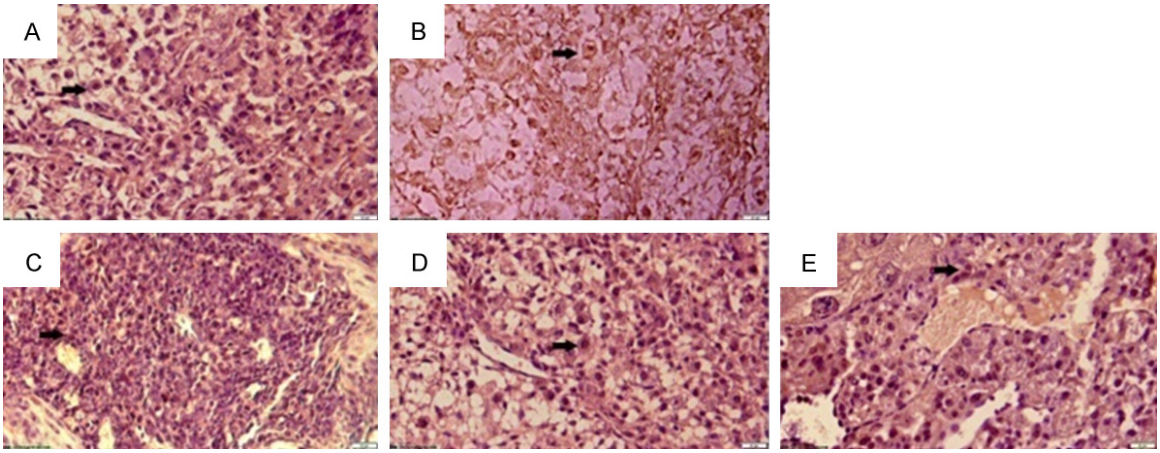

Figure 9. Expression of FasL in the uterus of early pregnant mice. A: Control group; B: Model group (20 mg/kg PFOA); C: Low group (20 mg/kg PFOA+10 mg/kg Lycopene); D: Medium group (20 mg/kg PFOA+20 mg/kg Lycopene); E: High group (20 mg/kg PFOA+40 mg/kg Lycopene). Bar = 20 μm.

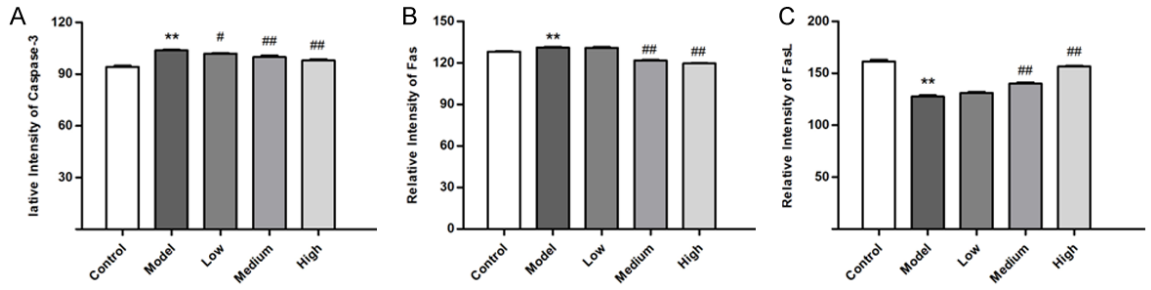

Figure 10. Effects of lycopene on expression of Caspase-3, Fas, and FasL. Mice were given PFOA (20 mg/kg) with low, medium, or high doses of lypocene. Expression of Caspase-3 (A), Fas (B), and FasL (C) was measured. $^{**}P<0.01$, compared with the control group. $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with the model group.

can generate anomalous changes of Bcl-2/Bax ratios, finally leading to pregnancy failure. Caspase-3 is closely related to apoptosis, not only the core enzyme mediating apoptosis but also the most crucial executing enzyme in the whole apoptotic process. Additionally, abnormal expression of Fas and FasL may lead to apoptosis exception, ultimately affecting immune response and embryo implantation and leading to occurrence of recurrent miscarriage [29]. Results of the present study showed that PFOA could upregulate Bax, Caspase-3, and Fas expression of the uterus, downregulate Bcl-2 and FasL expression, and decrease the ratio of Bcl-2/Bax, indicating PFOA continuous exposure can cause uterine apoptosis on early pregnancy. However, lycopene intervention could significantly increase uterine indexes and average uterine weights, upregulate ratios of Bcl-2/Bax and FasL expression, and reduce levels of Caspase-3 and Fas. Thus, the effects were most significant in medium and high groups. Lycopene could dose-dependently inhibit uterine damage and uterus apoptosis caused by PFOA exposure on pregnancy, thereby playing a role in protecting the fetus.

Lycopene could dose-dependently ameliorate liver damage caused by PFOA on early pregnancy, inhibit the apoptosis of uterine cells, and reduce miscarriage. Lycopene may play a role in protecting the liver and fetus, laying the foundation for further study of the impact of PFOA exposure on offspring during pregnancy and the protective effects of lycopene.

Acknowledgements

This study was financially supported by the National Natural Science Foundation of China (No. 31502111) and Natural Science Foundation of Hebei Province (No. C2016204097).

Disclosure of conflict of interest

None.

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