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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Histological and morphological characteristics of placenta in the rats administrated with *Glycyrrhiza glabra* extract

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Abstract

There is growing interest in the use of herbs to aid in the maintenance of health. Licorice root (Glycyrrhiza) has been employed in traditional Iranian medicine to treat infectious diseases. To do the experiments, a total of 40 Male and 40 female Balb/C mice aged 9 to 10 weeks were purchased from Razi Institute (Karaj, Iran). For mating, one female was placed into cage of one male overnight (12 h). Day 0 gestation was determined by a sperm-positive vaginal smear following copulation. The pregnant mice (n = 32) were randomly allotted into four equal groups (n = 8) in which mice were exposed to different doses of *Glycyrrhiza glabra* (50,100 and 200 mg/kg/day). On the 17th day of pregnancy, all of the animals were euthanized by chloroform. In the *G. glabra* treated mice compared to control, trophoblastic giant cells were significantly decreased in the number. In addition, massive hyperemia was seen in the labyrinth interhemal membrane compartment of placenta in the *G. glabra* treated mice. Significant increase in the placental weight as well as in the placental index (the ration of placental to foetal weight) were found in the treated groups with *G. glabra* extract in comparison to the control mice. Also, the thickness of placenta and its diameter was not affected by *G. glabra* exposure. In addition, the weight of fetuses in treated mice was decreased significantly. The results showed that the effects of *G. glabra* on the placental tissue are not dose-dependent.

Keywords: *Glycyrrhiza glabra*, histology, rat

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Introduction

Licorice, the root of the *Glycyrrhiza* species, is one of the most frequently employed botanicals in traditional medicine. The history of licorice, as a medicinal plant, is very old and has been used in many societies throughout the millennia (Wang, 2001). There are many useful compounds in licorice root such as, glycyrrhizin (saponin- like glycoside-50 times sweeter than sugar) and its aglycone, glycyrrhetinic acid which are clinically used for hyperlipidemia (Tamir et al., 2001). Licorice flavonoid constituents mainly include flavones, flavonals, isoflavones, chalcones, bihydroflavones andbihydrochalcones. Pharmacological investigations indicate that they have antioxidant, antibacterial and anti-inflammatory activities (Vaya et al., 1997). Comparative studies of pregnant women suggest that excessive amounts of liquorice (100g a week) may also adversely affect both intelligence quotient and behaviour traits of offspring. The placenta, in addition to its myriad of functions during development, is recognized as a target for the toxic actions of chemicals (Foster et al., 2008). With respect to the disposition of xenobiotics in the placenta, the most crucial factor is that the placenta is a two-way monitor and controller of flux for xenobiotics. (Myllynen et al., 2005; Foster et

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al., 2008). In this study, we investigated probable effect of administration *Glycyrrhiza glabra extract* on histology of placenta in mice.

Materials and Methods

Animals and experimental design

Experimental procedures were performed in accordance with institutional guidelines for animal care and use at the University of Ilam. The study was approved by the local ethics committee. A total of 40 Male and 40 female Balb/C mice aged 9 to 10 weeks were purchased from Razi Institute (Karaj, Iran). The animals were maintained in a controlled environment at a temperature of 23±1ºC, a humidity of 45±5% and natural 12:12 h light-dark cycle and had ad-lib access to drinking water and food. Animals were allowed to be acclimatized to the laboratory environment at least 7 days before commencement of testing. For mating, one female was placed into cage of one male overnight (12 h). On day 0, gestation was determined by a sperm-positive vaginal smear following copulation. The pregnant mice (n = 32) were randomly allotted into four equal groups (n = 8) in which mice were exposed to different doses of *Glycyrrhiza glabra*.

Histo-morphological assessment of placenta

On the 17th day of pregnancy, all of the animals were euthanized by chloroform. The uterus was opened and the placentas were removed. In order to study the morphological changes of placenta, immediately after opening the abdominal cavity, the placentas were removed, washed thoroughly in the phosphate buffer solution and were dried with tampons. Also, weight of placentas, weight of foetus and ratio of placental to foetal weight were measured by using an electronic scale by precision of 0.01 grams (A&D, Japan). Then diameter and thickness of placentas were measured by using a Caliper (NSK, MAX15 & MAX20, Japan).

For microscopic study, the placenta fixed with 10% formalin, the middle part of the placenta between the bottom and the top was sectioned (6µm), and mounted on microscope slides. The sections were subjected to hematoxylin and eosin (H&E) staining, and then studied by light microscope (Nikon Eclipse E800). Appropriate photographs were taken with a digital camera (COOLPIX 950, Nikon, China) and stored.

Data analysis and statistics

Computations were performed using the SPSS statistical package (version 16). All results were presented as mean ± standard error (S.E.M.). For each specific morphological parameter, a one-way analysis of variance (ANOVA) was carried out to test for differences in mean ± S.E.M. among the different groups. If a significant F value was obtained, complementary analyses (Tukey-Kramer´s multiple comparison tests) were performed. P-values less than 0.05 were considered as significant.

Results

Histological findings

The results showed that the effects of *G. glabra* on the placental tissue are not dose-dependent. The number and size of the spongiotrophoblastic cells as well as trophoblastic glycogen cells in the basal zone of the placenta was not different between various groups. But, in the *G. glabra* treated mice compared to control, trophoblastic giant cells were significantly decreased in the number (Figure 1b). In addition, massive hyperemia was seen in the labyrinth interhemal membrane compartment of placenta in the *G. glabra* treated mice (Figure 2b).

Morphological findings

Significant increasing in the placental weight as well as in the placental index (the ration of placental to fetal weight) were found in the treated groups with *G. glabra* extract in comparison to the control mice ($p<0.05$) (Table 1). Also, the thickness of placenta and also its diameter was not affected by *G. glabra* exposure. In addition, the weight of foetus in treated mice were decreased, statistically ($p<0.05$) (Table 1). Furthermore, regarding morphological parameters, there were no any significant differences in the effects of *G. glabra* exposure among various experimental groups.

Table1: Morphological parameters (Mean ± S.E.) of the placenta in the control and treated mice with different concentrations of *G. glabra* extract

Groups/Parameters	Control	50 mg/kg/day *G. glabra*	100 mg/kg/day *G. glabra*	200 mg/kg/day *G. glabra*
Placental weight (g)	$0.19\pm0.04^{*a}$	0.43 ± 0.06^{b}	0.44 ± 0.03^{b}	0.39 ± 0.01^{b}
Placental thickness (mm)	0.1 ± 0.08^{a}	0.11 ± 0.01^{a}	0.2 ± 0.09^{a}	0.2 ± 0.07^{a}
Placental diameter (mm)	$0.\ 8\pm0.03^{a}$	0.77 ± 0.09^{a}	0.72 ± 0.07^{a}	0.8 ± 0.04^{a}
The ratio of placental to foetal weight (%)	9.10^{a}	44.00^{b}	63.00^{b}	39.00^{b}
Foetal weight (g)	2.08 ± 0.05^{a}	0.97 ± 0.06^{b}	0.69 ± 0.01^{b}	0.98 ± 0.02^{b}

*Different letters over values indicate significant differences with control group ($P<0.05$)

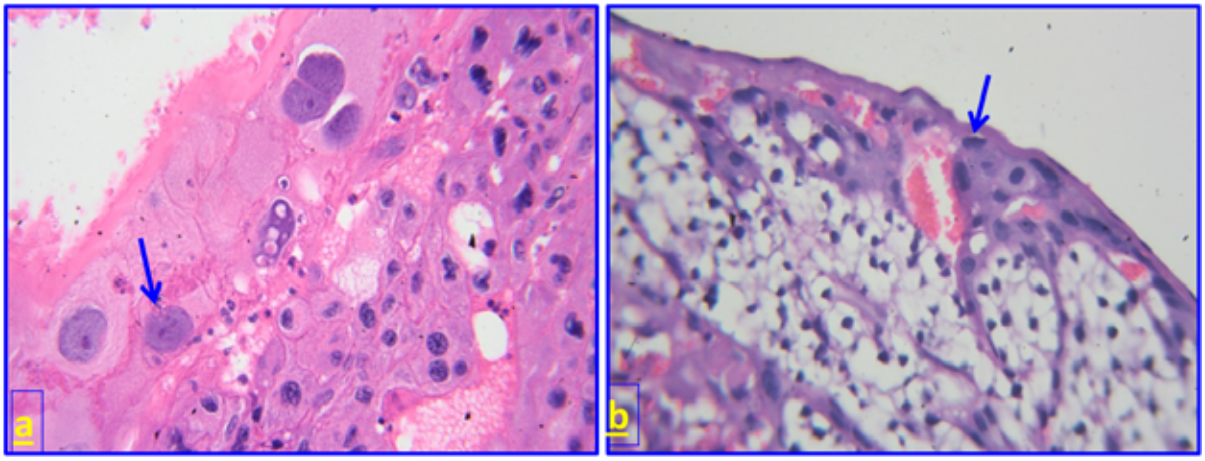

Fig. 1a: Transverse sections of the placenta of control, the normal number and large size of the trophoblastic giant cells (arrow) *G. glabra* treated mice. Fig1b: Decrease in the number and size of trophoblastic giant cells (arrow). (Haematoxylin and Eosine stain) (× 400)

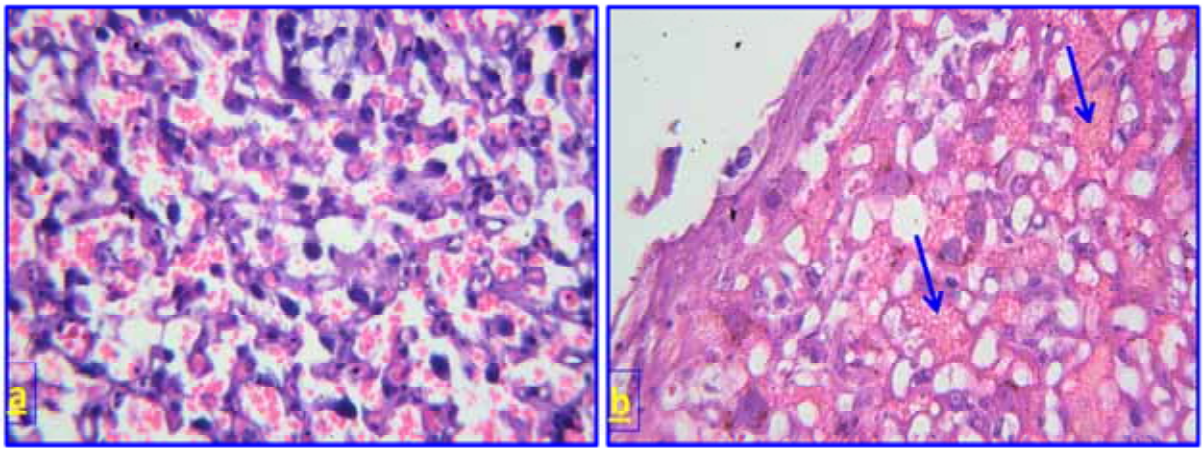

Fig. 2a: Normal blood supplies in the labyrinth interhemal membrane of placenta in control group (control). Fig2b: Massive hyperemia (arrows) in the labyrinth interhemal membrane of placenta in the *G. glabra* treated mice (*G. glabra* treated mice). (Haematoxylin and Eosine stain) (× 400).

Discussion

Toxic and/or foreign compounds may interfere with placental function at many levels and any deviation from normal development may constitute a potential threat to placental function, resulting in preterm delivery, congenital malformation or abortion. (Myllynen et al., 2005). With the resent awareness that an adverse intrauterine environment may predispose the fetus to development of disease in adult life, an understanding of the placental structure in relation to foetal growth and development is crucial. In the present study, the weight of foetus in treated mice decreased. Conversely a study showed that heavy glycyrrhizin exposure during pregnancy did not significantly affect birth weight (Strandberg et al., 2001). Our investigation indicated that glycyrrhizin glabra can cause an increase in placenta weigh which may be induced by inflammation process in placenta. We detected massive hyperemia in the labyrinth interhemal membrane of placenta in the *G. glabra* treated mice that can be one of the signs of inflammation process.

Conclusion

We concluded that Glyrrhiza glabra consumption can cause some risky consequences in histological and morphological characteristics of placenta in rats and these effects on the placental tissue are not dose-dependent.

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