Original Article

The Effect of Aspartame on Albino Rat's Placenta and the Possible Protective Effect of Ginger: Histological and Teratogenic Study

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ABSTRACT

Introduction: The placenta act as barrier that protects the embryo against injurious agents. Aspartame, an artificial sweetener used throughout the world, its metabolites can cross the placenta and cause change on its structure. Ginger is a plant used in the treatment of vomiting during pregnancy. Its extracts were reported to have an antioxidant action.

Aim of the Work: Assessing the histopathological and the teratogenic effect of Aspartame on the placenta and fetuses of albino rat and study the possible protective effect of Ginger.

Materials and Methods: Fifty female albino rats were divided into four groups: Control group: received distilled water. Aspartame group received 265 mg/kg body weight (b.w) Aspartame, Ginger group received 200 mg/kg b.w Ginger extract and Aspartame-Ginger group received 265mg/kg b.w Aspartame then 200 mg/kg b.w Ginger extract by orogastric tube on gestational days from one to 18. Placenta weighted and processed for histological examination and immunohistochemical staining to detect vascular endothelial growth factor (VEGF) expression. Fetuses were weighted and examined for anomalies.

Results: Aspartame group showed a significant decrease in the mean maternal weight gain, placental weight, fetal weight and length. Additionally, a decreased basal zone and an increased labyrinth zone thickness were detected. Moreover, this group showed placental structure alternations on the light and the electron microscopic level, an increase in the interhemal membrane thickness and a significant increase in the VEGF expression in the labyrinth zone. Ginger improved all fetal and maternal parameters and ameliorated the Aspartame induced effect on the placenta.

Conclusion: Aspartame has a deleterious effect on albino rat's placenta and fetus, adding Ginger ameliorate this impact.

Received: 23 February 2022, **Accepted:** 22 March 2022

Key Words: Aspartame; ginger; placenta; teratogenicity.

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ISSN: 1110-0559, Vol. 46, No. 3

INTRODUCTION

Aspartame is an artificial sweetener. It has a sweetening effect two hundred times higher than sucrose and in comparison to saccharine it does not have a bitter or metallic after taste. For this, Aspartame is wide used as an artificial sweetener and a flavor enhancer. As more than ninety countries use Aspartame as a sweetening agent in drinks contain low-calories and more than six thousand products contain Aspartame throughout the world[1].

After oral intake, Aspartame is being absorbed from the gastrointestinal tract and metabolized in its wall into three major metabolites which might be blamed for its damaging effect; methanol (10 %), aspartic acid (40 %), and phenylalanine (50 %). Additionally, it increases oxidative stress and markedly decreases the action of glutathione reductase and catalase in the kidney and liver of rats which act as antioxidant enzymes[2].

Ingestion of Aspartame with high dose was associated with some neurological symptoms including insomnia, headaches, memory impairments, and seizure[3]. Some authors stated that the incidence of cancer increased in rats treated with Aspartame such as brain cancer, leukemias, lymphomas and mammary gland cancer.[4]

Ginger is a plant that has many uses. It is used in managing vomiting of pregnant females because of its antiemetic effect. Likewise, it is used in controlling motion sickness, as it reported that it decreases dizziness, nausea, and vomiting associated with it[5,6]. Moreover, it is stated that Ginger improves the cardiac muscle function and blood circulation in our bodies[7]. Besides, it decreases blood glucose level in diabetic rats[8]. Some authors published that Ginger has anti-carcinogenic effect against cancer colon and mammary gland cancer, as it plays an important role in suppressing cancer cells by enhancing apoptosis[9,10].

Gingerols are considered the major active ingredient of Ginger which have a strong anti-inflammatory effect, by inhibiting the synthesis of certain inflammatory mediators as prostaglandin and leukotriene[11].

 DOI: 10.21608/ejh.2022.123588.1644

Similarly, polyphenol was derived from roots of Ginger express a strong antioxidant effect. The antioxidant activity of Ginger is one of the most important criteria that make Ginger highly useful in the treatment of multiple diseases[12].

The placenta is one of most important organs that plays vital role in the development of embryos at mammals. It exhibits endocrine, excretory, nutritional, immunological, and barrier functions. It is considered a target for many drugs and chemicals that may cause it harmful effects[13].

Thus, our study was aimed to assess the histological and teratogenic effect of Aspartame on the placenta and fetuses of albino rats, and to study the possible protective effect of Ginger.

MATERIALS AND METHODS

Drugs and chemicals

Aspartame (20mg) tablet was obtained from (El-Ameriya Company for drugs). Ginger was obtained from Botany department, Faculty of Science, Alexandria University. Vascular endothelial growth factor (VEGF) monoclonal antibody was obtained from Lab Vision Corporation (Thermo Fisher, Fremont, USA). Other chemicals were bought from local commercial providers.

Animals

50 female and 25 male Wistar albino rats, nearly 6-8 weeks old (150 ± 20 gram) were obtained from Animal house, Physiology Department, El-Mowssat, Faculty of Medicine, Alexandria University, Egypt. The experimental methods followed the code of research ethics approved by the Research Ethics Committee, Alexandria Faculty of Medicine. All rats were examined for health level, acclimatized to the laboratory environment for 2 weeks in a room temperature and kept at twenty-five degrees 25◦C on a 12:12 hour light: dark cycle. Foods was administrated following the Egyptian Institute of Nutrition (EIN) recommendations. The animals were given food and water without restrain during the experimental period. Adult virgin female rats were mated with adult males (one male/ two females). The day one of gestation was considered by the appearance of the vaginal plug.

Experimental Design

The mated female albino rats were divided into four groups: Control group: Included ten rats which received distilled water. Aspartame group: Included twenty rats received 265 mg/kg body weight (b.w) Aspartame per day. Ginger group: Included ten rats received 200 mg/kg b.w Ginger extract per day. Aspartame-Ginger group: Included ten rats received 265mg/kg b.w Aspartame and 200 mg/ kg b.w Ginger extract per day. All tested compounds were given by orogastric tube on gestation day (GD) from one to 18.

Mated pregnant females were assessed daily throughout the experimental period for any signs of behavioral changes or abortion. The maternal weight was recorded at GD1 and GD19 to calculate average weight gain during pregnancy for each group. Cesarean sections were done on GD 19 under anesthesia. The uterine horns were examined for the number, location of fetuses and resorption sites.

After opening of uterine horns, fetuses were carefully removed from the uteri. Each litter were divided in terms of lived and dead fetuses then lived fetuses were weighted by mini scale and their lengths were recorded.

Histological and Morphometric Analysis

Placenta was dissected, weighted and examined by the naked eye for any abnormalities and then divided into two parts. One part was fixed in 10 % formol saline and processed to get paraffin sections of 3-5 µm thick for H&E staining to detect placental histological alterations and immunohistochemical staining for detecting VEGF expression[14,15]. The other part was instantly cut into small pieces 0.5-1 mm^3, fixed in 3% glutaraldehyde solution and processed to obtain ultrathin sections for transmission electron microscope (TEM) examination[16].

For immunohistochemical studies, sections were placed on positively charged slides. Immunostaining procedure was performed after the streptavidin–biotin-immunoenzymatic antigen detection method, according to the manufacturer's protocol. The primary antibodies used was VEGF monoclonal antibody (Rabbit polyclonal, Thermo, diluted at 1:100). Positive and negative controls were included in each run. Sections without primary antibodies used as negative controls. While the positive control was human angiosarcoma[14].

Digital images from H&E and immunohistochemically stained sections were taken using (Olympus DP20) camera connected to (Olympus CX41) microscope. The images from H&E-stained placental sections were taken at magnification (40X), they were used to determine the thickness of the basal (BA) and labyrinth (LB) zone. On the other hand, the images from the immunohistochemically stained sections were taken at magnification (400X) and used to determine the mean area percentage of VEGF expression in the labyrinth zone. TEM (JEM-1400 plus; Japan) was used to take images for the placental ultrastructure. These images were used to measure the thickness of the interhemal membrane. Results were measured using NIH Image j (v1.49) software.

Statistical analysis of the data

Data were supplied to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were expressed using number and percent. The Kolmogorov-Smirnov test was utilized to validate the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 0.05% level.

RESULTS

Maternal observations

No deaths nor behavioral changes were recorded in all groups. Food and water intake weren't significantly different in the Ginger group (15.15 ± 0.78) gm, (19.65 ± 0.71) ml, but they were significantly decreased in the Aspartame group (10.28 ± 0.80) gm, (11.95 ± 0.83) ml, compared to the Control group (15.15 ± 0.58) gm, (21.10 ± 2.84) ml. There was a significant increase in food and water intake in the Aspartame-Ginger group (13.65 ± 0.67) gm, (16.85 ± 0.63) ml, compared to the Aspartame group, but both food and water intake in the Aspartame-Ginger group were still below the Control group with a significant difference (Table 1).

The mean maternal weight gain was not significantly changed in Ginger group (61.0 ± 2.45) gm, but it was significantly decreased in Aspartame group (42.35± 2.01) gm in comparison to Control group (62.30 ± 2.58) gm. For the Aspartame-Ginger group, there was a significant increase in the mean maternal weight gain (52.40 ± 1.71) gm when compared to Aspartame group but was significantly lower than Control group (Table 1).

Fetal Observations

There was no significant difference in the mean number of implants per litter and nor external congenital anomalies in all experimental groups. Comparing to the Control group, the mean fetal weight of Ginger group (4.32 ± 0.24) gm, showed no significant changes. While Aspartame group (2.55 ± 0.20) gm, was significantly decreased (4.34 ± 0.27) gm. On the other hand, the Aspartame-Ginger group (3.52 ± 0.16) showed significantly higher results when compared to Aspartame group. At the same time these results were still lower than Control group with a significant difference (Table 2).

For the mean fetal length, it was not significantly changed in Ginger group (3.91 ± 0.14) cm, but it was significantly decreased in Aspartame group (2.48 ± 0.21) cm, in comparison to Control group (3.98 ± 0.16) cm. For the Aspartame-Ginger group, there was a significant increase in the mean fetal length (3.37 ± 0.13) cm when compared to Aspartame group but was significantly lower than Control group (Table 2).

Placental observations

Placental weight

Comparing to the Control group, the mean placental weight of the Ginger group (0.65 ± 0.09) gm, showed no significant changes. While Aspartame group (0.38 ± 0.02) gm, was significantly decreased (0.67 ± 0.06) gm. On the other hand, the Aspartame-Ginger group (0.50 ± 0.02) gm, showed significantly higher results when compared to Aspartame group. At the same time these results were still lower than Control group with a significant difference (Table 2).

Light microscopic and placental thickness results

The hematoxylin and eosin-stained placenta of Control group and Ginger group showing normal placental structure. It showed three differentiated zones, the labyrinth, basal and decidual zone. The labyrinth zone was comprised of a network of maternal blood sinuses and fetal capillaries lined by endothelial cells. Cytotrophoblast and syncytiotrophoblast cells were revealed in this layer. Spongiotrophoblast cells with their basophilic cytoplasm and glycogen cells with a clear and vacuolated cytoplasm were seen in the basal zone. Giant trophoblasts with large nuclei were noticed between the basal and the decidual zone (Figure 1).

Alternatively, Aspartame group of placental sections showed apparent decrease in the basal zone and an increase in the labyrinth zone thickness. In addition, the basal zone revealed degenerating glycogen cells, giant trophoblasts and spongiotrophoblasts with dark small nuclei. The labyrinth zone showed interrupted interhemal membranes, cytotrophoblasts with darkly stained nuclei and syncytiotrophoblast with dark small nuclei (Figure 2).

Interestingly, placental sections of the Aspartame-Ginger group showed mostly normal placental structure with few areas of abnormalities, as the basal zone was still showing few degenerating glycogen cells, few giant trophoblasts and spongiotrophoblasts with dark small nuclei. Likewise, it showed almost normal labyrinth with some interrupted interhemal membranes and few cytotrophoblasts with darkly stained nuclei (Figure 3).

For the mean thickness of the basal zone, it was not significantly changed in Ginger group (416.8 ± 77.17) µm, but it was significantly decreased in Aspartame group (347.5 ± 60.24) µm, in comparison to the Control group (427.5 ± 142.1) µm. For the Aspartame-Ginger group, there was a significant increase (373.5 ± 52.77) µm when compared to Aspartame group but was significantly lower than Control group (Figure 4).

The mean thickness of the labyrinth zone showed no significant change in Ginger group (2196.8 ± 84.29) µm, but it was significantly increased in Aspartame group (2799.8 ± 122.0) µm, when compared to the Control group (2162.9 ± 271.0) µm. For Aspartame-Ginger group the mean thickness of the labyrinth zone (2047.7 ± 174.1) µm was comparable to the Control group values (Figure 4).

Immunohistochemical results and area percentage of VEGF expression

Examining the labyrinth of placental sections immunohistochemically stained with VEGF monoclonal antibody (a marker for angiogenesis) showed, few areas of cytoplasmic VEGF expression in the trophoblasts of the Control and Ginger groups. On the other hand, the Aspartame group showed wide areas of cytoplasmic VEGF expression in the labyrinth. Aspartame-Ginger group shows improvement of VEGF expression in the cytoplasm of trophoblasts which nearly similar to normal. This was

confirmed by morphometric analysis, as the mean area percentage of VEGF expression in the Ginger group (2.36 ± 0.40) showed no significant difference when compared to the Control group (2.59 ± 0.62). While the Aspartame group values (8.42 ± 1.40) were significantly higher than that of the Control group. For the Aspartame-Ginger group the results (3.04 ± 0.74) were significantly lower than Aspartame group and comparable to the Control group without a significant difference (Figures 5,6).

Electron microscopic and interhemal membrane thickness results

The transmission electron microscopic assessment of the placenta ultrathin sections of the control and Ginger groups showed normal organization of labyrinth zone of rat placenta with three trophoblasts layers; cytotrophoblast (layer I) and syncytiotrophoblast (layer II and III). Layer I cytotrophoblast facing maternal blood, with numerous microvilli, fenestrations, and cytoplasmic protrusions with vesicles in them. Desmosomes are noticed between trophoblast layers. In syncytial layer II, trophoblast contain infoldings and microplicae, endocytic vesicles and glycogen granules are noticed. The fetal capillaries are noticed lined by endothelial cells. The interhemal membrane between the maternal and fetal blood, formed of three layers of trophoblasts and the endothelial cell is present with normal thickness (Figures 7,8).

On the other hand, placental ultrathin sections of the Aspartame group showed abnormal organization of labyrinth zone of rat placenta. In the syncytial layer, increased infoldings of microplicae, vacuoles with heterogenous substance in it, increased glycogen deposition, dilated rER, degenerating mitochondria with absent cristae, increased lipid droplets and collagen deposition were revealed (Figure 9).

Electron micrograph of placenta of Aspartame-Ginger group showed areas of normal organization of the rat placenta but some abnormalities were still present. As few vacuoles, glycogen deposition, dilated rER, degenerated mitochondria with absent cristae and collagen fibrils were revealed in the syncytial layers II and III (Figure 10).

The mean thickness of the interhemal membrane showed no significant change in Ginger group (4.53 ± 1.03) µm, but it was significantly increased in Aspartame group (8.36± 1.57) µm, when compared to the Control group (4.11 ± 0.44) µm. For Aspartame-Ginger group the mean thickness result (4.05 ± 1.01) µm was significantly lower than Aspartame group and comparable to the Control group values (Figure 11).

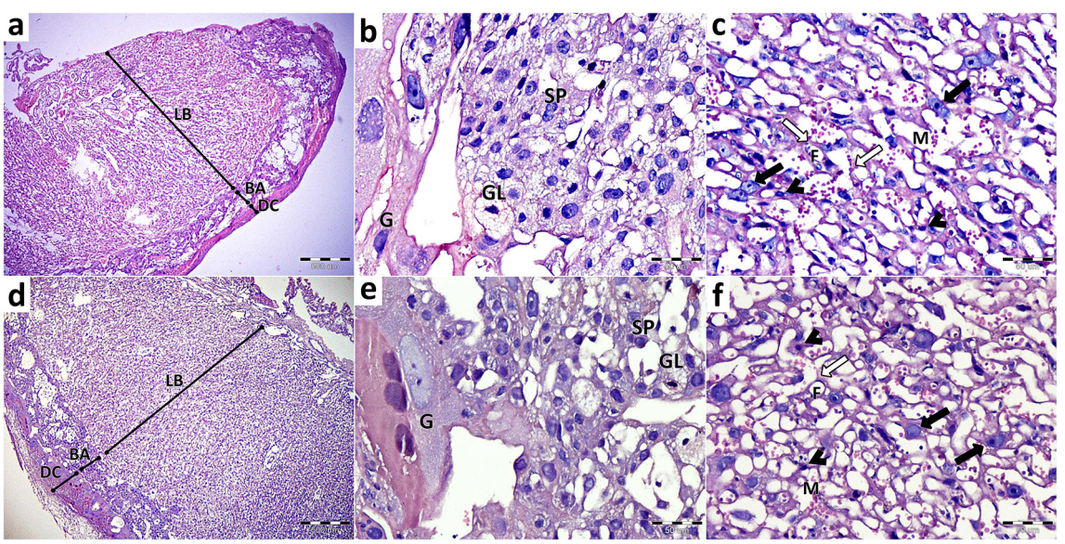

Fig. 1: Photomicrographs of placenta of Control group (a-c) and Ginger group (d-f) showing normal placental structure. a&d: showing different parts of the placenta. labyrinth zone (LB). basal zone (BA). decidual zone (DC). b&e; showing the basal zone. spongiotrophoblast (SP), glycogen cell (GL). giant trophoblast (G). c&f; showing the labyrinth zone with intact interhemal membrane (thick white arrow), cytotrophoblast (thick black arrow) and syncytiotrophoblast (black arrowhead). maternal blood spaces (M). fetal capillaries (F). (H&E stain. a,d; 40X Magnification - b,c,e,f; 400X Magnification).

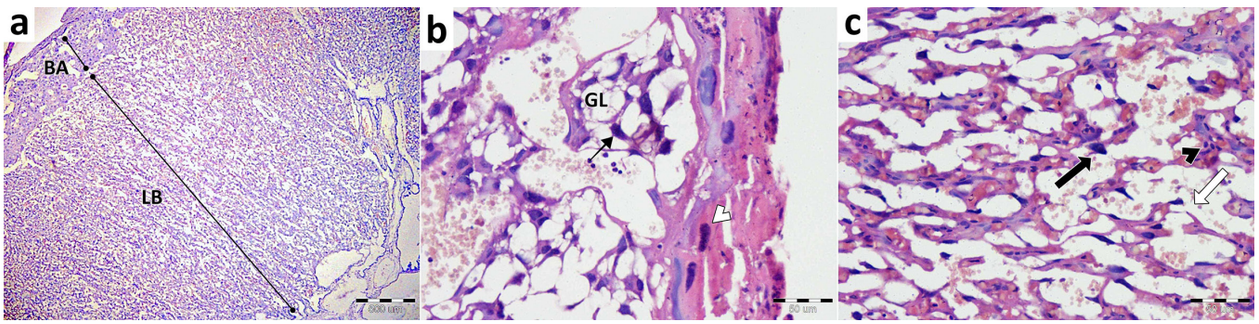

Fig. 2: Photomicrographs of placenta of Aspartame group showing a; showing an increase in labyrinth zone (LB) and a decrease in the basal zone (BA) thickness. b; showing basal zone with degenerating glycogen cells (GL), giant trophoblasts (white arrowhead) and spongiotrophoblasts (thin black arrow) with dark small nuclei. c; showing the labyrinth with interrupted interhemal membrane (thick white arrow), cytotrophoblasts (thick black arrow) with darkly stained nuclei and syncytiotrophoblast with dark small nuclei (black arrow head). (H&E stain. a; 40X Magnification - b,c; 400X Magnification).

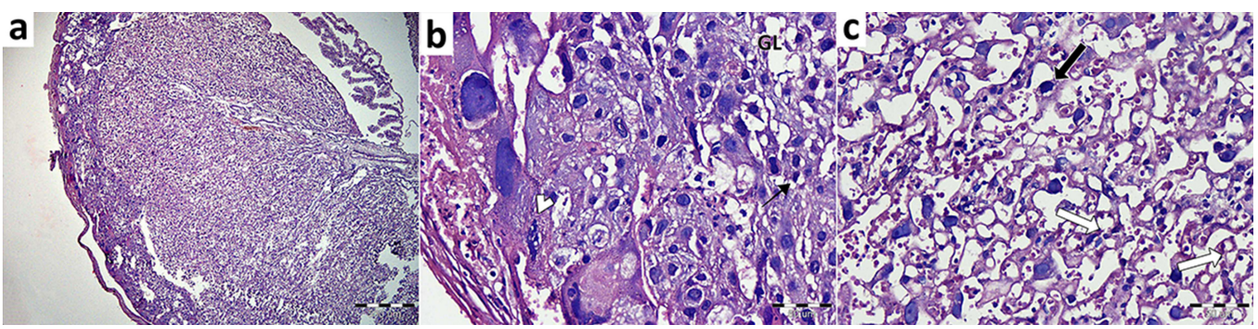

Fig. 3: Photomicrographs of placenta of Aspartame and Ginger group (a-c) showing mostly normal placental structure with few areas of abnormalities. b; basal zone still showing few degenerating glycogen cells (GL), few giant trophoblasts (white arrowhead) and spongiotrophoblasts (thin black arrow) with dark small nuclei. c; Notice almost normal labyrinth with some interrupted interhemal membrane (thick white arrow) and few cytotrophoblasts (thick black arrow) with darkly stained nuclei. (H&E stain. a; 40X Magnification - b,c; 400X Magnification).

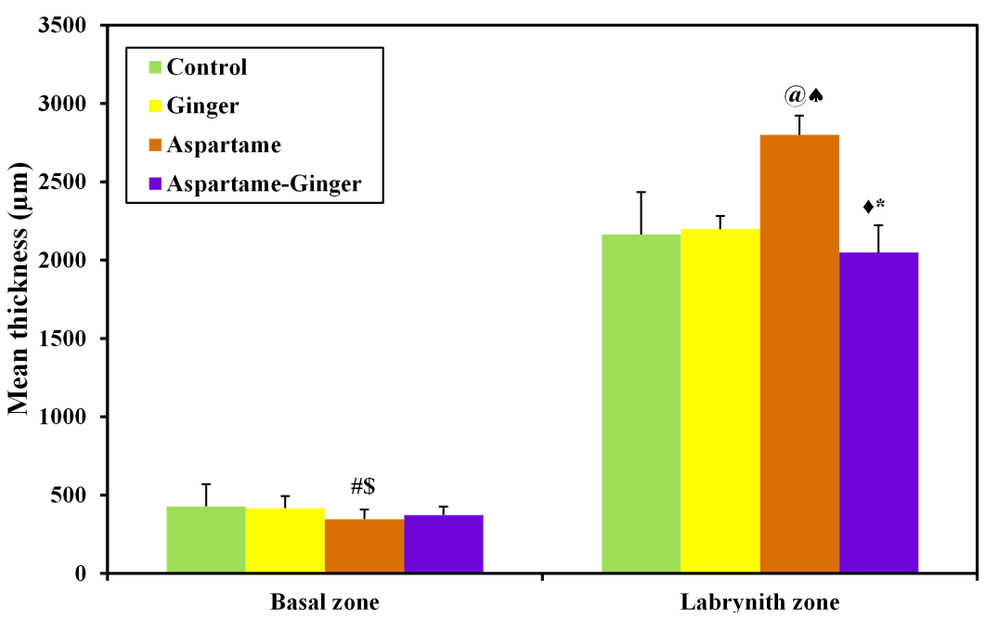

Fig. 4: Morphometrical analysis of mean thickness of basal and labyrinth zones
Basal zone #: p=0.005* between Control and Aspartame, $: p=0.018* between Ginger and Aspartame, Labyrinth zone @: p<0.001* between Control and Aspartame, ♠: p<0.001* between Ginger and Aspartame ♦: p=0.008* between Ginger and Aspartame-Ginger, *: p<0.001* between Aspartame and Aspartame-Ginger.

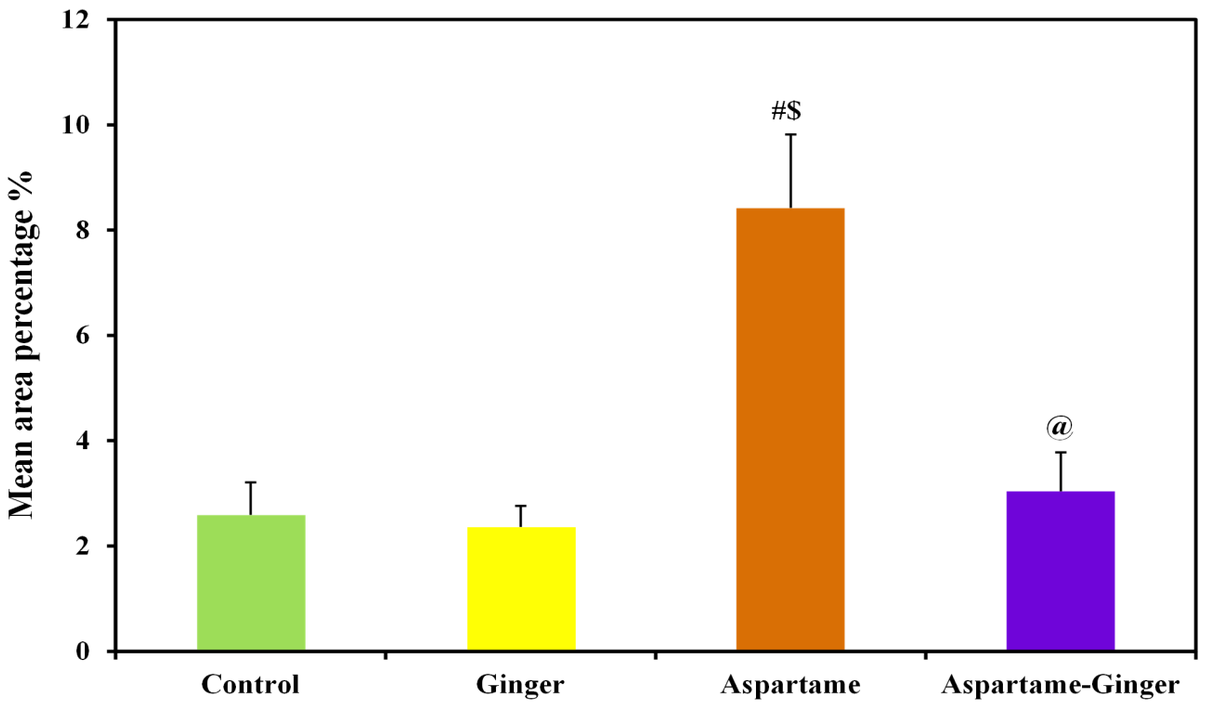

Fig. 5: Comparison between the different studied groups according to VEGF immunopositive reaction.
#: $p<0.001$* between Control and Aspartame, $: $p<0.001$* between Gingar and Aspartame, @: $p<0.001$* between Aspartame and Aspartame-Gingar

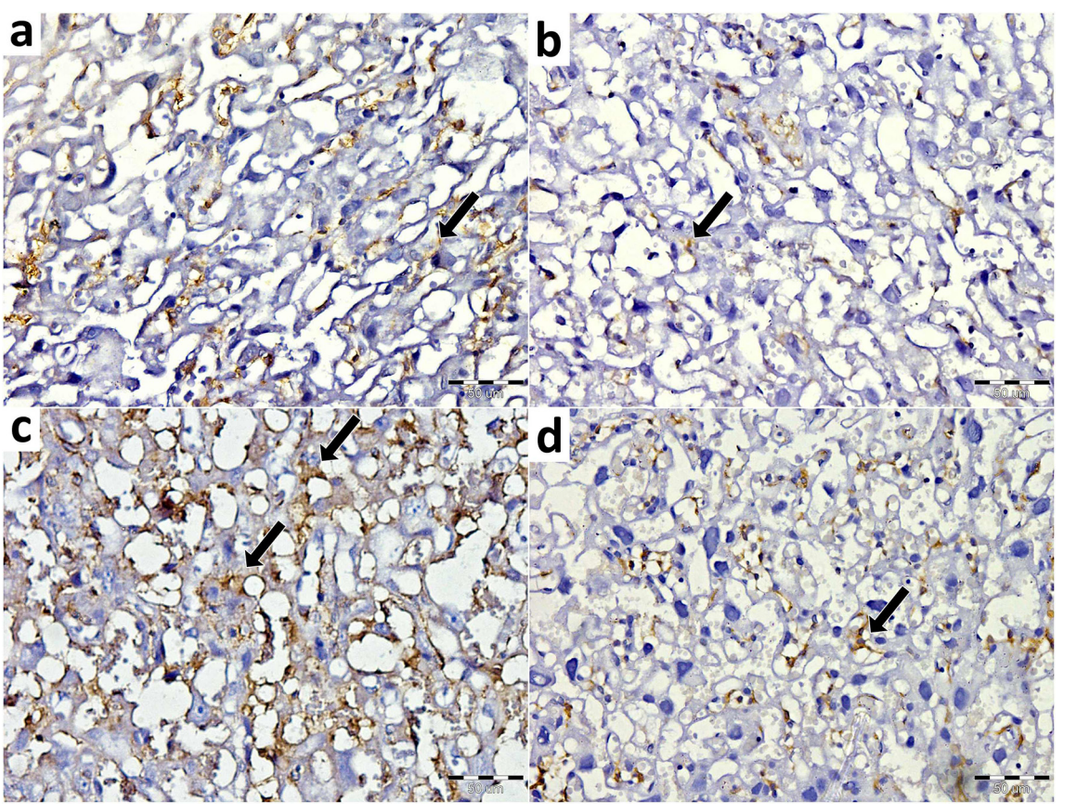

Fig. 6: Photomicrograph of the labyrinth of placental sections stained with VEGF monoclonal antibody, (a,b) Control group and Ginger groups, respectively, showing few areas of cytoplasmic VEGF expression in the trophoblasts (black arrow). (c) Aspartame group shows many areas of cytoplasmic VEGF expression (black arrow). (d) Aspartame-Ginger group shows improvement of VEGF expression in the cytoplasm of trophoblasts which is nearly similar to normal. (Mag. X400).

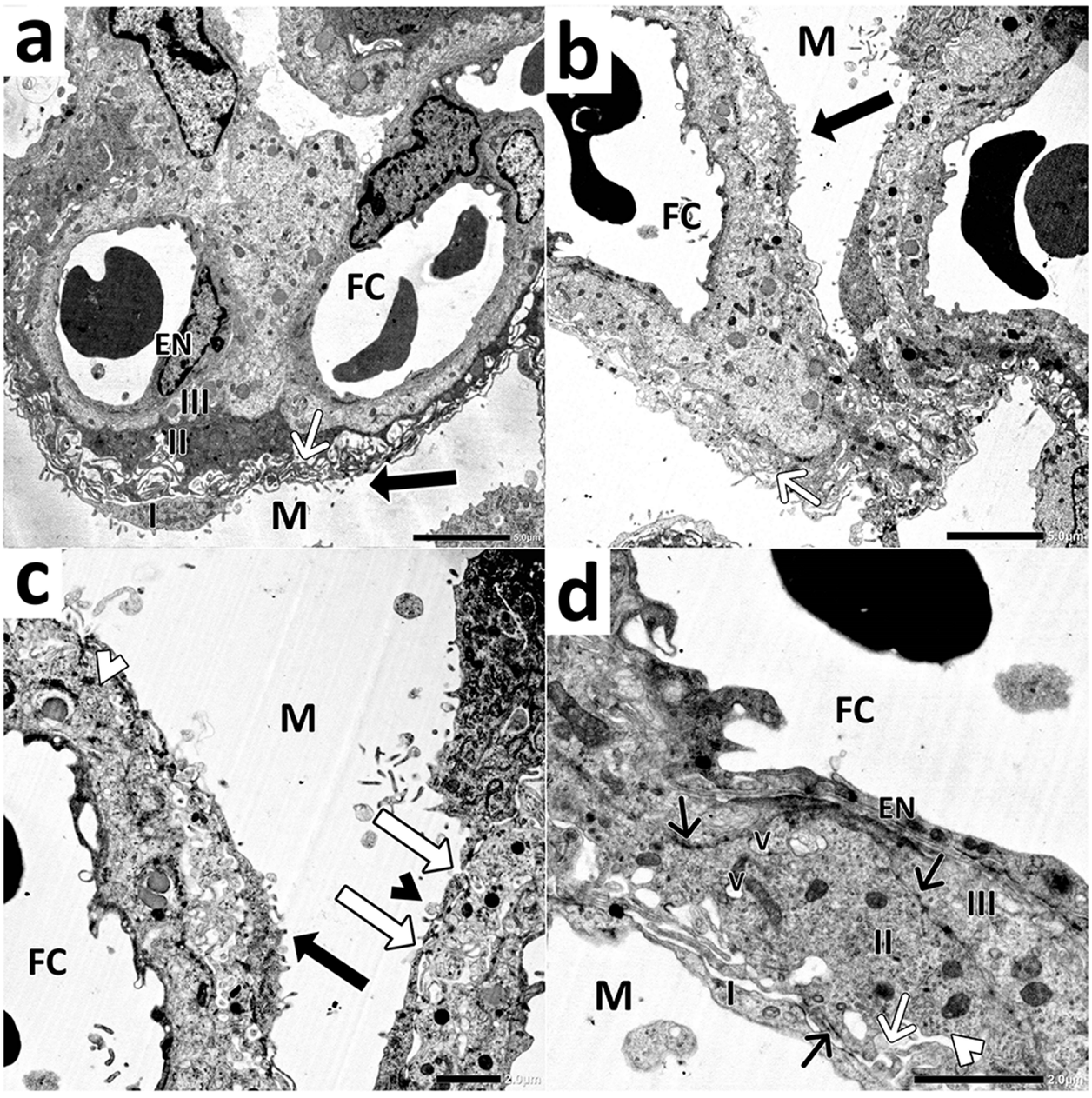

Fig. 7: Electron micrograph of placenta of Control group, (a-d) showing normal organization of labyrinth zone of rat placenta with three trophoblasts layers; cytotrophoblast (layer I) and syncytiotrophoblast (layer II and III). Notice fetal capillaries (FC) lined by endothelial cells (EN). Notice layer I cytotrophoblast facing maternal blood (M), with numerous microvilli (thick black arrow). Notice in syncytial layer II trophoblast; infoldings and microplicae (thin white arrow), endocytic vesicles (V) and glycogen granules (white arrowhead). (c) is a higher magnification of (b) showing cytoplasmic protrusions with vesicles in them (black arrowhead) and fenestrations (thick white arrow). (d) Notice desmosomes between trophoblasts layers (thin black arrow). Notice interhemal membrane with normal thickness formed of three layers of trophoblasts and endothelial cell. (Uranyl acetate/lead citrate stain) (a; Magnification X1200. b; Magnification X1200. c; Magnification X2000. d; Magnification X4000).

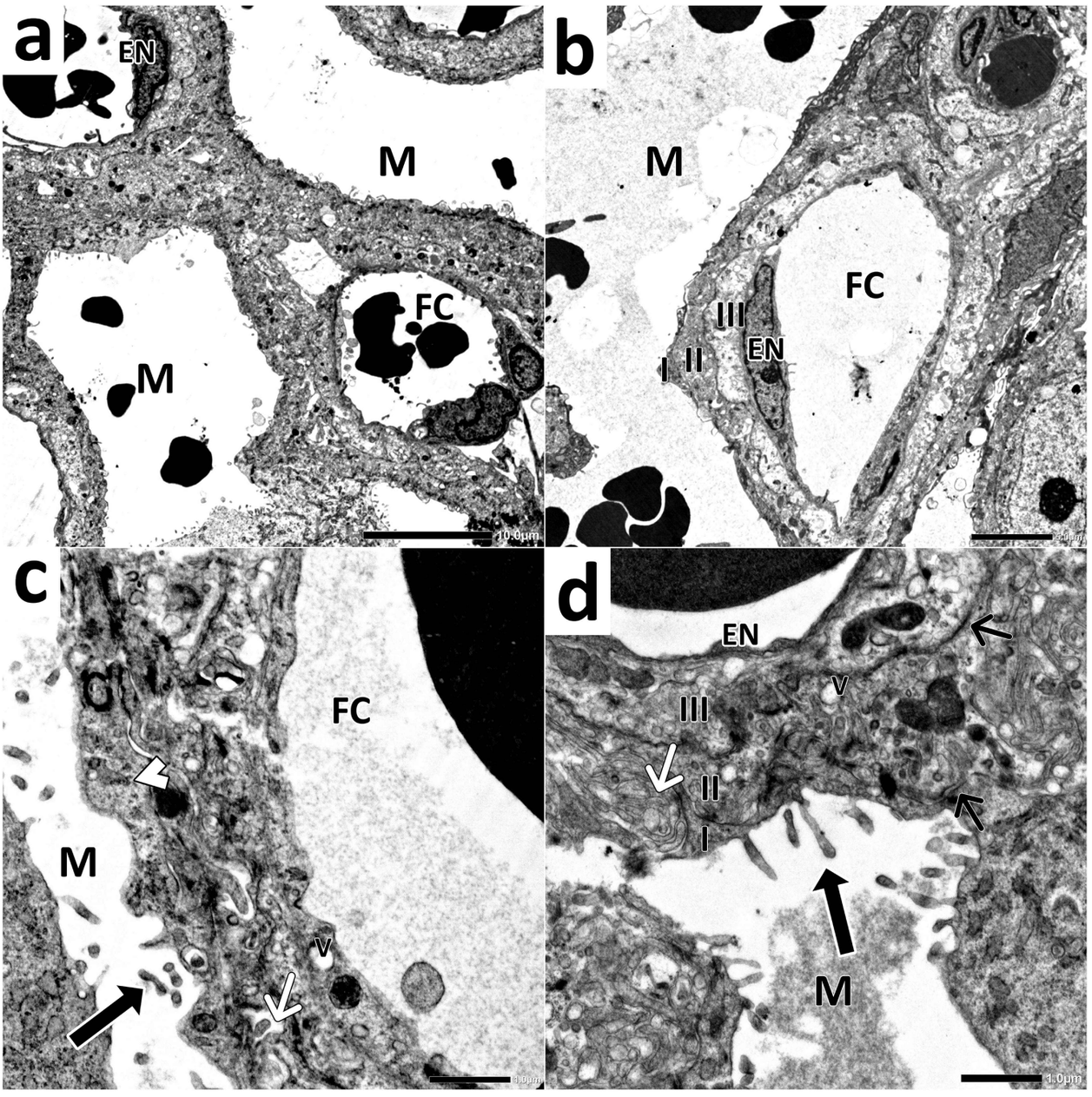

Fig. 8: Electron micrograph of labyrinth zone of placenta of Ginger group, (a-d) showing normal organization of rat placenta with three trophoblasts layers; cytotrophoblast (layer I) and syncytiotrophoblast (layer II and III). Notice fetal capillaries (FC) lined by endothelial cells (EN). Notice layer I cytotrophoblast facing maternal blood (M), with numerous microvilli (thick black arrow). Notice in syncytial layer II trophoblast; infoldings and microplicae (thin white arrow), endocytic vesicles (V) and glycogen granules (white arrowhead). (d) Notice desmosomes between trophoblasts layers (thin black arrow). Notice interhemal membrane with normal thickness formed of three layers of trophoblasts and endothelial cell. (Uranyl acetate/lead citrate stain) (a; Magnification X800. b; Magnification X1000. c; Magnification X5000. d; Magnification X5000).

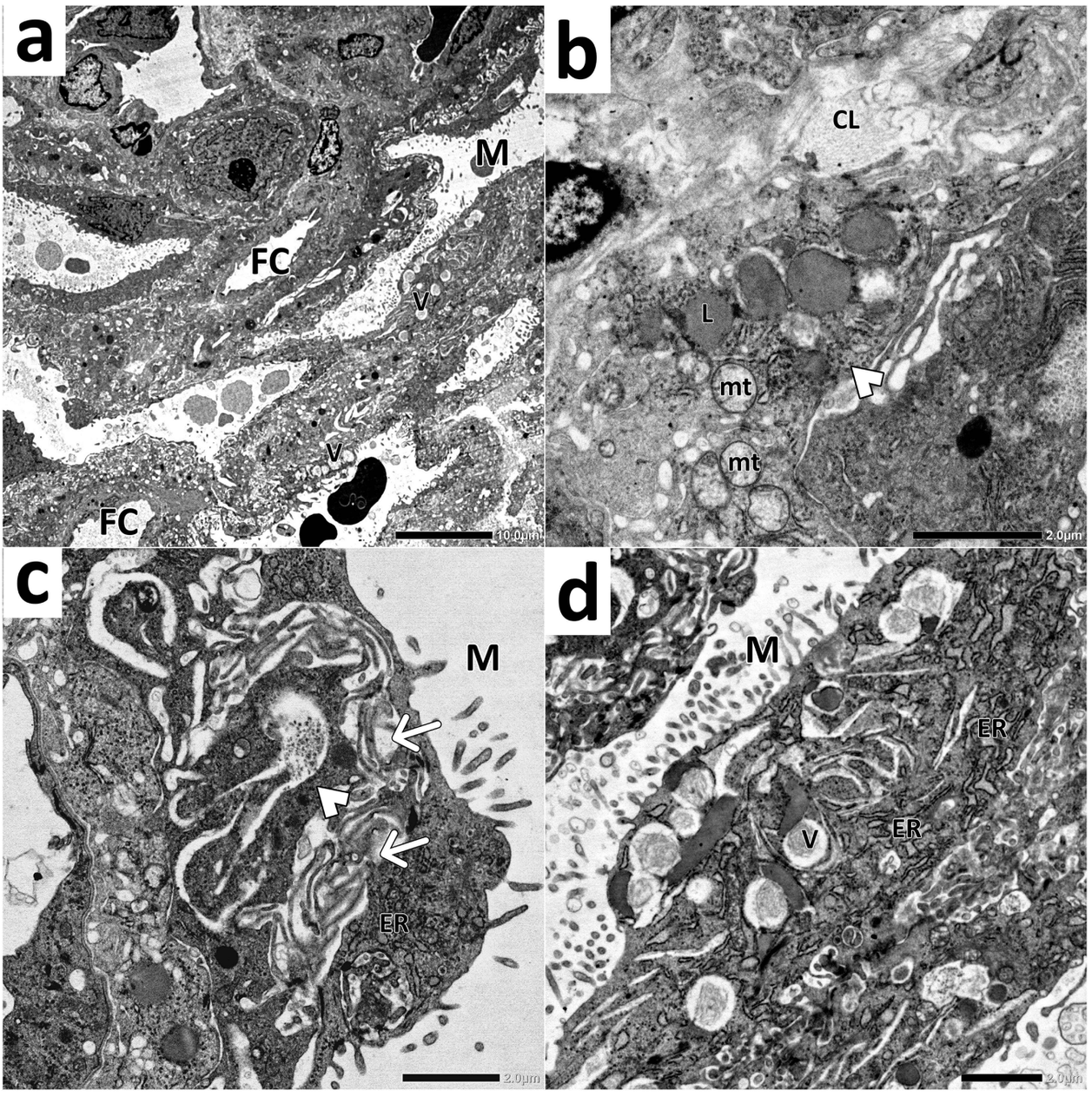

Fig. 9: Electron micrograph of labyrinth zone of placenta of Aspartame group, (a-d) showing abnormal organization of rat placenta. Notice in the syncytial layers; increased infoldings of microplicae (thin white arrow), vacuoles with heterogenous substance (V), increased glycogen deposition (white arrowhead), dilated rER (ER), degenerating mitochondria with absent cristae (mt), increased lipid droplets (L) and collagen fibrils (CL). maternal blood (M). fetal capillary (FC). (Uranyl acetate/lead citrate stain) (a; Magnification X600. b; Magnification X4000. c; Magnification X3000. d; Magnification X2500).

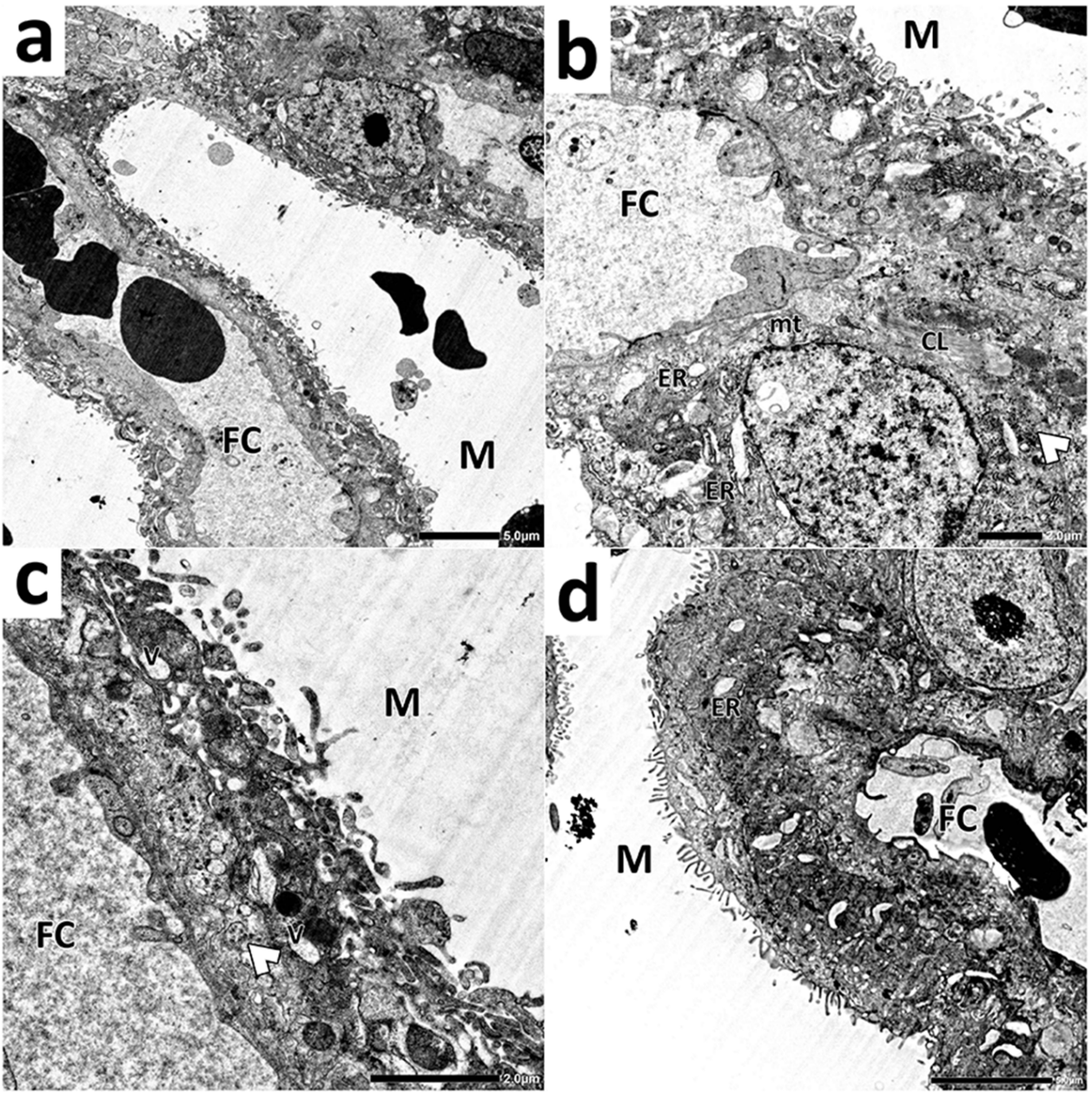

Fig. 10: Electron micrograph of placenta of Aspartame and Ginger group, showing areas of normal organization of rat placenta where some abnormalities are still present. (a) shows normal placental tissue organization. (b-d) show some persistent abnormalities like increased vacuoles (V), glycogen deposition (white arrowhead), dilated rER (ER), degenerating mitochondria with absent cristae (mt) and collagen fibrils (CL) in the syncytial layers. (d) Notice interhemal membrane with normal thickness. M; maternal blood. FC; fetal capillary. (Uranyl acetate/lead citrate stain) (a; Magnification X1000. b; Magnification X2000. c; Magnification X4000. d; Magnification X1500).

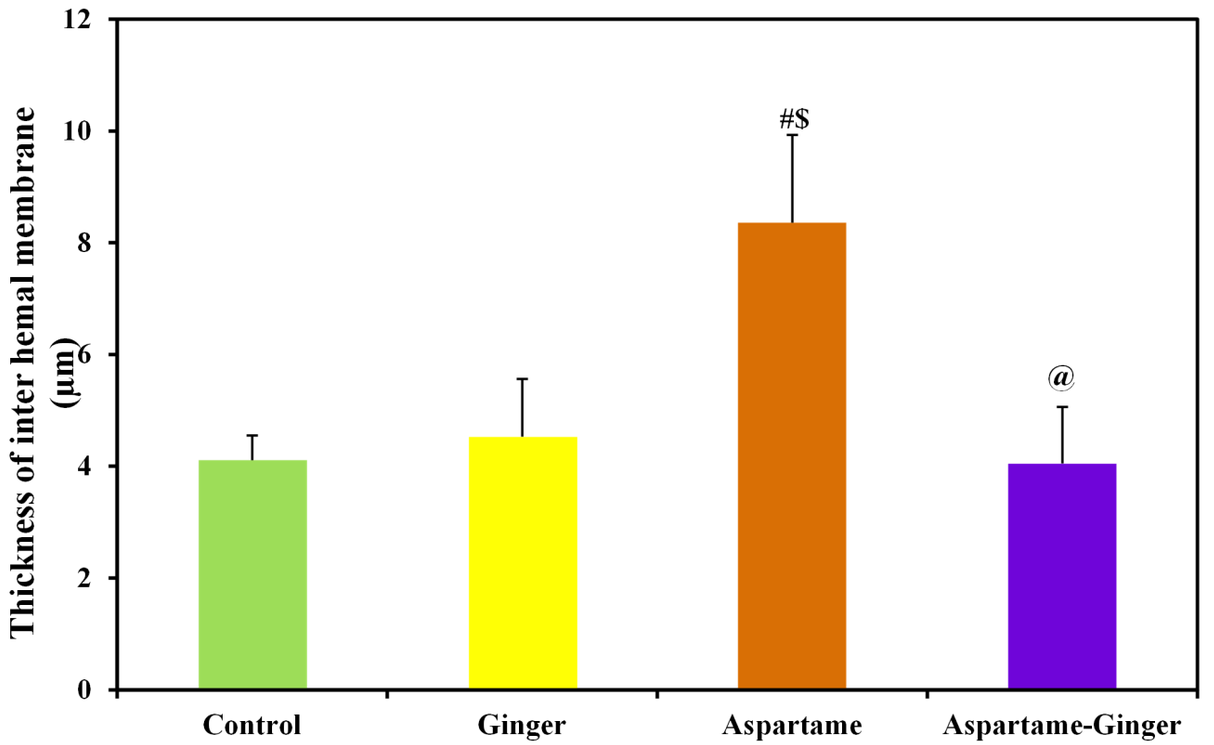

Fig. 11: Comparison between the different studied groups according to thickness of interhemal membrane.
#: p<0.001* between Control and Aspartame, $: p<0.001* between Ginger and Aspartame, @: p<0.001* between Aspartame and Aspartame-Ginger

Table 1: Comparison between the different studied groups according to different parameters

	Control (n = 10)	Ginger (n = 10)	Aspartame (n = 20)	Aspartame-Ginger (n = 10)	p
Diet consumption (gram)	15.15 0.58 ±	15.15 0.78 ±	10.28#@ 0.80 ±	13.65$♥♣ 0.67 ±	<0.001*
Water intake (ml)	21.10 2.84 ±	19.65 0.71 ±	11.95#@ 0.83 ±	16.85$♥♣ 0.63 ±	<0.001*
Maternal weight gain (gram)	62.30 2.58 ±	61.0 2.45 ±	42.35#@ 2.01 ±	52.40$♥♣ 1.71 ±	<0.001*

Data was expressed by using Mean ± SD.
#: p<0.001* between Control and Aspartame
$: p<0.001* between Control and Aspartame-Ginger
@: p<0.001* between Ginger and Aspartame
♥: p<0.001* between Ginger and Aspartame-Ginger
♣: p<0.001* between Aspartame and Aspartame-Ginger

*: Statistically significant at $p \leq 0.05$

Table 2: Comparison between the different studied groups according to different parameters

	Control (n = 10)	Ginger (n = 10)	Aspartame (n = 20)	Aspartame-Ginger (n = 10)	
Fetal weight (gram)	4.34 0.27 ±	4.32 0.24 ±	2.55#@ 0.20 ±	3.52$♥♣ 0.16 ±	<0.001*
Fetal length (cm)	3.98 0.16 ±	3.91 0.14 ±	2.48#@ 0.21 ±	3.37$♥♣ 0.13 ±	<0.001*
Placental weight	0.67 ± 0.06	0.65 ± 0.09	0.38#@ ± 0.02	0.50$♥♣ ± 0.02	<0.001*

Data was expressed by using Mean ± SD.
Data was expressed by using Mean ± SD.
#: p<0.001* between Control and Aspartame
$: p<0.001* between Control and Aspartame-Ginger
@: p<0.001* between Ginger and Aspartame
♥: p<0.001* between Ginger and Aspartame-Ginger
♣: p<0.001* between Aspartame and Aspartame-Ginger

*: Statistically significant at $p \leq 0.05$

DISCUSSION

The placenta is an impermanent organ which performs a fundamental role in fetal development during gestation. It is responsible for nutrition, respiration, excretion, and hormonal production for the embryo[13]. Aspartame as an artificial sweetener, has sweetening effect 200 times higher than sucrose[3]. For the favor of its low caloric content it is widely applied by diabetic people and by people working on decreasing their weights[1]. Ginger is one of plants used in the management of vomiting accompanied with pregnancy because of its antiemetic effect. It is also reported that Ginger is beneficial in the treatment of many diseases because of its antioxidant activity[5].

Thus, the aim of our study was to assess the histological and teratogenic effects of Aspartame on the placenta and fetuses of albino rats, and to study the possible protective effect of Ginger.

In the present study, Aspartame caused a significant decrease in the mean maternal weight gain. This is in agreement with Aboshanady *et al*[17] who showed highly significant reduction of maternal weight gain in Aspartame treated rats. This reduction was recorded in both groups received low and high doses of Aspartame (14 mg/kg and 40 mg/kg), respectively. Additionally Abd Elfatah *et al*[18] stated that the treated animals showed body weight reduction.

These results discussed by many researchers who explained the mechanisms by which body weight reduction induced by Aspartame. Rogers *et al*[19] stated that Aspartame causes satiety in human resulting into weight loss. Hall *et al*[20] reported that the satiety caused by Aspartame due to the increased level of phenylalanine at blood causing inhibition of food intake in mammals and promote the secretion of cholecystokinin which inhibit gastric emptying.

Furthermore, we observed that Aspartame decreased food consumption and water intake by the pregnant rats. These results disagree with the results obtained by Yang *et al*[21] who reported that Aspartame causes weight gain and increased appetite.

Alternatively, the Aspartame-Ginger group showed significant increase in the mean maternal weight gain when compared to Aspartame group. This findings were in accordance with many studies which confirmed the ability of Ginger in recovering the loss of body weight[22]. Several researches approved the ability of Ginger in the prevention of weight loss in diabetic rats in comparison to non-treated diabetic groups, and they described this mechanism by the hypoglycemic effect of Ginger[23]. Besides, it includes vitamin B6 which leads to weight gain by induction of the synthesis of protein[5]. On the contrary, Weidner M. and Sigwart[24], stated that oral administration of Ginger from 6 - 15 GD didn't change the weight gain in rats.

For the mean fetal weight and length, there was significantly decreased in the Aspartame group compared to Control group. Reduction of fetal body weight and length are signs of retardation of fetal growth[25]. These results were associated with the decrease in maternal weight gain. This might be due to maternal stress, which impact the growing fetuses resulting in fetal growth retardation. In addition to that, this also may be due to the decrease thickness of basal zone of placenta of rats treated by Aspartame[25], as Woods *et al*[25] reported that the fetal growth is influenced by the size of the developing basal zone. Obviously, this impact is associated to the basal zone and not the whole placenta, as fetal growth retardation is present in animals showed changes of the basal zone rather than that of the labyrinth. Basal zone impairment not only can locally alter endocrine environment of the placenta, but also have multiple systemic effects on both mother and fetus. This decrease in the basal zone thickness was revealed in the Aspartame group in our study. On the other hand, there was an increase in the thickness of the labyrinth zone compared to Control group with significant difference.

Reduction of fetal body weight can be explained by the deprivation of the fetuses requirements needed for normal growth such as glucose, as it will decrease in the blood of maternal rats that ingest sweetener[17]. This agrees with the results reported by Portela *et al*[26] who recorded a decreased fetal weight in Aspartame treated rats.

Alternatively, the mean fetal weight and length was significantly increased in Aspartame-Ginger group compared to Aspartame group. This is in consistent with Abd El-Aziz *et al*[27] who stated that Ginger administration with cadmium presented by fetal weight improvement.

This ameliorative effect of Ginger may be attributed to its antioxidant effect[28]. As, related reports have showed that treatment with antioxidant can reduce malformations and growth retardation[29]. In line with that, it was reported that co-administration of vitamin E with valproic acid and phenytoin significantly improved the fetal weight, length and malformations[30]. Moreover, the study of Abd El-Aziz *et al*[31] reported that vitamin E administration with methylmercury was linked to an enhancement of the fetal parameters as; crown-rump length, length of head, body weight, and biparietal diameter of the rat fetuses.

The mean of placental weights, in our study was significantly decreased in Aspartame group compared to Control group. This agrees with the results obtained by Portela *et al*[26]. While in our study, the Aspartame-Ginger group, showed significant increase in the mean of placental weights when compared to Aspartame group. This is in accordance with El-Borm and Attallah[32], who revealed that the placental weight significantly increased in Ginger and Labetalol group in comparison to the labetalol group (Labetalol; drug used for high blood pressure management). Furthermore, Abd El-Aziz *et al*[27] reported that pregnant rats treated with 250 mg/kg b.w of Ginger improved the weight of placenta in cadmium induced toxicity.

Light microscopic results of Aspartame group showed interrupted interhemal membrane in the labyrinth zone, this may be due to lipid peroxidation occurred by Aspartame which was recorded in mice received 40 mg/kg Aspartame for six weeks[33]. Moreover, Rey *et al*[34] reported that the interhemal membrane damage was not only due to a mechanical injury through the dilation of the maternal sinusoids but also result from the cellular changes like cytoplasmic vacuolization.

Additionally, degenerating glycogen cells, giant trophoblasts, spongiotrophoblasts and cytotrophoblasts with darkly stained nuclei, were revealed. These degenerative changes can result from the direct toxicity of Aspartame on the placenta of rats or as complication of free radicals release and production of Aspartame metabolite such as; aspartic acid and methanol. The methanol increases the production of hydrogen peroxide and superoxide anion at mitochondria which lead to cell membrane damage after ingestion of Aspartame[35].

Glycogen cells (GCs) are a major component of the basal zone. The area of the basal zone depends on the percentage of GCs it contains, and their content of glycogen which decreases when pregnant rats are exposed to dietary restriction[36]. Thus, decrease in the thickness of basal zone may be linked to degeneration of GCs and this was revealed in our study. The capacity of GCs to release and store glycogen in huge amount suggests they play an essential role in supplying fetuses with energy source as stated by Shalaby *et al*[35].

Moreover, the spongiotrophoblast, giant trophoblast and cytotrophoblast cells depicted darkly stained nuclei, indicating cell death. These findings were attributed to the increased accumulation of methanol and formaldehyde resulted from Aspartame metabolism. These products produce their cellular toxicity through the structural and functional changes of proteins and mutations caused to DNA, thus resulting into cell death or malignancies[18].

Placental ultrastructural assessment of the Aspartame-treated group in this work revealed multiple degenerative signs such as increased infoldings of microplicae, vacuoles with heterogenous substances, increased glycogen deposition, dilated rER, degenerating mitochondria with absent cristae, increased lipid droplets, collagen deposition and thickened interhemal membrane. Such degenerative changes have been revealed in studies of diabetes, preeclampsia, and hypertensive diseases during pregnancy, suggesting that these cellular damage are resulted from placental injury[37,38].

Increased folding in syncytial layer seen in the current study has been noticed with other complications such as pre-eclampsia and diabetes but more studies are needed to quantify this finding[38]. The increase of cytoplasmic vacuolation with heterogenous material might be due to intralysosomal proteolysis inhibition[39]. For the increased glycogen deposition, similar findings were revealed in a study for the effect of hyperthermia on the placenta of rats[40] This increase could represent a limitation of glycogen transfer to the fetal compartment or alteration of its metabolism inside the trophoblasts at the labyrinth zone[40].

In our study we revealed an increase in collagen fibrils deposition. Collagen play essential role during pregnancy, as it is capable of organizing the trophoblasts' biological behavior. Collagen protein regulate the adhesion, invasion and proliferation of these cells. Additionally, the abnormal collagen expressions and its fragments are linked to certain disorders during pregnancy such as; recurrent abortion, and preeclampsia[41]. Likewise, increased collaged deposition is revealed in the placenta of mice with diabetes[42].

The thickness of placental interhemal membrane is an essential parameter to placental function[42]. In the present work, a significant increase in the thickness of interhemal membrane in the placenta of Aspartame group was revealed. Increased thickness of interhemal membrane associated with increased distance of diffusion between the fetal and maternal blood. These alternation caused reduction of nutrient diffusion from the maternal circulation to the fetal blood leading to decrease the fetal growth and weight gain[25,43].

In the current work, immunohistochemical staining for VEGF showed an increased expression in the labyrinth zone in Aspartame group. Normal expression of VEGF supports that normal angiogenesis of placenta is occurring, which is important for successful pregnancy. Aspartame is accused to increase oxidative stress, which stimulate vascularization and abnormal angiogenesis resulting in alternation of the placental circulation and impairment of exchanges between maternal and fetal blood[44]. This was confirmed by, Alleva *et al*[45] who reported that Aspartame is a strong angiogenic factor that increases production of radical oxygen species (ROS), thus enhance secretion of a number of growth factors, from them is the VEGF and its soluble receptors by the endothelial cells.

Additionally, the VEGF is reported to stimulate proliferation of vascular smooth muscle at hypoxic cells[46]. On the other hand, it is stated that hypoxia which resulted from increased interhemal membrane thickness interfere with gases exchanges between fetal and maternal blood played essential role in increasing VEGF expression. Worth to mention that increased VEGF expression was reported in the labyrinth zone during studying the impact of diabetes on rats placenta[45].

The current study showed the ameliorative effect of Ginger against Aspartame by improving the thickness of the basal zones, labyrinth zones and interhemal membrane and also showed improvement in the expression of VEGF at labyrinth and better structure of placenta by light and electron microscope. This agrees with Abd El-Aziz *et al*[27] who reported that Ginger showed improvement of cadmium induced toxicity of kidney and liver in both mothers and their fetuses. Similarly, Badawy *et al*[22] reported that Ginger administration with gabapentin improve the morphological and skeletal anomalies induced by gabapentin.

All these ameliorative and protective effects of Ginger could be associated with its antioxidant characteristics[28]. Its antioxidant activity has been linked to its active phenolic component, especially, Gingerol, and shogaol[47]. Furthermore, Ginger showed to suppress oxidative stress by reduction of lipid peroxidation and protein damage as sources of free radical production and increase the expression of enzymes responsible for the antioxidant defense mechanism[28].

CONCLUSION

Aspartame has harmful impact on both pregnant rats and their fetuses. This was represented in our study, by the decreased fetal weight and length, placental weight reduction and alter normal placental structure. Co-administration of Ginger with Aspartame ameliorate its effect and improve all fetal and maternal parameters. Based on these results, we advise not to use aspartame and manufactured food containing it, even within a small dose, during pregnancy. Moreover, further studies are needed to be done on animals and human to assess the safety and benefits of Ginger during pregnancy.

ACKNOWLEDGMENTS

The authors are thankful to the Human Anatomy and Embryology Department, Faculty of Medicine, Alexandria University.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير الأسبارتام على مشيمة الجرذان البيضاء والتأثير الوقائي المحتمل للزنجبيل: دراسة نسيجية وماسخة

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الخلفية: تعمل المشيمة كحاجز يحمي الجنين من العوامل الضارة. الأسبارتام هو مُحلي صناعي يستخدم في جميع أنحاء العالم ، يمكن أن تعبر نواتج الأيض الخاصة به عبر المشيمة وتسبب تغيرًا في بنيتها. الزنجبيل نبات يستخدم في علاج القيء أثناء الحمل. تم الإبلاغ عن أن مستخلصاته لها تأثير مضاد للأكسدة.

هدف العمل: تقييم التأثيرات النسيجية المرضية والتأثيرات المسخية للأسبارتام على مشيمة وأجنة الجرذان البيضاء ودراسة التأثير الوقائي المحتمل للزنجبيل.

المواد والطرق: تم تقسيم خمسين جرذاً من الجرذان البيضاء إلى أربع مجموعات: المجموعة الضابطة: تلقيت الماء المقطر. تلقت مجموعة الأسبارتام ٢٦٥ ملجم / كجم من وزن الجسم من الأسبارتام ، وتلقت مجموعة الزنجبيل ٢٠٠ ملجم / كجم من وزن الجسم ، بينما تلقت مجموعة الأسبارتام-الزنجبيل ٢٦٥ ملجم / كجم من وزن الجسم من الأسبارتام ثم مستخلص الزنجبيل ٢٠٠ ملجم / كجم من وزن الجسم عن طريق الأنبوب الفموي في أيام الحمل من من ١ إلى ١٨. تم وزن المشيمة ومعالجتها للفحص النسيجي والهستوكيميائي للكشف عن عامل نمو بطانة الأوعية الدموية. تم وزن الأجنة وفحصها بحثًا عن التشوهات.

النتائج: أظهرت مجموعة الأسبارتام انخفاضاً ملحوظا في متوسط زيادة وزن الأم ووزن المشيمة ووزن وطول الجنين. بالإضافة إلى ذلك تم الكشف عن نقص في سمك المنطقة القاعدية وزيادة في سمك منطقة المتاهة. أظهرت هذه المجموعة تغيرا في تركيب المشيمة بالمجهر الضوئي و الإلكتروني ، وزيادة في سمك الغشاء البيني وزيادة ملحوظة في انتاج عامل النمو البطاني الوعائي في منطقة التيه في منطقة المتاهة. حسّن الزنجبيل جميع مؤشرات الأم والجنين وخفف من تأثير الأسبارتام على المشيمة.

الخلاصة: الأسبارتام له تأثير ضار على جنين ومشيمة الجرذان البيضاء ، وأن الزنجبيل يخفف من هذا التأثير.