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Histological and Morphological Characteristics of Placenta in the Rats Administrated with *Glycyrrhiza glabra* Extract

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Abstracts: It had been demonstrated that *Glycyrrhiza glabra* extract has biological beneficial impacts include anti-inflammatory as well as anti-allergic properties, although there is little published data about its probable side effects on the placental integrity. Since, there is not enough data regarding the impacts of *G. glabra* on the placental histo-morphology. Therefore, this study was carried out on thirty-two pregnant Sprague-Dawley rats which were randomly distributed into four main groups (n=8). Experimental groups were injected intra-peritoneally with aqueous extract of *G. glabra* at 50, 100 and 200 mg/kg/day, respectively, during the organogenesis period (6^{th} to 16^{th} day of gestation) while control group was injected only with tap water. On the 17^{th} day of gestation, all of the animals were euthanized; their fetuses and placentas were removed and histomorphological study was performed. The obtained results showed that in the *G. glabra*-treated rats compared to control, trophoblastic giant cells were significantly decreased in the number and size. In addition, massive hyperemia was seen in the labyrinth interhemal membrane compartment of placenta in the *G. glabra* treated rats. Also, significant increase in the placental weight as well as in the placental index was found in the treated groups in comparison to the control rats. From this study, it is concluded that *G. glabra* extract administration has harmful effects on the placental structure and therefore popular consumption of this plant should be reconsidered.

Key words: *Glycyrrhiza glabra* · Histology · Placenta · Rat

INTRODUCTION

It has been noted that *G. glabra* L. (family:Fabaceae:Leguminosae) is an herbaceous perennial, with pinnate leaves and purple to whitish blue flowers which has been used as a medicinal plant for thousands of years in the traditional medicine. Licorice, the root of the *Glycyrrhiza* species, is one of the most frequently employed botanicals in traditional medicine [1, 2]. Also, pharmacological investigations indicate that licorice flavonoid constituents have antioxidant and antibacterial activities [3]. In addition, anti-inflammatory as well as anti-allergic activities have been attributed to one of its main constituents, glycyrrhizin [2].

The placenta, in addition to its myriad of functions during development, is recognized as a target for the toxic actions of chemicals [4]. 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. [4, 5]. On the other hand, it has been shown that paternal exposure to some herbal plants includes *Carthamus tinctorius* could result in adverse outcomes on the placental histomorphology and survival of the neonates [6]. In fact, 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 [5]. Since there is not enough data regarding the impacts of *G. glabra* on the placental histo-morphology; this study was done.

MATERIALS AND METHODS

Plant Extraction: The *G. glabra* (Licorice) root was purchased from Emam-Reza medicinal plants market (Ilam, Iran) and botanical identification was confirmed at the herbarium of Ilam University (Exsiccatae number: 132-4-91). For extraction preparation, the roots of plant was washed with sterile water, dried in shade at room temperature for about 3 weeks and ground them in an

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electric mill to obtain particles smaller than 4 mm. This material was then extracted by maceration in 70% methanol solution at 50 °C for 2 hours. The extract was filtered through a Whatman ?1 paper and evaporated to dryness in a rotary evaporator under reduced pressure. The dried material was then stored under refrigeration at 4-8 °C until its use.

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. To do the experiments, a total of 40 male and 40 female Sprague-Dawley rats aged 9 to 10 weeks were purchased from Razi Institute (Karaj, Iran). The animals were then 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). Day 0 gestation was determined by a sperm-positive vaginal smear following copulation. The pregnant rats (n = 32) were randomly allotted into four equal groups (n = 8). Experimental groups were injected intra-peritoneally with aqueous extract of *G. glabra* at 50, 100 and 200 mg/kg/day, respectively, during the organogenesis period (6^{th} to 16^{th} day of gestation) while control group was injected with tap water only.

Histo-Morphological Assessment of Placenta: On the 17^{th} 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 fetuses and ratio of placental to fetal weight were measured by using an electronic scale by precision 0.01 grams (A and D, Japan). Then diameter and thickness of placentas were measured by using a caliper (NSK, MAX15 and MAX20, Japan).

For microscopic study, the placenta fixed with formalin10%, 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 and 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.). For each specific morphological parameter, a one-way analysis of variance (ANOVA) was carried out to test for differences in mean±S.E. 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 rats compared to control, trophoblastic giant cells were significantly decreased in the number and size (Fig. 1b). In addition, massive hyperemia was seen in the labyrinth interhemal membrane (LIM) compartment of placenta in the *G. glabra* treated rats (Fig. 2b).

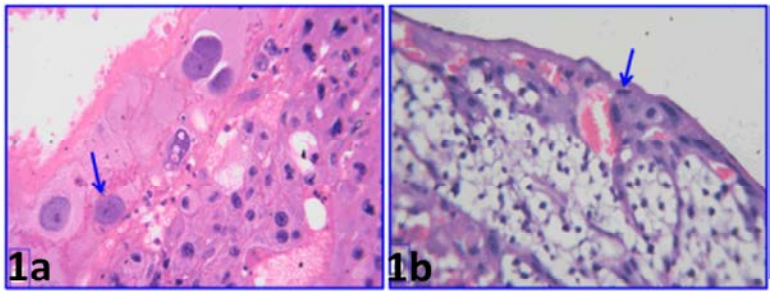

Fig. 1: Transverse sections through the placenta of control (a) and *G. glabra* treated rats (b). The a part of fig. shows the normal number and large size of the trophoblastic giant cells (arrow), but in the b part of fig. the decreasing in the number and size of trophoblastic giant cells (arrow) is obvious. (Haematoxylin and Eosine stain) (a, b,: × 400).

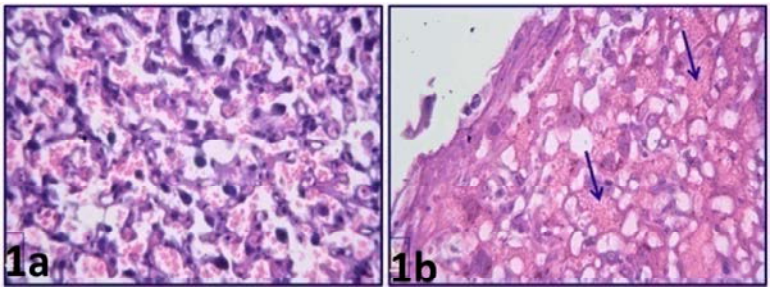

Fig. 2: Transverse sections through the placenta of control (a) and *G. glabra* treated rats (b). The a part of fig. shows normal blood supplies in the labyrinth interhemal membrane of placenta in control group; whereas the b part indicate massive hyperemia (arrows) in the labyrinth interhemal membrane of placenta in the *G. glabra* treated rats. (Haematoxylin and Eosine stain) (a, b,: × 400).

Table 1: Morphological parameters (Mean±S.E.) of the placenta in the control and treated rats 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±0.04 [*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±0.03 [a]	0.77±0.09 [a]	0.72±0.07 [a]	0.8±0.04 [a]
The ration of placental to fetal weight (%)	9.1[a]	44 [b]	63 [b]	39 [b]
Fetal weight (g)	2.08±0.05 [a]	0.97±0.06 [b]	0.69±0.01 [b]	0.98±0.02 [b]

[*]Different words indicated significant differences with control group as $p<0.05$

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 rats ($p<0.05$) (Table 1). Also, the thickness of placenta and also its diameter was not affected by *G. glabra* exposure. In addition, the weight of fetuses in treated rats 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 (Table 1).

DISCUSSION

Herbal medicine is a complementary therapy that uses plants to treat disorders. In various countries throughout the world, a large number of plants have been used as therapeutic agents in the traditional medicine [7], but there are no enough documents in the literature about their probable toxic effects. With the recent 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 fetal growth and development is crucial [5].

Our findings showed that administration of different concentrations of the *G. glabra* extract in the rats could produce significant changes in the placental histo-morphology and also fetal weight. It has been demonstrated that herbal toxicity clearly represents a serious human health threat and is an important issue that to be tackled [8]. Also, herbs have a variety of complex chemical constituents that act on the body as a whole or on specific organ and systems. Some of the chemical constituents are mild and safe even in large doses while, some act more strongly or toxic in large doses or when taken continuously [8].

In the present study, the placental trophoblastic giant cells in the treated rats with different concentrations of the *G. glabra* extract were poorly developed. Similar results have been reported by other researchers about different plant extracts [6]. It is suggested that trophoblastic giant cells participate in a number of processes essential to a successful pregnancy including blastocyst implantation, remodeling of the maternal deciduas and secretion of hormones that regulate the development of both the fetal and maternal compartments of the placenta [9].

In the current work, massive hyperemia was seen in the labyrinth interhemal membrane (LIM) compartment of placenta in the *G. glabra* treated rats. The integrity of the LIM is a critical parameter for placental [10]. Thus, in the present study, massive hyperemia of LIM in the placentas of *G. glabra* treated rats, confirming an abnormality

in the maternal-fetal blood barrier. These changes are associated with reduced nutrient supply from the maternal to the fetal circulation [11, 12] and are in line with significant decreasing in the fetal weight.

There are no exact mechanism(s) of toxic action for *G. glabra* on the placental tissue in the literature. But it has been reported that *G. glabra* plant has a variety of complex chemical constituents including flavonoides, glycosides, glycyrrhizin, saponin, glabrene, starches and yellow coloring matter that could be responsible for its toxic properties [2]. Previously, the mechanism of action was considered to be identical to that of glucocorticoids. This assumption was based on the structural resemblance between P-glycyrrhetinic acid and corticosteroids. Recently, it was postulated that the anti-inflammatory activity of /1- glycyrrhetinic acid is probably due to inhibition of the enzyme 1 lfl-hydroxysteroid hydroxygenase [13]. When glycyrrhizin is administered orally, its aglycon fl-glycyrrhetinic acid is the major metabolite [14]. Fl-Glycyrrhetinic acid exhibits anti-inflammatory properties in different animal models [15, 16, 17]. Its mode of action, however, is as yet unknown. Oral administration of either f3-glycyrrhetinic acid or glycyrrhizin was found to increase significantly the plasma levels of hydrocortisone and prednisolone, respectively [13]. Because of this property, a novel application of P-glycyrrhetinic acid has been suggested based on the potentiating of the activity of glucocorticoids by inhibiting their metabolism. Also, a potentiating of hydrocortisone activity has been observed in skin and lung tissue after co-medication with Jglycyrrhetinic acid [13, 18].

Based to these findings, it is concluded that *G. glabra* extract exposure has harmful effects on the placental structure and therefore popular consumption of this plant should be reconsidered.

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