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Original Article

Can Excessive Consumption of Celery Threaten Continuation of pregnancy :a Biometrical and Histopathological Study of Mice Placenta

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ABSTRACT

Background: Placenta has a strategic role in fetal/maternal exchange. A healthy placenta is essential for normal fetal development. Avoiding consumption of some foods and vegetables is suggested during pregnancy due to the risk of placental disorders. Celery is a high consumed vegetable that some studies concern about its adverse effect on pregnancy. The present study focused on its consumption side effects in different trimesters of gestation on mouse placenta.

Methods: Duration of pregnancy in mice is three weeks. Five groups of 5 mice were considered. Groups I, II and III received celery hydro-alcoholic extract only during the 1st, 2nd and 3rd weeks of pregnancy, respectively and group IV received it during all the weeks of gestation. The 5th group was considered as control and just received placebo. The biometrical sizes of placenta (weight, length and diameter) were measured and histopathological analysis was done.

Results: Celery consumption decreased the weight of placenta in groups II, III and IV. Also, placental length and diameter decreased in groups I and IV. Histopathological examination showed decrease of placenta trophoblastic giant cells and increase of trophoblast glycogen cells in the basal layer. Generally, morphological and pathological changes in the 4th group showed more deviations from the control group. Basal and Labyrinth layer thickness decreased in the experimental groups. Also, hyperaemia was observed in labyrinth layer of the experimental groups.

Conclusion: Due to the decrease of placenta biometrical sizes and histo-pathological adverse effects, it is advised that celery should be used with more precaution, during pregnancy.

Keywords: Placental Weight, Placental Length, Placental Diameter, Celery, Pregnancy, Trophoblast Glycogen Cells

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Introduction

Fertility is defined as the ability to produce the gametes and a healthy fertilized egg, also a normal embryo development and a successful implantation as well as carrying a full-term pregnancy. In other words, not only a normal embryo and fetus development is necessary, but also a healthy placenta is essential to reach a live birth (1,2). So, any disorders in implantation and placenta may compromise the pregnancy process. Disorders of placenta is one of the etiologies of female infertility due to implantation disorders, miscarriage or abortion (3,4). Placenta is a functional unit between mother and fetus with a key role in the development of fetus. A normal placenta is essential for normal fetal development. Normal morphology and histology of placenta is necessary for a successful ongoing pregnancy (5,6). According to a systematic review in 2018, placental histopathology is associated with placenta-related pregnancy complications (7).

Several factors can affect placental morphology and cause missed, incomplete and complete abortion (8). Some of these factors are chemotherapy drugs, antibiotics, toxins, pesticides, radiation, air pollution and lack of adequate vitamins. It is known that these factors can create free reactive oxygen species (ROS). Subsequently, produced ROS cause the oxidation of cell components, which make the placenta leading to the impairment of placental cell's quality and function (9). Pathak *et al.* reported that the macroscopic and morphological shape of the placenta cannot precisely predict histological placental lesions (10). But, quantitative assessment of placental morphology can predict the causes of stillbirth. So quantitative as well as qualitative assessments help to predict the reason of abortion or stillbirth (11).

On the other hand, herbal medicine has traditionally some applications in the treatment of diseases and based on the available evidences, administration of medicinal plants for the improvement of fertility has a long history (12). For example, some plant extracts like ginger have being used in the treatment of morning sickness symptoms and pregnancy nausea and vomiting (13). Clery is a medicinal plant which is also proposed for prevention of nausea and vomiting (14).

But, despite the numerous medicinal effects of plants, their un-prescribed and abused consumption could have serious side effects. Hence, taking them during pregnancy can jeopardize the placenta and the fetus health (12). Also, inappropriate or excessive consumption of some herbs, vegetables and herbal compounds may cause unwanted adverse effect during pregnancy. Overall, maternal diet may be a risk factor for gestation especially for women who are unaware of their pregnancy (15).

Celery (*Apium graveolens*) from Apiaceae family is a stimulating factor for appetite and sexual power (16). It has a very wide range of usages in food and pharmaceutical industries (15). Also, it is used for morning sickness, nausea and vomiting (14). But, celery may causes womb contraction, miscarriage or preterm labour (17) and uterine stimulation (18). It has many phytoestrogen compounds like coumarin phytoestrogens and flavonoid which can affect reproductive endocrine system and cause reduction of fertility (19).

Also, in case of chronic or high concentration use, it has inhibitory effects on fertility (20).

These compounds affect the cells in the hypothalamus, inhibit the secretion of hypothalamic gonadotropin and stop pituitary-gonadal axis (19). According to the traditional medicine, celery leaves may induce abortion, so consuming too much celery during pregnancy and lactation is not recommended (21). In contrast, some studies reported that aqueous extract of celery can increase the delivery rate in rat (22). Controversially, Kooti *et al.* investigated the effect of Celery extract on Wistar rats (100 and 200 mg/kg/BW), and their results showed no significant effect on number of the delivery and number of newborns in rats; even though, they reported decreased weight of infants (19). Therefore, still, it is not clear how much celery consumption during pregnancy is safe and at what time of pregnancy it exhibits more adverse effects? Also, no study is present, regarding the exact effect of hydro-alcoholic extracts of celery leaves on pathological and morphological changes of placenta in mouse. Therefore, the present study aimed to evaluate the effect of celery leaves extract on biometry and histology of mouse placenta.

Materials and Methods

Animals

The research was approved by ethic committee of "Yazd International Campus, Shahid Sadoughi University of Medical sciences", Yazd, Iran (IR.SSU.MEDICINE.REC.1400.366). NMRI

female mice were obtained from "Yazd International Campus" animal house. The research was conducted under the "Animal Research: Reporting of *in Vivo* Experiments" (ARRIVE) guidelines 2.0 (https://arriveguidelines.org/arrive-guidelines). Free access to water and food and cage cleaning was provided for all mice. They were fed with standard diet and fresh water and grown under 22±2°C, humidity (45 ± 5%) and 12 h light/dark. The female mice with the weight of 20-30 gr (mean=25 gr) were used.

Celery leaves Hydro-Alcoholic Extract Preparation

Fresh celery leaves were purchased from "Institute of medicinal plant", Yazd Agriculture Jihad. The Apium graveolens leaves were characterized by a herbalist. The fresh leaves were collected and after shadow drying and milling, the powder was stored at 4 °C. After that, 50 g of the produced powder was mixed with 200 ml of 70% ethanol. Prepared solution was stored at room temperature for 4 days with subsequent agitation. After 72 hours, the solution was passed through the filter paper, followed by solvent evaporation in water bath at a 40° C. The obtained extracts were diluted with normal saline to get the required concentration. The dose of 200 mg/kg was used because in the previous studies, 200 mg/kg of Celery extract had abortive effect in mice and did not cause mice death (23, 24).

Also, Kooti *et al.* reported that with the administration of 200 mg/kg/BW of celery extract in rat, the number of the delivery rate did not significantly change, but the weight of infants showed a significant decrease, compared to the control (25).

Study Design

The mice were randomly distributed into 5 groups of five and let to mate. The next morning, observing the vaginal plug was considered as the first day of pregnancy. The pregnant mice were selected and categorized into 5 groups as follows: The mice in the 4 experimental groups underwent oral gavage of the extract. Briefly, the experimental groups I, II and IV received 200 ml/kg of extract solution during the 1st, 2nd and 3rd weeks of pregnancy, respectively. The 4th experimental group (IV) received 200 ml/kg of extract, during all the time of the pregnancy. The mice in group 5 received normal drinking water by gavage, and were considered as the control group.

Evaluation of Placental Biometry

Considering the time of 21 days for mouse pregnancy, the mice were euthanized with cervical dislocation, on the 21st day. The abdomen was cut and the placentas were transferred into a sterile dish. Then, the placenta weight, length and diameter were measured, based on Heerwagen *et al.* study (26). Briefly, the diameter of several parts of placenta was measured and the average was considered as placenta diameter (Figure 1B). Also, the placenta length was measured from one side to the end of the other side, according to the figure 1 (Figure 1A).

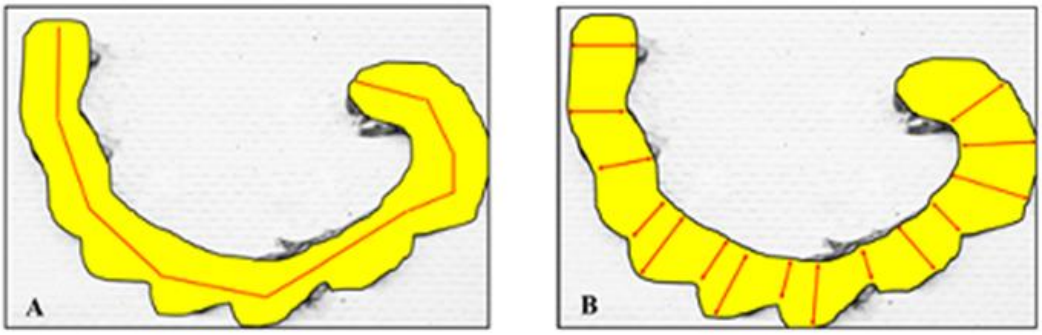

Figure 1. Measurement of placenta diameter and length.

Histological Evaluation of placenta

For histopathological evaluation, the placentas were fixed in 10% formalin solution overnight. After that fixation, dehydration, clearing, embedding, ten sections with a thickness of 5 micrometers were made from each placenta and staining with H& E was done. All histological sections were reviewed by one histologis. The hemorrhagic areas, vessels distribution and the other disorders were

recorded. The evaluations were performed using light microscope. Spongio trophoblast, trophoblast giant cells, maternal and fetal arteries and labyrinth layer were evaluated (23).

Statistical Analyses

The results were presented as (Mean ± SD). Statistical test of one-way analysis of variance (ANOVA), was used followed by post hoc Least Significant Difference (LSD) test. When significant main effects were revealed in the ANOVA, post hoc comparisons were done with LSD, using software SPSS v.20. P value less than 0.05 was considered as significant between the experimental and control groups.

Results

The effect of Celery extract on placental weight

According to one-way ANOVA, followed by Post Hoc LSD Test, $P \leq 0.05$ was considered as significant difference between the experimental groups and control group (Table 1).

Table 1. Placental weight changes after the treatment with Celery extract

	Control Group	Group I	Group II	Group III	Group IV
Control Group		0.290	0.006**	0.040*	0.001**
Group I	0.290		0.002**	0.033*	0.000***
Group II	0.006**	0.002**		0.002**	0.005**
Group III	0.040*	0.033*	0.002**		0.011*
Group IV	0.001**	0.000***	0.045	0.011	

*. ($P \leq 0.05$). **($P \leq 0.01$) and ***($P \leq 0.001$) was considered as significant difference between the experimental groups and control group.

So, compared to the control group, the placental weight in groups II, III and IV was significantly decreased (P value = 0.006, 0.040 and 0.001, respectively). But, the placental weight in the first group had no significant difference with that in the control group (P value = 0.290). This means that the placental weight of group I was close to its value in the control group.

Also, according to one-way ANOVA, followed by Post Hoc LSD Test, P values $\leq$ 0.001 were considered as significant difference between the experimental groups. So, the placental weight in groups I and IV had significant difference with each other; so that, placental weight in group IV decreased significantly, compared to the group I (p value = 0.000) (Table 1 and table 4).

The effect of Celery extract on placental length

According to one-way ANOVA, followed by Post Hoc LSD Test, $P \leq 0.05$ was considered as significant difference between the experimental groups and control group (Table 2). All groups showed significant difference with the control group (P value ≤ 0.05); that is, compared to the control group, the placental length of groups I and IV was significantly decreased (P value = 0.034 and 0.02, respectively). While, the placental length in groups II and III was significantly increased (P value = 0.041 and 0.046, respectively). Although, all the groups showed significant difference with the control group (P value $\leq$ 0.05), the means of the placental length in groups II and III were close to the corresponding value in the control group (0.041 and 0.046 respectively).

Also, according to one-way ANOVA, followed by Post Hoc LSD Test, P values $\leq$ 0.046 were considered as significant between the groups and accordingly, all groups had significant difference with each other, except groups II and III (P value = 0.05). It means that the placental length of group IV was significantly decreased, compared to the groups I (P value = 0.031), II (P value = 0.03) and III (P value = 0.005). The placental length of group I was significantly decreased, compared to the groups II (P value = 0.034) and III (P value = 0.024). The placental length of group II was decreased compared to the group III (P value = 0.05), but not significantly (Table 2, Table 4).

Table 2. Placental length changes after the treatment with Celery extract

	Control Group	Group I	Group II	Group III	Group IV
Control Group		0.034*	0.041*	0.046*	0.021*
Group I	0.034*		0.034*	0.024*	0.031*
Group II	0.041*	0.034*		0.050	0.030*
Group III	0.046	0.024*	0.050		0.005**
Group IV	0.021*	0.031*	0.03*	0.005**	

*. All groups have significant difference with the control group ($P \leq 0.05$). ** Significant difference between III&IV groups vs control group ($P \leq 0.01$).

The effect of Celery extract on placental diameter

For comparison of placental diameter between the groups, according to ANOVA analysis (Table 3), p value ≤ 0.05 was considered as significant for comparison between the experimental groups vs. the control group. Accordingly, all the groups showed significant difference with the control group. It means that compared to the control group, the placental diameters in groups I and IV were decreased significantly (P value = 0.033 and 0.01, respectively). While, in Group II (P value = 0.011) and group III (P value = 0.047), the placenta diameters were increased significantly, compared to the control group (Table 3 and table 4).

Table 3: Placental diameter changes after the treatment with Celery extract

	Control Group	Group I	Group II	Group III	Group IV
Control Group		0.033**	0.011**	0.047**	0.01**
Group I	0.033*		0.010**	0.010**	0.037
Group II	0.011*	0.010**		0.010**	0.014**
Group III	0.047*	0.010**	0.01**		0.01**
Group IV	0.01*	0.037	0.014**	0.01**	

*. All the groups show significant difference with control group ($P \leq 0.05$). **. Groups I & II, II & III, II & IV and III & IV showed significant difference with each other ($P \leq 0.01$).

Also, according to one-way ANOVA, followed by Post Hoc LSD Test, P values $\leq$ 0.018 were considered as significant among the experimental groups. So, groups I & II (P value = 0.01), groups II & III (P value = 0.01), groups II & IV (P value = 0.014) and groups III & IV (P value = 0.01) showed significant difference with each other. It means that the placental diameter of group I was significantly decreased compared to the groups II and III (P value = 0.01). The placental diameter of group II was significantly decreased compared to group III (P value = 0.01). The placental diameter of group IV was significantly decreased compared to the group II (P value = 0.014) and group III (P value = 0.01).

Generally, about the biometry, the deviation from the control group was more sever in the group IV which received celery in all trimesters of pregnancy. Overall, the exact values of (mean ± SD) of placental weight, length and diameter (mean ± SD) are presented in table 4 (Table 4).

Table 4. The average of placental weight, length and diameter (mean ± SD).

Groups	The mean of placenta weight (mg)	The mean of placenta length (mm)	The mean of placenta diameter (mm)
Control	19± 2.24	3.4 ± 0.516	7.3 ± 0.674
Group I	19.3 ± 2.98	3.3 ± 0.674	7.2 ± 1.135
Group II	16.5 ± 1.433	3.6 ± 0.516	7.5 ± 0.527
Group III	17.4 ± 0.699	3.8 ± 0.632	8 ± 0.666
Group IV	16 ± 1.054	3	7 ± 0.666

Histopathological Evaluation

The histopathological feature of placenta in the control group was a characteristic of a normal structure of placenta. It means that normal number of the placental cells was observed. The H & E staining showed basal decidua and

labyrinth layers of placenta. Also, the basal decidua lined under the implanted ovum and trophoblastic giant cells and glycogen trophoblastic cells of decidua basalis were formed (Figure 2 A). Figure 2B showed the labyrinth layer of placenta in the control group with detailed view. As it is clear, normal trophoblastic cells accompanied with expected number of blood cells are present (Figure 2B).

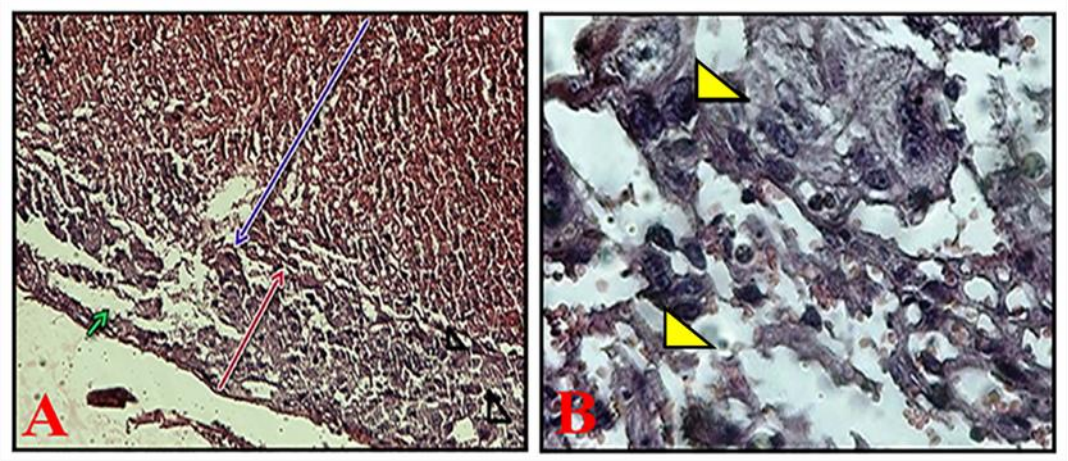

Figure 2: A). H & E staining of placenta in the control group
A) Placenta in the control group: Normally, the fetal part of placenta consists of the basal (red arrow) and labyrinth (blue arrow) zones. Red arrow shows basal decidua or decidua basalis which lies beneath the implanted ovum. The giant trophoblastic cells are visualized with arrow head. The green arrow shows the decidua. B) The labyrinth layer of placenta in the control group. Normal trophoblastic cells are showed with yellow arrow head. Normal and expected number of blood cells is present. (Stained: H & E, magnification ×400).

The microscopic examination of labyrinth and basal zones of placenta in different experimental groups demonstrated some histopathological changes. Receiving celery extract caused reduction in the number and size of the placenta trophoblastic giant cells and increase of trophoblast glycogen cells in the basal placental zone in each of the experimental groups (especially in group II). In all of the experimental groups, shrinkage of nucleus was observed in trophoblastic giant cells. Also, the celery extract led to placental structural & cellular disarray and mislocalization of the cells which was more sever in group III. The upper-mentioned changes were more severe in groups III and VI. Labyrinth and basal layer thickness was decreased in experimental groups, but the basal layer of groups III and IV tolerated the most thickness decline. On the other hand, despite normal diffusion of blood in the control group, in labyrinth layer of the experimental groups, the engorgement of vascular bed and hyperaemia was observed. The mentioned changes were more emerged in groups III and IV (Figure 3).

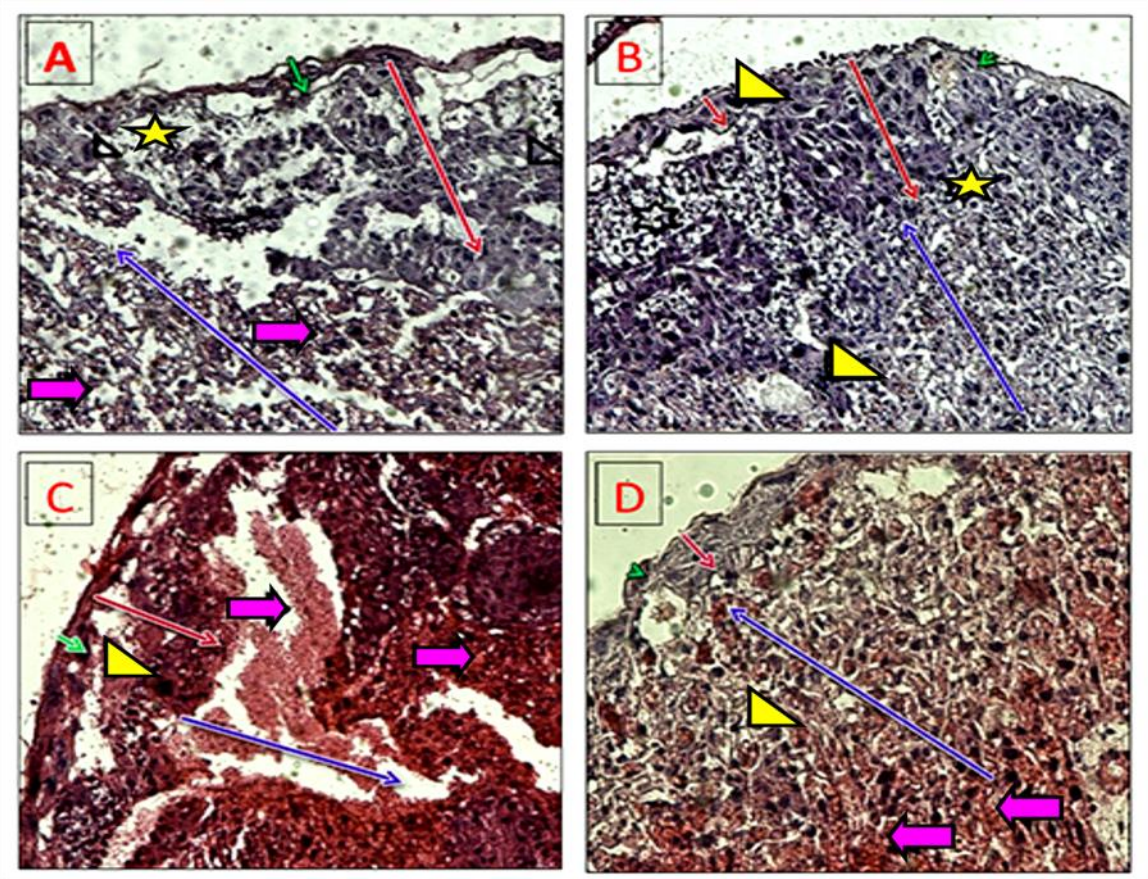

Figure 3. The microscopic examination of labyrinth and basal zones of placenta in different experimental groups
Red arrow shows basal layer and blue arrow points labyrinth zone. The giant trophoblastic cells are shown with yellow arrow head. The decidua is shown with green arrow. The glycogen trophoblastic cells are visualized with yellow star. The blood cells are marked with pink arrow. A) Placenta cross section of the experimental group I (receiving the celery extract in the first week). B) Placenta cross section of the experimental group II (receiving the celery extract in the 2nd week). C) Placenta section in the experimental group III (receiving the celery extract in the third week). D) Placenta section in the experimental group IV (recipients the celery extract during the whole time of pregnancy). (Stained: H & E, magnification ×400).

Evaluation of labyrinth zone in all experimental groups showed diffuse hyperaemia in all experimental groups, especially groups II, III and IV. In general, the pathological changes were more sever in the group that received celery in the whole period of pregnancy (Figure 4).

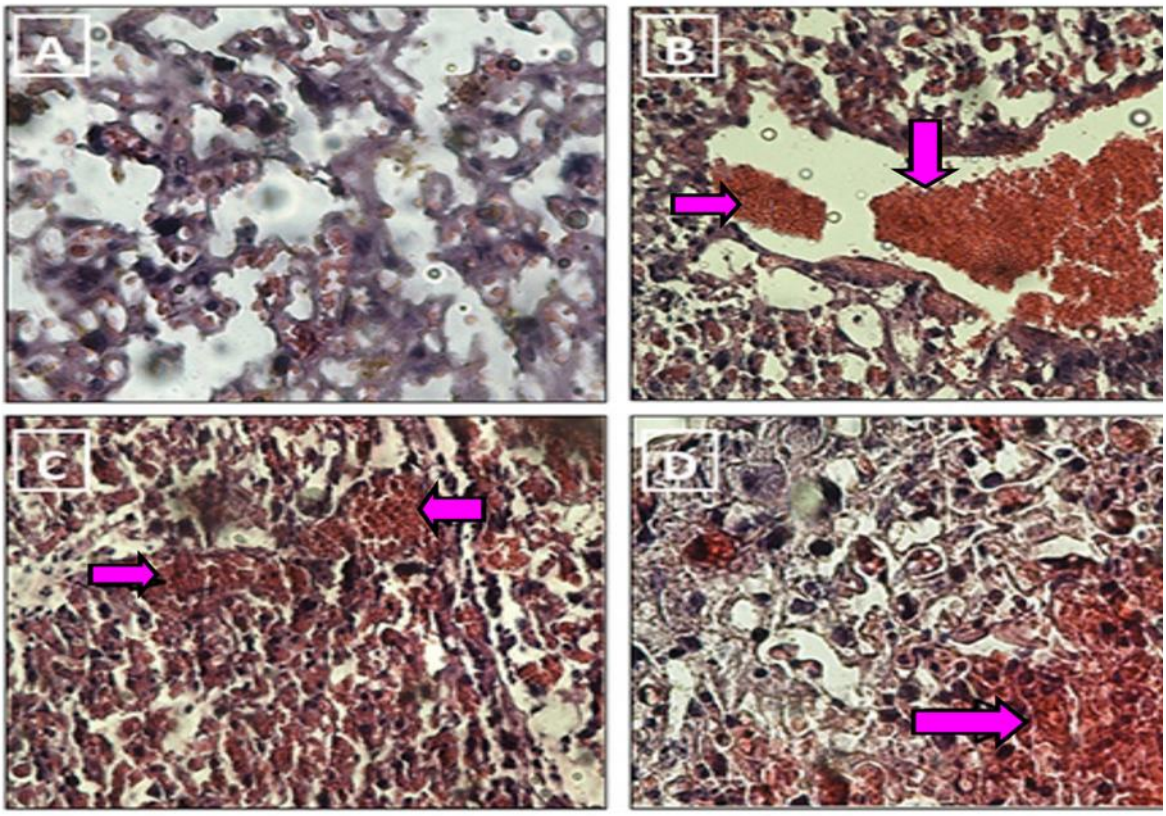

Figure 4. Microscopic examination of placental labyrinth zone in different experimental groups
A) experimental group I, B) experimental group II, C) experimental group III and D) experimental group IV. Diffuse congestion (arrow head) was seen in all experimental groups, especially groups II, III and IV. (Stained: H & E, magnification ×400).

Discussion

In this study, the measurement of placental biometry in experimental groups, which received celery extract at different trimesters of pregnancy, demonstrated deviations in the placenta weight, diameter and length in experimental groups compared to the control group. Briefly, morphological and pathological changes were more sever in the 4th group, which received celery in the whole period of pregnancy. About the other groups, regarding the weight of placenta, we observed that groups II, III and IV tolerated more decrease of placental weight. While, about placental length and diameter, the 1st and 4th groups exhibited more decrease. Also, the microscopic examination of placenta in different experimental groups showed reduced number of placenta trophoblastic giant cells and increase of trophoblast glycogen cells in the basal layer. Placental structural & cellular disarray occurred and labyrinth and basal layer thicknesses were decreased in the experimental groups. As well, in group IV, the changes compared to the control group, were more considerable than the other groups.

Placenta, a temporary important organ during pregnancy, interacts between the mother and the developing embryo/fetus. It has some important functions such as anchoring the fetus to uterine, mediating immune tolerance, exchanging of O2/CO2, providing nutrients and secreting hormone (27). One of the most critical placental function is providing a barrier against xenobiotics and toxic materials (28). Therefore, evaluation of the placental biometry, morphology, histology and toxicology can provide more precautions about exposure and consumption of different materials through gestation, which is important for the prevention of maternal and fetal toxicology (29).

In our study, we observed that consumption of celery extract through the 2nd and 3rd trimesters and especially through the whole period of gestation, led to the significant decrease of placental weight. Previously, Hutcheon *et al.* reported that placental weight is a risk factor for adverse perinatal outcomes, low birth weight and stillbirth, but its mechanism requires further elucidation (30). Also, there is a strong correlation between placental weight and birth weight (31). Birth weight/ placental weight (BW/PW) ratio is an indicative of placenta

efficiency. In mice, it was shown that reduced placental weight and small placenta up-regulates the placental nutrient transfer performance to prevent fetal sub-growth, but it still needs more evaluation in human (32). *Herman et al.* reported that in human the BW/PW ratio increases significantly after the 39th week of gestation. Placental weight is probably a short term risks factor for newborns (33). The placenta has a key role in control of intrauterine fetal growth. It was shown that intrauterine growth restriction is significantly related to smaller placenta (34). In reverse, large placenta is associated with pregnancy obesity and excessive gestational weight gain (35). Therefore, exposure to celery may increase the risk of pregnancy complications and malicious outcomes. Also, Salafia *et al.* reported that weight of placenta is only a crude marker for placenta function and other characteristics like the placental lateral growth can be more important, because it reflects the number of maternal spiral arteries that supply the placenta (36). Therefore, the length of placenta can be a landmark for better placental function.

In our study, about placental length, more decrease of placental length was observed in the group that received celery during all the time of pregnancy. Also, compared to the control group, in the 1st trimester of gestation, celery extract resulted in decrease of placental length, although it was not significant. Also, group IV showed significantly the most reduction of placenta length. On the other hand, although the pathological results showed that the number of the cells decreased in the 2nd and 3rd groups, the placental lengths were increased in these two groups. The reason may be due to the increase of blood engorgement and hyperemia in these two groups.

Alwasel *et al.* noted that, the surface of the placenta is measured by its breadth and length and tissues along the breadth are responsible for mother's nutritional state. Therefore, placental length along with placental other parameters is an important factor for fetal growth. Also, the weight, breadth and length of placenta are positively associated with fetal size (37). The size of the breadth correlates with neonatal body size. On the other hand, although, it seems that breath is more important than length, the placental breadth and length are highly correlated with each other. Placenta parameters such as weight, length and breadth have close correlation with newborn parameters such as weight, length and Apgar score (38). Therefore, placental length can be considered as a landmark for placental healthy function and flawless gestation (39).

Moreover, in the present study, placental diameter was decreased in the 1st and 4th groups. But, in the 2nd and 3rd groups, the diameter was increased. It was shown that placental diameter is a parameter, which reflects the extent of the placental/ uterine surface area or lateral placental dimension (40). It is not still clear whether small lateral placental dimension affects placental function or not? Also, there is a trend toward a significant correlation between the placental diameter and composite adverse pregnancy outcome and small for gestational age (41). But, it was shown that smaller diameter of the placental surface is correlated with hypertension (42). The diameter of the chorionic disk increases between the end of the first trimester and the end of the gestation (41), while in our study by administration of celery in wholetime of gestation, the placental diameter was decreased. Sprouting of the villi is contributed to placental thickness (43) and any defects in early placental growth, affects early sprouting of the villi rather than the inability of placenta to lateral expantion (44). Therefore, any imperfections in early placental growth and development may affect villus and leads to defect in embryo normal development (41).

Rats and mice have a discoid and hemotrichorial placenta. Their placenta is composed of labyrinth zone, basal zone, decidua and metrial glands (45). The labyrinth zone has three layers of trophoblast cells which separate the maternal blood from the fetal blood including the outer trophectoderm which is referred to as cytotrophoblasts and the next are two layers of syncytiotrophoblasts (46). The basal zone of placenta has three types of cells including spongiotrophoblasts, trophoblastic giant cells and glycogen cells; eventhough, the functions of both cell groups are unknown (47). The spongiotrophoblasts are located immediately above the layer of trophoblastic giant cells. The glycogen cells construct multiple cell masses. They develop into glycogen cell islands in the midgestation and the majority of them disappear before parturition (45, 48). Overall, Caon *et al.* reported that the junctional zone of the mouse placenta has two trophoblast population cells including glycogen cell and spongiotrophoblasts (45, 49).

Our histopathological results in the control group showed normal and expected number of the placental cells, while labyrinth and basal zones of placenta in different experimental groups showed some histopathological changes, compared to the control group, such as decrease of placental trophoblastic giant cells and increase of trophoblast glycogen cells in the basal placental zone.

Mouse and human placentas are hemochorial (50). The functional part of mouse placenta is labyrinth layer, where nutrition exchange occurs. Three layers seperat mother blood from fetal blood including: syncitiotrophoblast I and II and sinusoidal giant cells. The supportive junctional zone contains spongiotrophoblast and glycogen trophoblasts. Spongiotrophoblast cells give rise to trophoblast giant cells (51).

Two processes play role in placenta formation in mouse including cell fusion and endoreduplication (DNA synthesis without nuclear division) (52) . Cell fusion in placenta leads to the formation of syncytiotrophoblast cells in interhemal compartment. The interhemal compartment forms the labyrinth and "chorionic villi" layers in mouse and human, respectively. In mouse, endoreduplication makes hyperdiploid trophoblast cells leading to giant nuclei, therfore they are called ''trophoblast giant cells'' (52,51).

Glycogen cells are analogous to the human intrestitial invasive extra villus trophoblast cells which invade the maternal spiral arteries and lead to the implantation. As a result, nutrients, blood flow and oxygen increase in implantation site. Therefore, beside, synthesis of protocadherin12 and accumulation of glycogen, they are important for fetal survival (50 51).

But, their importance for gestation is a two-edged razor, because it is supposed that in mouse, glycogen cells are required for triggering of parturition (53). They produce high levels of cyclooxygenases in late gestation. These oxidative stress markers cause hypoxia reoxygenation injury. This process is a part of labor (54 55). Therefor, their increase in our study may be a risk factor for preterm parturition.

In our study, celery extract administration led to the decrease of trophoblast giant cells. These cells are endocrine in nature and are the first terminally differentiated cells, formed during embryogenesis in pregnant rodents. They are vital for embryo implantation and maternal adaptations to pregnancy. They are very important for the establishment of fetal-maternal interface (52). Their important functions are as fololows: 1) Adhesion to the uterine epithelium, 2) Production of progestron and regulation of uterine changes and various maternal physiological interaction, 3) Poroduction of paracrine factors for regulation of feto-maternal interface (56, 4) Decidualization of the uterine stromal cells, which is controled by production of progesterone, 5) Mediation of attachment of blastocyst to the uterine epithelium, 6) Vasculature anastomose to form the yolk sac placenta. 7) Expression of several integrins (52). Therefore, any factors that decrease the number of trophoblast giant cells may interface with abovementioned proceses.

The mechanisms behind the detrimental effect of celery is known. Maybe, they can be asoossiated to the presence of some chemical coumpounds, present in celery. It was shown that celery is rich in perfluoroalkyl acids (PFAAs) which are transferred into the food chains through the soil. Celery uptakes them and has a highest amount of PFAAs (57). These compounds cause the increase of several lipid classes which interfere with membrane lipids. They alter cellular lipid pattern at high concentrations and significantly inhibit aromatase activity in placental cells (58). Different kinds of celery estrogenic favonid like IP-Zhenin are related to the cell cycle arrest in G2/M phase and decrease of cell growth and division (59), which is responsible for small size of placenta in the experimental groups. On the other hand, Tootian *et al*. reported that heroin, by changing the cell cycle and inducing apoptosis, can reduce placental diameter and weight (58). Therefore, probably the same mechanisem is responsible for placental weight and diameter decrease in our study. Flavonid, Alkaloids and anti-estrogenic compounds cause endometrial atropgy, insuficciant placental blood supply and decrease of placenta and fetus growth (60). Therefore, flavonoid compounds of celery cause decrease of placental growth. In the current study, the celery extract administration caused decrease of pacental growth. As, the blood conection betwean mother and fetus is completed toward the end of gestation, any disorders in this way may interface with placental and fetus growth (61). The other issue is decidua thichness. During an ongoing pregnancy, the thickness of decidua decreases gradually (23), while in our study, celery extract resulted in the increase of decidua thichness. In

our study, hyperaemia in the experimental groups was related to abnormal vasculature formation in labyrent layer, due to blood fetal/maternal barrier (61). All toghether, the labyrinth layer integration is one of the essential parameters for placental function and the hyperaemia, observed in this layer showed the presence of abnormality in fetal-maternal blood flow. Therefore, decline of placental biometrical size may be due to decrease of nutrient and oxygen.

Conclusion

Altogether, the present study showed that celery extract consumption can lead to placenta tissue destruction; therefore, its uptake during pregnancy should be done with more caution, due to the risk of abortion. Also, long time consumption and exposure to the extract can exacerbate the pathological changes of placenta structure. Although, the exact mechanisms of these changes still need more evaluations, it is suggested that the pregnant women take more precaution, regarding celery consumption during their pregnancy, especially in the 2nd and 3rd trimesters of gestation.

Data availability

We ensure the data underlying the findings of this paper can publicly be available from the corresponding author, if required.

Conflicts of Interest:

No

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