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Protective effects of *Zingiber officinale* extract on myocardium and placenta against labetalol-induced histopathological, immune-histochemical, and ultrastructural alterations in pregnant rats

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Abstract

Background: Labetalol is an antihypertensive drug commonly used in obstetrics for both long-term treatment and the acute management of severe maternal hypertension. However, there have not been published articles about the effects of labetalol on the myocardium and the placenta. This study aimed to estimate the histological, immune-histochemical, and ultrastructural cardio- and placental-toxicity of labetalol administration and the effectiveness of ginger against this toxicity in pregnant rats. Labetalol was daily administrated orally with or without ginger at a dose of 300 mg/kg and 200 mg/kg, respectively, during the gestation days 6 to 20.

Results: In the labetalol-administrated group, the myocardium displayed histological and ultrastructure destructive changes and a significant increase in caspase-3 expression. Labetalol also decreased the placental weight compared with the control group, caused marked degeneration and disorganization of their architecture, and increased caspase-3 expression. Co-administration of ginger after labetalol highly ameliorates the adverse effect of labetalol on both cardiac and placental tissues.

Conclusions: It is concluded that ginger can mitigate cardiac and placental toxicity induced by labetalol administration into pregnant rats.

Keywords: Wistar rats, Heart, Placenta, Labetalol, Ginger, Caspase-3, Pregnancy

Background

Several classes of antihypertensive drugs have been investigated in treatment of hypertensive disorders during pregnancy including the β-adrenoceptor blockers [such as propranolol, metoprolol, oxprenolol, pindolol, labetalol and atenolol (Braunthal & Brateanu, 2019; Shekhar et al., 2016)], α-methyldopa (Leal et al., 2020), calcium channel blockers (Butalia et al., 2018), and hydralazine (Magee et al., 2003). The potential harmful effects of antihypertensive therapy on both mother and developing fetus, especially for β-blockers remains unclear. Labetalol is widely used for management of essential hypertension during pregnancy. However, labetalol has been demonstrated to cross the placental barrier and are present in varying concentrations in the fetal circulation (Khedun et al., 2000). The antihypertensive action of labetalol is considered to be due to its dual α- and β-adrenoceptor antagonism (Turbeville & Sasser, 2020). Labetalol therapy has many side effects including

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dizziness, gastrointestinal disturbances, tiredness, headache, scalp tingling, skin rashes, urinary retention and impotence. Other side effects related to the β-adrenoceptor blocking effect of labetalol, including asthma, heart failure and Raynaud's phenomenon, have been reported in rare instances (Podymow & August, 2011). Labetalol is used alone or together with other medicines to treat high blood pressure. The latter adds to the workload of the heart and the arteries. This can damage the blood vessels of the brain, heart, and kidneys, resulting in a stroke, heart failure or kidney failure (Campese & Krol, 2002). As with all β blockers, labetalol has negative inotropic effects and has the potential to cause acute left ventricular failure if given in sufficiently large enough doses to patients who have impaired function of the left ventricle (Facchini et al., 2015). Moreover, adverse cardiac effects related to β-adrenergic receptor blockade myocardial infarction and congestive heart failure (Rehsia & Dhalla, 2010). Recently study by El-Borm et al. (2021) reported that labetalol had severe pathological effect on the rat fetal heart. Other studies on different antihypertensive drugs during pregnancy suggested the negative effect of these drugs on the placenta as it can reduce the placental blood flow, placental weight and caused many pathological effects in the placenta (Furukawa et al., 2011; Said et al., 2019). Labetalol is designated as category "C," which states that human studies are lacking, animal studies are either positive for fetal risk or are lacking, and the drug should be given only if potential benefits justify potential risks to the fetus (Umans, 2007). So, according to the available knowledge, studies on the effect of labetalol on the architecture of the internal organs is needed.

Active oxygen species and free radicals play an important role in the pathogenesis of several human diseases, such as cardiovascular diseases, rheumatoid arthritis, and cancer (Bahorun et al., 2006). The antioxidant defense mechanism has been suggested to play an important role in maintaining the physiological levels of oxygen and hydrogen peroxide and eliminating peroxides generated from exposure to drugs. Medicinal plants, especially those with traditional use, have always been considered as a rich source of antioxidants (Galal et al., 2013). Ginger (*Zingiber officinale* Roscoe) belongs to the family *Zingeberaceaehas* has been used extensively in traditional medicine for treatment a wide range of health problems including respiratory disorders, stomach pain, nausea, asthma, and diarrhea (Grzanna et al., 2005). Ginger contained several bioactive compounds, including 6-gingerol, 6-shogaol, 10-gingerol, gingerdiones, gingerdiols, paradols, 6-dehydrogingerols, 5-acetoxy-6-gingerol, 3,5-diacetoxy-6-gingerdioal, and 12-gingerol, which contribute to its biological activities. However, the primary active compounds in ginger are gingerol and shogaol (Tanaka et al., 2015). Due to its bioactive compounds and constituents, ginger has shown various types of therapeutic effects, including antibacterial (Chakotiya et al., 2017), anti-inflammatory (Ezzat et al., 2018), antidiabetic (Alshathly, 2019), gastroprotective (Liju et al., 2015), antioxidant (Si et al., 2018), hepatoprotective (Beklar et al., 2020) and cardioprotective activities (Galal et al., 2013). The objective of the present study was, first, to study the cardiac and placental toxicity of labetalol, and second, to investigate the possible protective effect of ginger administration during pregnancy.

Methods

Labetalol

Labipress tablets (each tablet contains labetalol hydrochloride 100 mg) were manufactured by DBK for pharmaceutical industries, Cairo, Egypt. Tablets were ground and dissolved in distilled water (1 ml/rat) and orally administrated daily from the 6th to the 20th day of gestation. The applied dose was 300 mg/kg body weight which is equivalent to the recommended human dose (Mahmoud et al., 1993).

Water extraction of ginger

Ginger rhizomes were purchased from a local market at Shebeen El-Koom, Menoufia, Egypt. They were shade dried at room temperature and then crushed to powder. 125 g of powder was macerated in 1000 ml of distilled water for 12 h at room temperature and filtered. Accordingly, concentration of the obtained extract was 24 mg/ml and equal to 120 mg/kg (Kamtchouing et al., 2002). Ginger extract was orally given one hour after labetalol administration at a dose of 200 mg/kg body weight (Abd El-Aty & Morgan, 2011).

Animals

The study was carried out on 40 mature virgin females and 20 fertile males of Wistar albino rats (*Rattus norvegicus*), with weights 220 ± 10 g and ages 17 ± 1 weeks. Rats were purchased from Hellwan Farm, Ministry of Health, Cairo, Egypt. They were kept in well-ventilated cages at room temperature and under controlled conditions of ambient temperature and allowed free access to food and water at the animal house, Faculty of Science, Menoufia University. Mating was induced by housing females and males at a ratio of 2:1, respectively, overnight. Females were checked daily in the morning for the presence of copulatory plug and the presence of sperms in unstained native vaginal smears. The day at which vaginal smear was positive has been considered as the day zero of pregnancy. Day 20 was determined as the end point for experimentation (El-Borm et al., 2021).

Experimental design

The selected pregnant females were divided equally (10 in each group) into four groups as follows:

1. Control group was given orally distilled water (1 ml).
2. Ginger group given oral administration of ginger (200 mg/kg) (Abd El-Aty & Morgan, 2011).
3. Labetalol group given oral administration of labetalol (300 mg/kg) (Mahmoud et al., 1993).
4. Labetalol and ginger group received oral administration of labetalol first followed by ginger (200 mg/kg) 1 h later.

The experiment initiated from the 6th day (beginning of organogenesis) to the 20th day (late gestation) (Abdelrahman et al., 2017; Said et al., 2019). On the 20th day, the pregnant females were anesthetized using ether, killed, dissected, the heart and the placenta were removed, and the placenta was weighted.

Investigated parameters

Placental weight

The weight (g) of the placenta of both control and experimental groups was recorded.

Histological investigation

Specimens from the maternal heart were immediately fixed in 10% neutral formalin. Paraffin sections of 5 μm thickness were prepared and stained by Harris' hematoxylin and eosin for the histological study according to Suvarna et al. (2018). Histological sections were examined, and some representatives were photographed using Olympus microscope (BX41, Japan).

Immuno-histochemical investigation

Method of avidin–biotin peroxidase was carried out for the immune-histochemical demonstration of the pro-apoptotic antigen caspase-3 (Sternberger, 2006). The paraffin sections were incubated with the primary rabbit anti-caspase-3 overnight at 4 °C, and the primary binding was detected using a horseradish peroxidase-conjugated goat anti-rabbit antibody (Vector Laboratories, Burlingame, CA, USA) and visualized by development with 3,3-diaminobenzidine (DAB, Sigma). Then sections were countered stained using hematoxylin stain.

Image analysis

Digital images were analyzed by a semiquantitative scoring system (Fiji-Image J software, Java based application for analyzing images). The brown-stained immune-histochemical expressions of caspase-3 were analyzed in heart and placenta; the percentage-colored stained area (area fraction) per field area was determined by measuring six randomly photographed high-power fields (X400 magnifications) (Schindelin et al., 2012).

Transmission electron microscopic investigation

Small specimens of maternal heart and placenta were excised and rapidly fixed in 2.5% glutaraldehyde for 24 h at room temperature. Then the specimens were washed in 0.1 M phosphate buffer and post-fixed in 1% osmium tetra-oxide for 3 h at 4 °C. The tissues were washed in phosphate buffer several times, then dehydrated in ascending grades of ethanol and transferred to propylene oxide solution for clearing. The specimens were then infiltrated in a mixture of clearing agent and embedding medium and embedded in epoxy resins. Semithin sections of 1 μm thickness were stained with toluidine blue for examination by light microscope. Ultra-thin (50 nm) sections were cut, mounted on copper grids, and stained with uranyl acetate and lead citrate (Kuo, 2007). The grids were examined and photographed with JEOL electron microscope (TEM-1400Plus, Japan), Electron Microscope Unit, Alexandria University.

Data evaluation and statistical analysis

All data were calculated as means ± SEM for each group. The data were statistically analyzed for normal distribution (independent samples *T*-test) and homogeneity of variances (Levene's test) using statistical package of social sciences (IBM SPSS) statistics software for windows, Version 22 (IBM corp., Armonk, NY, USA). Differences were considered in significant whenever $P > 0.05$. The significances of the obtained data were classified into two categories, i.e., $P < 0.001$ and $P < 0.05$ according to the obtained *P* values.

Results

Heart

Histological observation of heart

Microscopic investigations showed normal histological structure of the cardiac tissue in the control group. The myocardium appeared striated, branched and arranged in groups. They were joined together with intercalated discs. The cardiac muscle fibers were separated by narrow intercellular spaces with blood capillaries. The cardiomyocytes exhibited homogenous acidophilic sarcoplasm with central elongated nuclei (Fig. 1a). Histological examination of the heart tissue of ginger group revealed somewhat normal appearance of the cardiac muscle (Fig. 1b).

Labetalol-treated group showed obvious histological changes in the form of disorganization and contraction of cardiac muscle fibers. Congestion and dilation of blood vessels, cellular infiltration and increased intracellular spaces between myocardial fibers were detected. Also, perinuclear vacuolation and pyknosis

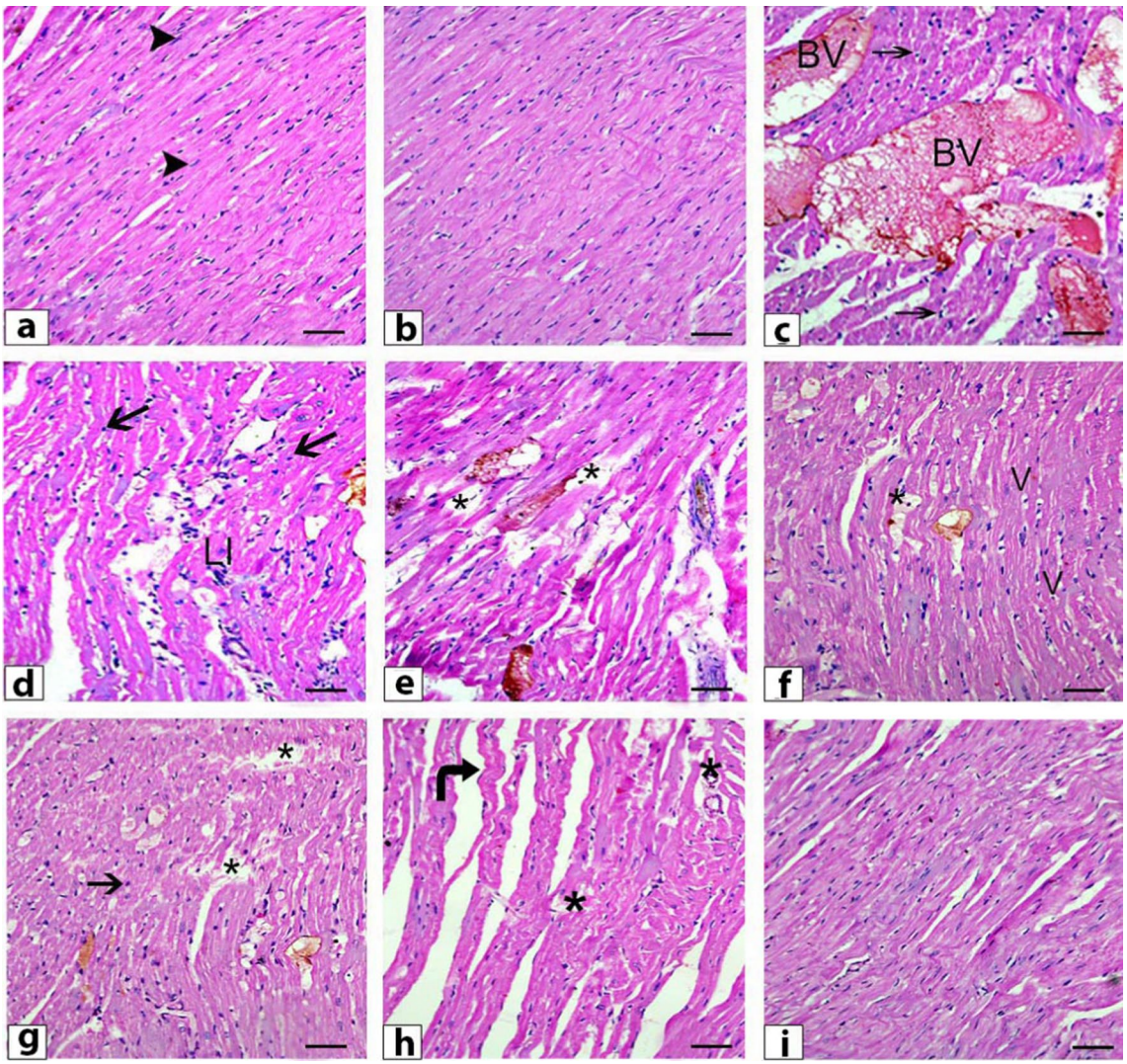

Fig. 1 Photomicrographs of longitudinal sections of the cardiac muscle of female rat. **a** Control group showing striated and branched myocardium with central nuclei (arrow head). **b** Ginger group. **c**–**h** Labetalol treated group showing congestion and dilation of blood vessels (BV), leuckocytic infiltration (LI), pyknotic nuclei (arrow), loss of cellular pattern (*), perinuclear vacuolation (V), wavy cardiac muscles (curved arrow) and widening of the endomysium. **i** Labetalol + ginger group showing restoration of the normal structure of the myocardium. (H&E, X400) Scale bar = 0.015 mm

were demonstrated. Necrotic areas which revealed loss of cellular pattern and fragmentation were noticed. The muscle fibers appeared wavy, separated and degenerated with widening of the endomysium (Fig. 1c–h).

On the other hand, tissue sections taken from the combined group, i.e., labetalol + ginger, exhibited an obvious recovery and restoration of the normal structure of cardiac tissue. The myocardium revealed minimal changes including little intercellular spaces in between muscle fibers (Fig. 1i).

Immuno-histochemical observation of heart

Examination of the expression of the pro-apoptotic protein marker caspase-3 in cardiac muscles of control and ginger administrated female rats showed very weak reaction. On the other hand, there was a highly significant increase in the optical density of caspase-3 positive cells in Labetalol-administrated rats compared with control group. However, combined group revealed mild immunostaining reaction compared with control

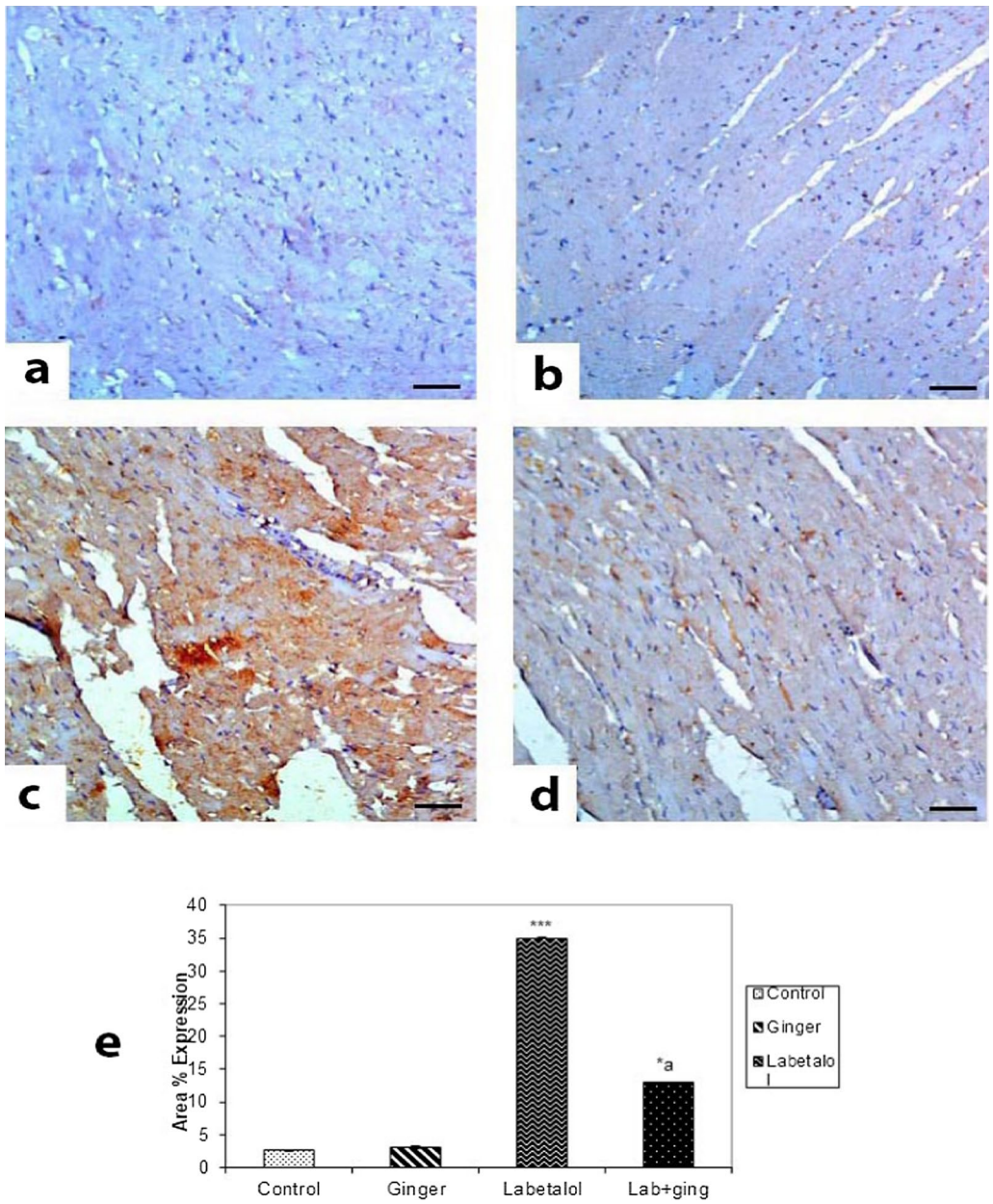

Fig. 2 Photomicrographs of longitudinal sections of cardiac muscles showing immune-histochemical caspase-3. **a** Control, **b** ginger, **c** labetalol-administrated and **d** labetalol + ginger-administrated groups. (X400) Scale bar = 0.015 mm. **e** Graph showing the mean area % of caspase-3 expression in the cardiac tissue of different groups. Data are represented as mean ± SEM. Asterisks (***$P > 0.001$, *$P > 0.05$) refer to the P value compared with the control group. a = highly significant ($P < 0.001$) compared with labetalol group

and highly significant decrease compared with labetalol group (Fig. 2a–e).

Ultrastructure investigation of heart

Examination of the cardiomyocytes of the control and ginger groups revealed the normal electron microscopic picture of the muscle fibers with oval euchromatic nucleus and a prominent nucleolus. The sarcoplasm showed myofibrils which limited by sarcolemma and arranged in striated bundles with alternating dark A and light I bands between the Z-lines. The myofibrils contained uniformly distributed mitochondria with closely packed cristae in rows and scattered glycogen granules in between them. The cardiac myocytes were joined together by intact intercalated discs with their transverse and lateral portions (Fig. 3a–e).

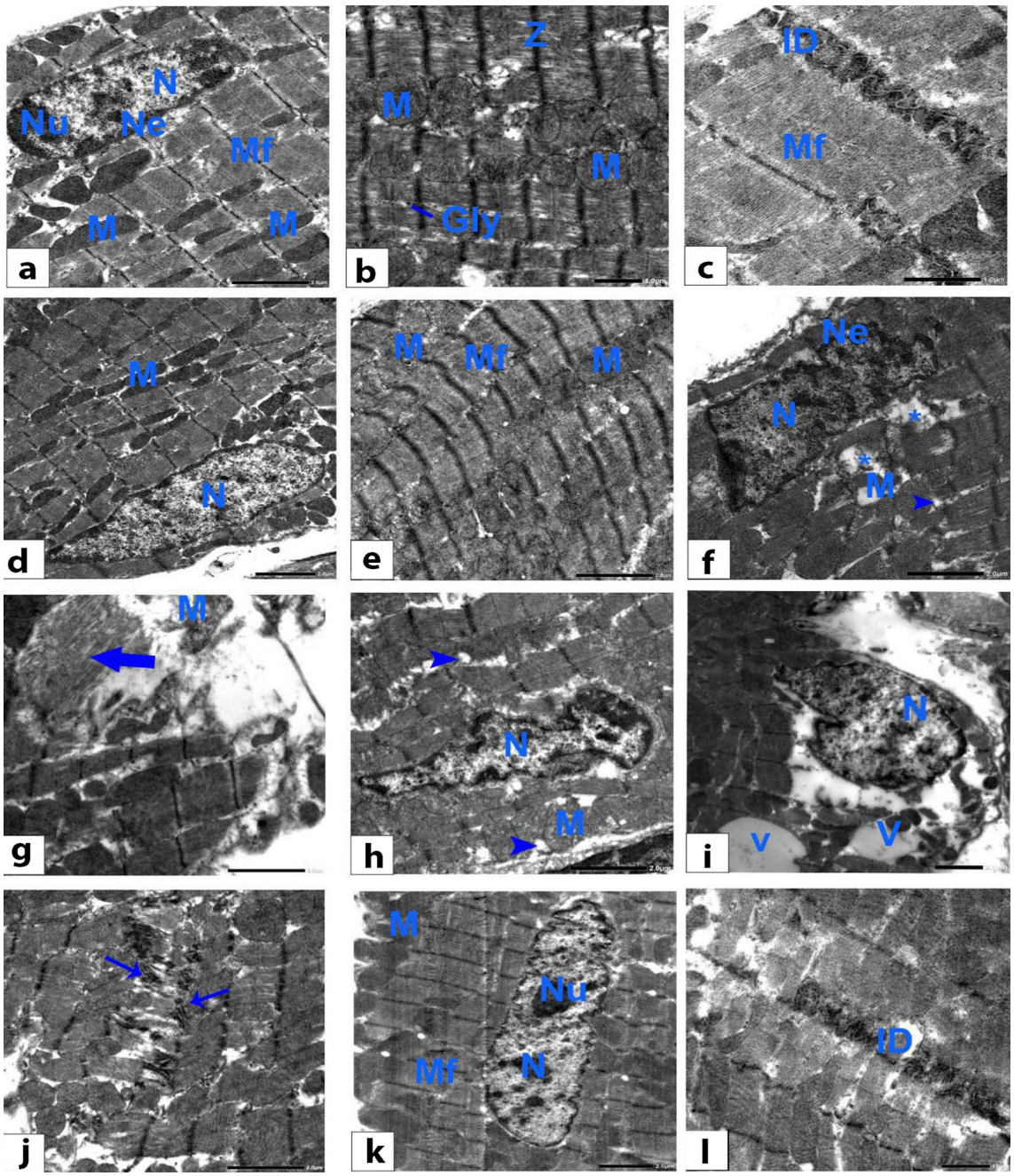

Fig. 3 An electron photomicrograph of the myocardium of female rats. **a**–**c** Control group showing oval euchromatic nucleus (N) with prominent nucleolus (Nu) and regular nuclear envelope (Ne), myofibrils striated in bundles with alternating dark A and light I bands between the Z-lines (Z), mitochondria packed in rows (M), glycogen granules (Gly) and intact intercalated discs (ID). **d**, **e** Ginger group. **f**–**j** Labetalol-treated group showing fragmentation and lysis of myofibrils (*), irregular, pyknotic and shrunken nuclei (N), destroyed mitochondria (M), collagen fibers (Thick arrow), dilated sarcoplasmic reticulum (arrow head), vacuolation (V), and destroyed intercalated discs (arrow). **k**, **l** Labetalol + ginger group. Scale bar = 2.0 µm for all except (**b**, **c**, **l**) = 1.0 µm

The cardiac muscle of labetalol-administrated group revealed focal damage and vacuolation of the cardiomyocytes. The nuclei showed irregular nuclear envelope with peripheral chromatin condensation, and the other appeared pyknotic and shrunken. The myocardium exhibited areas of fragmentation and lysis of some myofibrils. Moreover, the mitochondria showed disorganization and variety in size and some were destroyed.

In another section, the sarcoplasmic reticulum was dilated. Also, some collagen fiber bundles were noticed in the endomysium. The intercalated discs were highly destroyed and fragmented (Fig. 3f–j).

In the combined group, the cardiomyocytes revealed large euchromatic nuclei with well-defined nucleolus and dispersed euchromatin. The majority of myofibrils were well organized, while some appeared degenerated. Numerous mitochondria were recognized, and the intercalated discs with their transverse and lateral portion were intact (Fig. 3k, l).

Placenta

Placental weight

The placental weight of the labetalol-administrated group was highly significantly decreased compared with the control group, while there was highly significant increase in the placental weight of combined group compared with the labetalol group (Fig. 4).

Histological observation of placenta

The placenta of control rats was composed of three distinct zones, namely decidua basalis, basal or trophospongium zone and labyrinthine zone from the maternal to the fetal surface (Fig. 5a). The decidua basalis was consisted mainly of densely packed decidual cells embedded in homogenous ground substance. The trophospongium zone composed of mainly three types of cells which were giant cells (Fig. 5c), spongiotrophoblasts and glycogen cells which formed groups of small cell masses and develop into glycogen cell islands (Fig. 5d). The labyrinth zone consisted of fetal capillaries and scanty fetal mesenchyme, enveloped by a trichorial wall. Between the labyrinths are the maternal sinusoids (Fig. 5e). The placenta of the ginger-administrated group showed the same normal structure as control (Fig. 5b).

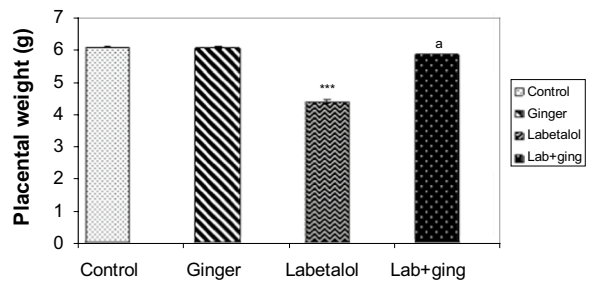

Fig. 4 Graph showing the mean weight of 20th day placenta of different groups. Data are represented as mean ± SEM. Asterisks (***$P > 0.001$) refer to the P value compared with the control group. a = highly significant ($P < 0.001$) compared with labetalol group

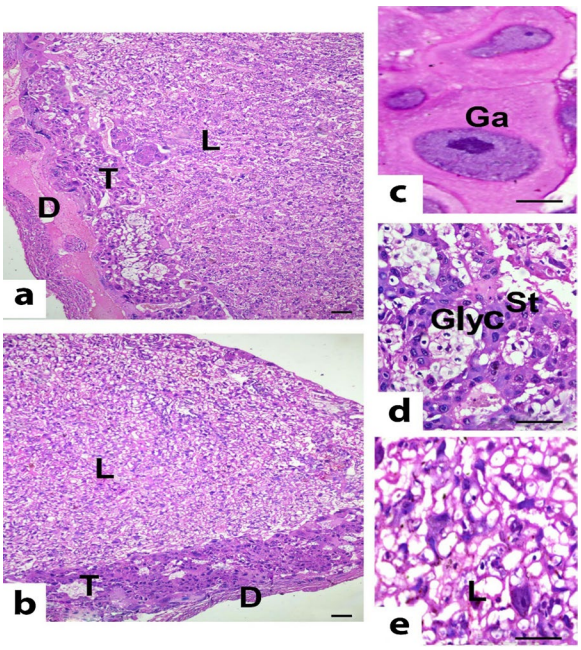

Fig. 5 Photomicrographs of transverse placental sections (GD 20) showing normal histological structure. **a** Control group. **b** Ginger group. (H&E, X400). **c**–**e** Higher magnification of **a**. Decidua basalis (D), Trophospongium zone (T), labyrinthine zone (L), Giant cells (Ga), Spongiotrophoblasts (St) and Glycogen cells (Glyc). (H&E, X1000) Scale bar = 0.059 mm (**a**, **b**) and 0.015 mm (**c**–**e**)

Light microscopic examination of the placenta of labetalol-administrated females showed marked degeneration and disorganization of their architecture (Fig. 6a). The cells of decidua basalis showed number of degenerative changes. Most decidual cells appeared fragmented with large vacuoles and pyknotic nuclei (Fig. 6b). The basal zone: The giant cells were reduced in frequency and size and had degenerating nuclei and their cytoplasm contained small vacuoles (Fig. 6c). Glycogen cells had cystic degeneration with cytoplasmic vacuolation, apoptotic cells with pyknotic nuclei, necrosis and hemorrhage between spongiotrophoblast cells (Fig. 6d, e). The labyrinth zone lost its normal architecture and showed congestion and extensive hemorrhage. Moreover, dilation of blood sinuses and disintegration of the barrier that separated the maternal blood from embryonic capillaries were observed. Degeneration of trophoblast cells and necrotic foci was also noticed (Fig. 6f, g).

The combined group displayed highly amelioration in the histological structure of the placenta after administration of ginger. The placenta appeared normal with its three zones. The glycogen cells, giant cells and labyrinthine wall restored their normal structure (Fig. 6h, i).

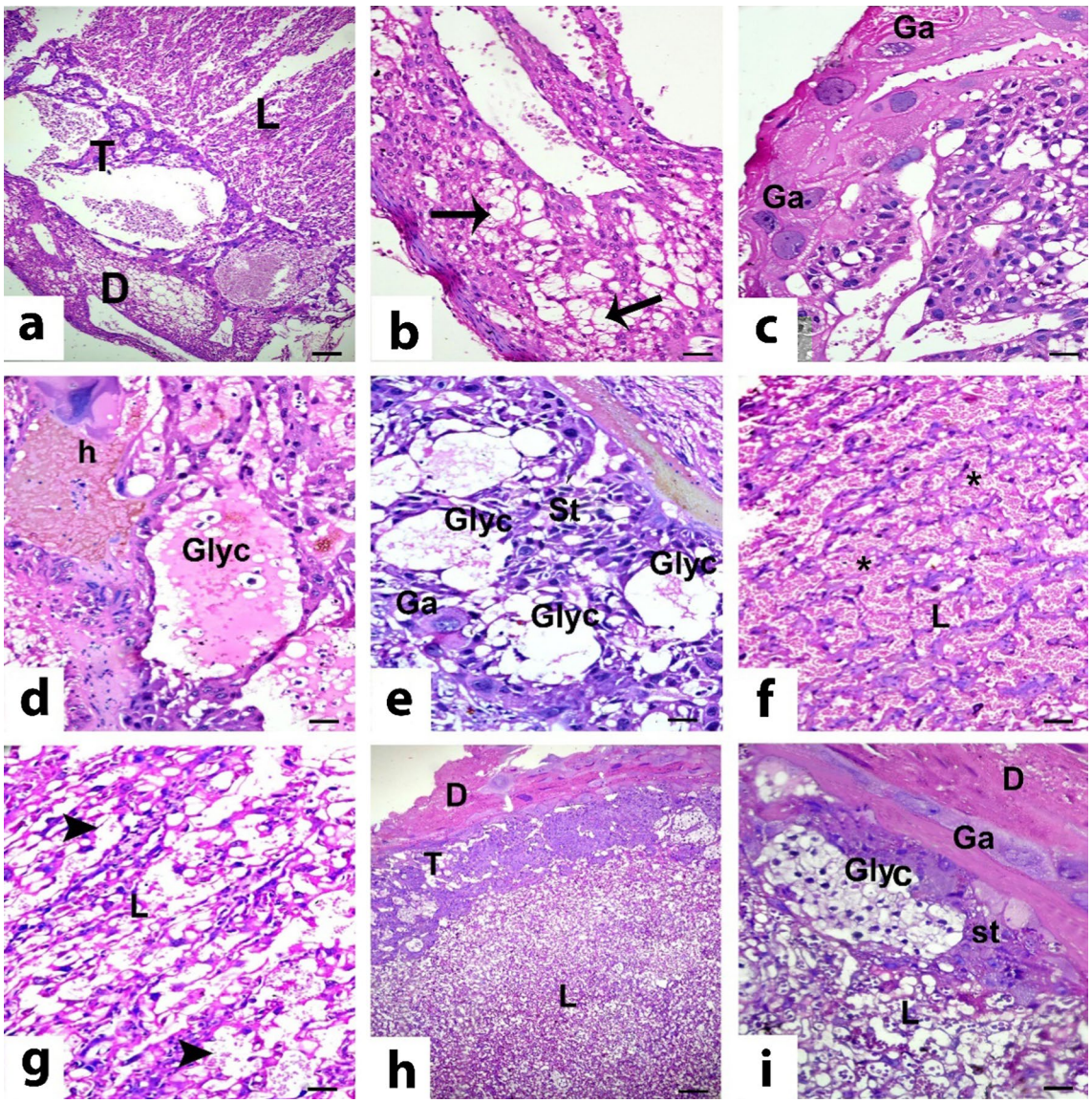

Fig. 6 Photomicrographs of transverse placental sections (GD 20) of placenta. **a**–**g** Labetalol group and **h**, **i** labetalol + ginger group showing: **a** degeneration and disorganization of decidua basalis (D), trophospongium zone (T) and labyrinthine zone (L); **b** fragmentation and vacuolation of decidual cells (arrow); **c** degenerated giant cells (Ga); **d**, **e** glycogen cells (Glyc) with cystic degeneration, vacuolation, pyknotic nuclei of spongiotrophoblasts (St), necrosis and hemorrhage (h); **f**, **g** labyrinth zone (L) lost its architecture, congestion (*), dilation of maternal blood sinuses (arrow head) and degeneration of trophoblast cells. **h**, **i** normal structure of the different placental zone. (H&E, X400) Scale bar = 0.059 mm (**a**, **h**), 0.015 mm (**b**–**g**, **i**)

Immuno-histochemical observation of placenta

Immuno-histochemical examination of the basal and labyrinth zones cells of placenta of the control and ginger groups showed mild reaction of caspase-3. The placental cells of the labetalol-administrated group revealed positive caspase-3 reaction which was highly significant compared with control group. On the other hand, administration of ginger with labetalol showed highly significant reduction of the caspase-3 immunoreactivity in the basal and labyrinth cells of placenta compared with labetalol group (Fig. 7a–e).

Ultrastructure investigation of placenta

Examination of control (Fig. 8a, b) and ginger Fig. 8c, d) administrated placenta by transmission electron microscope revealed normal structure. The labyrinth comprised a major part of placenta at 20th day of gestation; the labyrinthine wall between the maternal and fetal

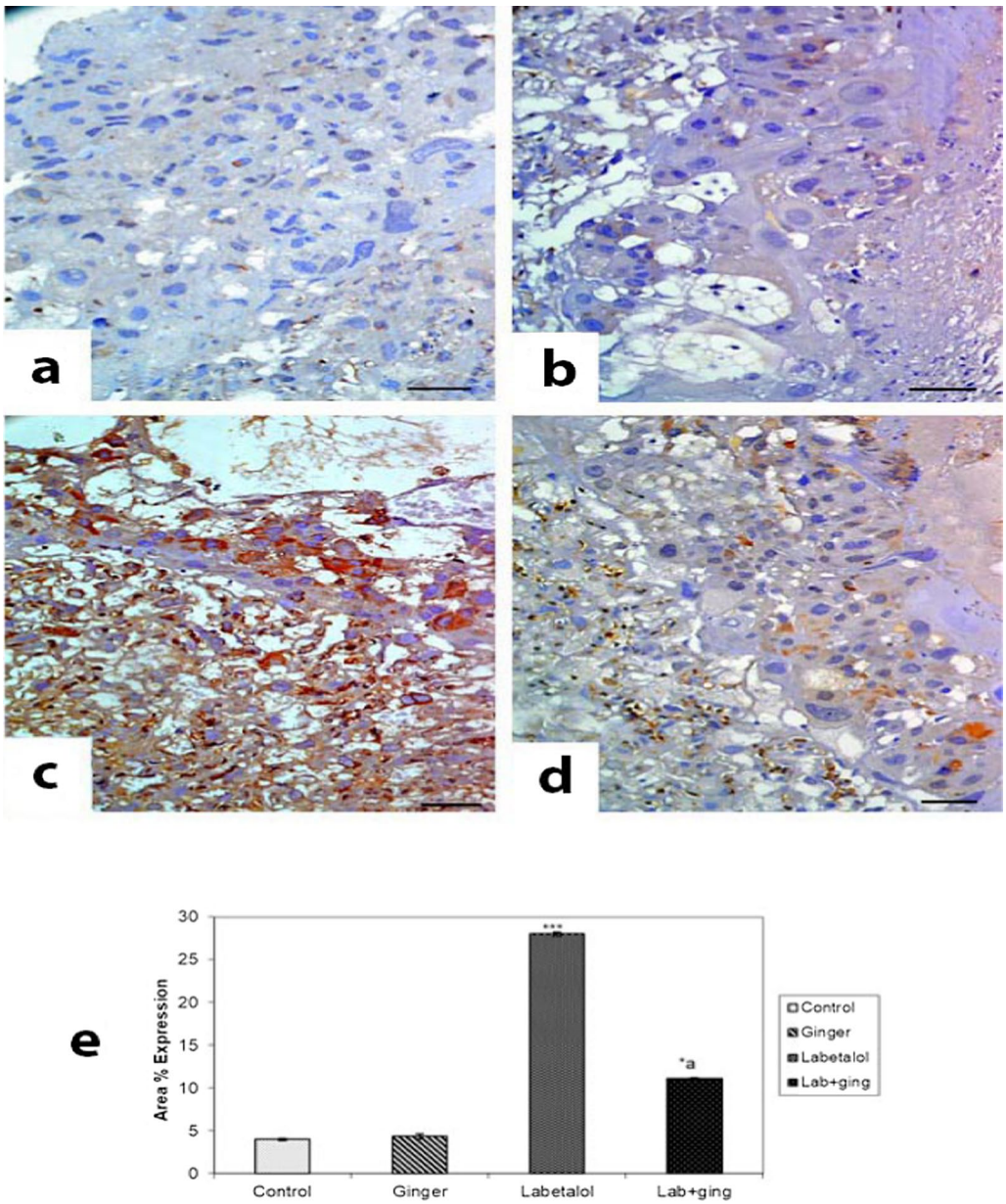

Fig. 7 Photomicrographs of transverse sections of the placenta showing immune-histochemical caspase-3. **a** Control, **b** ginger, **c** labetalol-administrated and **d** labetalol + ginger-administrated groups. (X400) Scale bar = 0.015 mm. **e** Graph showing the mean area % of caspase-3 expression in the placenta of different groups. Data are represented as mean ± SEM. Asterisks (***$P > 0.001$, *$P > 0.05$) refer to the P value compared with the control group. a = highly significant ($P < 0.001$) compared with labetalol group

circulations constituted of three trophoblast layers and endothelium of fetal capillaries. The layer I trophoblasts were cellular and called cytotrophoblasts which face the maternal blood. The cytotrophoblast was distinguished by its large nucleus and numerous microvilli. Underneath it, there were two layers of syncytiotrophoblasts (II & III). Basal laminae were located between the syncytiotrophoblast III layer and the fetal capillary endothelium. The spongiotrophoblast cells which comprised the main structural component of the basal zone showed normal structure with prominent euchromatic nuclei and homogenous cytoplasm.

Placenta of labetalol-administrated rats showed many destructive changes including irregular dilatation and fragmentation of maternal sinusoids which were filled with red blood cells. The endothelium-lined fetal

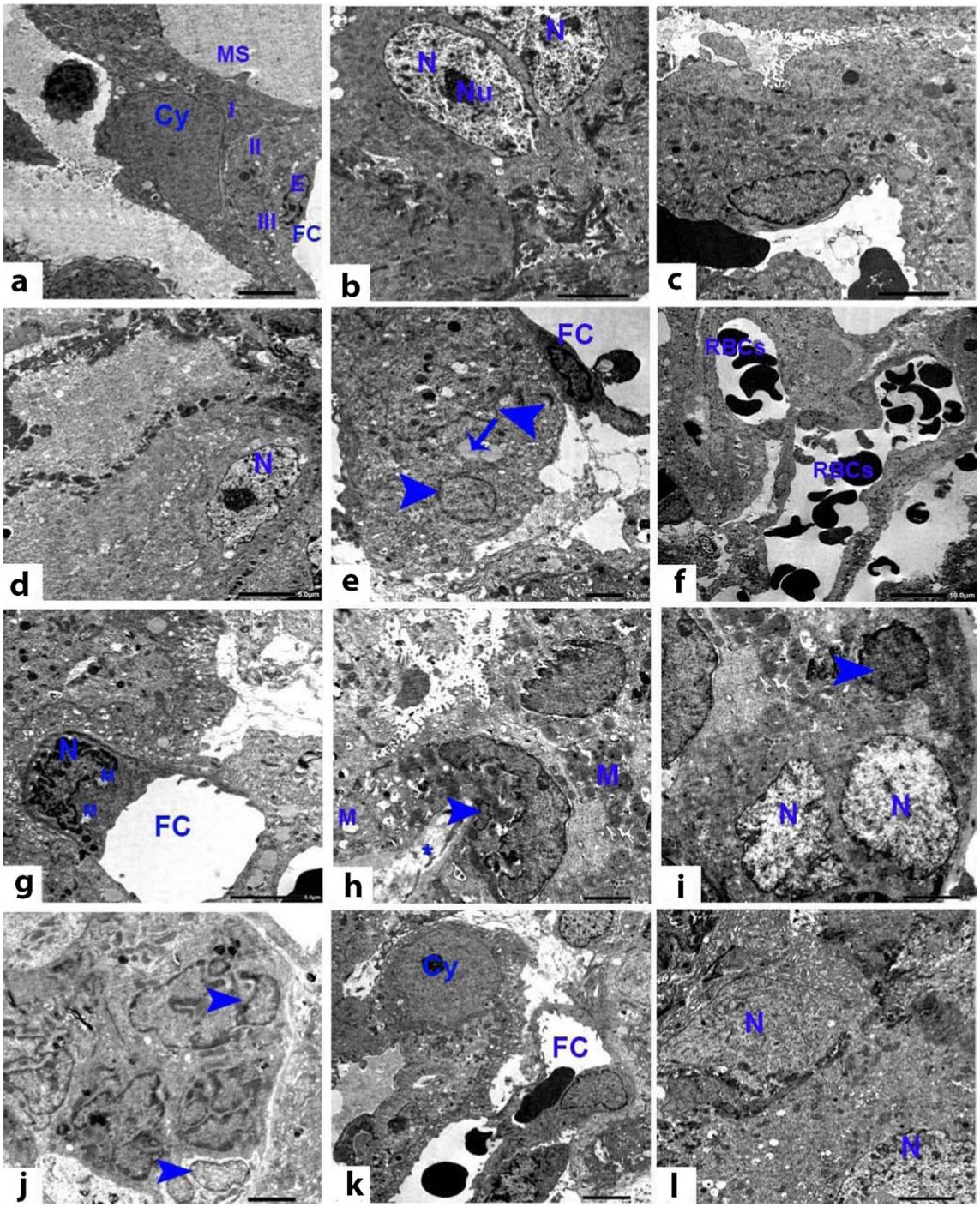

Fig. 8 An electron photomicrographs of the placenta (GD 20) of female rats: **a** Control labyrinth showing normal structure of the feto-maternal barrier, I, II and III trophoblastic layers, cytotrophoblasts (Cy), fetal capillary (FC), endothelium (E) and maternal sinusoids (MS); **b** control basal cells with normal euchromatic nucleus (N), Nucleolus (Nu) and homogenous cytoplasm; **c**, **d** normal labyrinth and basal cells of placenta of ginger group; **e**–**h** labyrinth cells of labetalol-administrated group showing many destructive changes including degenerated nuclei (arrow head) of the trophoblast of labyrinth wall, small droplets (Arrow), fragmentation of maternal sinusoids which filled with red blood cells (RBCs), rarified cytoplasm (*), many lysosomes, pyknotic nucleus (N) of endothelium of fetal capillary (FC) and degenerated mitochondria (M), **i**, **j** basal cells of labetalol-administrated placenta showing apoptotic spongiotrophoblast cells with pyknotic, vacuolated and fragmented nuclei (arrow head), **k**, **l** labetalol + ginger placenta showing normal structure. Scale bar = 5.0 µm (**a**–**d**, **g**, **k**, **l**), 10.0 µm (**f**), 2.0 µm (**e**, **h**, **i**, **j**)

capillaries revealed abnormal structure with pyknotic nucleus and degenerated mitochondria. The trophoblast cells of the labyrinth interhemal membrane showed degenerated nuclei which appeared fragmented or pyknotic. Other cells showed degenerated mitochondria, increase in the number of small droplets and lysosomes inside the rarified cytoplasm (Fig. 8e, h). Moreover, EM examination showed numerous apoptotic spongiotrophoblast cells with pyknotic, vacuolated and fragmented nuclei (Fig. 8i, j).

The placenta of the combined group showed no obvious degeneration in labyrinthine cells, fetal capillaries and spongiotrophoblast cells (Fig. 8k, l).

Discussion

The selection criteria for many antihypertensive drugs are somewhat unclear, and although vasodilator agents reduce the peripheral vascular resistance (e.g., methyldopa, hydralazine and labetalol) and have been accepted for general obstetric use, there are still many problems involved in calculating their benefits and potential hemodynamic hazards (Braunthal & Brateanu, 2019). Based on scarce information about the hazard effect of labetalol administration on tissue structure during pregnancy, this study described its effect on both heart and placenta of pregnant rats.

Histopathological results of the present study demonstrated that administration of labetalol during pregnancy induced many histological changes in the heart of the pregnant rats. The heart showed massive changes in the form of disorganization of cardiac muscle fibers, congestion and dilation of blood vessels, cellular infiltration, perinuclear vacuolation, pyknosis and necrotic areas. This result correlates well with the results of Goyal et al. (1996) who reported that atenolol treatment at a dose 10 mg/kg for six weeks in the diabetic rats showed distorted fibers with clustered nuclei, extensive vacuolation and disrupted intercalation myocardium. Moreover, Abed (2015) concluded that injection of one of β-blocker drugs as metoprolol at doses 10 and 15 mg/70 kg/b.wt caused histopathological lesions in the heart muscle of chicken embryos. This change includes infiltration of inflammatory cells, thickening in epicardium, vacuolation, edema, congestion in the blood vessels and hemorrhage. Similarly, El-Borm et al. (2021) reported that labetalol administration during organogenesis of rats caused many pathological changes in the fetal heart as hemorrhage, vacuolated cytoplasm, Pyknotic nuclei, fatty hydropic degeneration and mononuclear cellular infiltration between the muscle fibers.

Also, labetalol administration to pregnant rats in the present study caused highly significant increase in the pro-apoptotic protein marker caspase-3 of cardiac muscles. This is in agreement with our previous study which showed that labetalol administration during organogenesis caused an evident increase in the apoptotic cells of the fetal heart (El-Borm et al., 2021). Similarly, a study by Tea et al. (1999) has been found that a 4-week administration of different antihypertensive drugs such as renin-angiotensin system intervention and β-adrenergic blockade produced an increment of apoptosis.

As observed in the present study, labetalol was found to cause several ultrastructural changes in the cardiomyocytes. The nuclei appeared pyknotic and shrunken and the myocardium exhibited areas of fragmentation and lysis of some myofibrils. Also, some collagen fiber bundles were noticed in the endomysium. The intercalated discs were highly destroyed and fragmented. This is in agreement with Abdelmeguid et al. (2008) who found that irregular and raptured sarcolemma, myofibrils disorganization, decreased sarcomere length, raptured mitochondria, abnormal nuclei and hypertrophied Golgi elements of the myocardium were the most obvious ultrastructure changes after administration of antihypertensive drug (captropril) in mice. Also, Vulpis et al., (1994, 1995) reported that captropril-administration in both spontaneously hypertensive rats and Wistar-Kyoto rats (normal rats) caused ultrastructural alternation in myocardiocytes. Moreover, labetalol administration during rat organogenesis caused severe damage in myocardiocytes of 20-day fetuses, including extensive lysis of the myofibrils and disruption in the Z-line of fibrils, pyknotic, and irregular outlines of the nuclei, degeneration of mitochondria, vacuolation of the cytoplasm, degenerated intercalated discs, and accumulation of collagen fiber in some myocytes (El-Borm et al., 2021).

The placenta grows rapidly with high blood flow during pregnancy. Although the placenta is a temporary organ, it is an interface between the mother and the developing embryos. It performs multiple functions including anchoring the embryo to the uterine wall, barrier function, nutritional transport, gases exchange, endocrine action and drug metabolizing activity (Burton & Fowden, 2015). Consequently, placental injury have adverse effects on the maintenance of pregnancy and fetal development. Drug-induced histopathological changes in rat placenta are an important reason of developmental toxicity. Moreover, the detailed histopathological studies to the pathogenesis caused placental toxicity are considered to provide an important tool to understand the mechanism of developmental toxicity and teratogenicity with particular regard to embryo lethality and delayed development and could benefit reproductive toxicity studies (Furukawa et al., 2011).

The present study revealed that pregnant mother rats administrated with labetalol exhibited highly reduction in the placental weight and a marked histological and ultrastructure degeneration in the placenta. The cells of the placental three zones showed many destructive changes; the decidual cells were fragmented with large vacuoles and pyknotic nuclei. The giant cells were reduced in frequency with degenerating nuclei. Glycogen cells had cystic degeneration and cytoplasmic vacuolation. The spongiotrophoblast cells showed pyknotic nuclei, necrosis and hemorrhage between them. The labyrinth zone lost its normal architecture and showed congestion, extensive hemorrhage, irregular dilatation and fragmentation of maternal sinusoids. The trophoblast cells of the labyrinth showed degenerated nuclei and mitochondria. In addition, administration of labetalol caused highly significant increase in the caspase-3 expression in the three zones.

Chronic hypertension with or without treatment during pregnancy is considered a significant risk factor for adverse perinatal outcomes such as preterm delivery intrauterine growth restriction, small for gestational age, and intrauterine growth restriction (Orbach et al., 2013). A study by Gladstone et al. (1975) has been reported that perinatal problems including small placenta, fetal depression at birth and intrauterine growth retardation were associated with continuous antihypertensive propranolol therapy throughout pregnancy. Also, Furuhashi et al. (1991) showed that nifedipine (Ca antagonist) injection to normotensive Wistar Kyoto rats reduce the placental blood flow, fetal weight and placental weight. Placental weight reduction is a pathological effect observed as a small placenta. Degeneration, apoptosis and necrosis of trophoblasts, which were induced by direct placental injury or nonspecific effects associated with the conditions of an excessively unfavorable maternal environment, result in the inhibition of placental development, leading to a small placenta (Furukawa et al., 2011). Also, it has been found that atenolol (β-adrenoceptor antagonist) treatment in pregnancy resulted in impairment of placental physiologic function (Rubin et al., 1984). Moreover, a study by Said et al. (2019) has been reported that administration of verapamil (Calcium channel blockers) during gestation period (5th–19th) of rats caused evident decrease in the placenta weight and many histological changes including hypoplasia of the basal layer, cystic degeneration of glycogen cells, degenerated spongiotrophoblasts and giant cells, irregular dilation of maternal sinusoid with deposition of fibrin and necrotic trophoblasts cells of the labyrinth zone.

Placental necrosis macroscopically shows discoloration, thinning, or hemorrhage. In experimental animals, placental necrosis can be induced by many chemicals or drugs as ethanol (Akay & Kockaya, 2005), cadmium (Di Sant'Agnese et al., 1983), chlorpromazine (Singh & Padmanabhan, 1980), glucocorticoids (Graf et al., 1989) and streptozotocin (Padmanabhan et al., 1988). Histologically, placental necrosis appears more commonly in the trophoblasts of the labyrinth zone. There was a reduction in thickness and disruption of the trophoblastic septa and irregular dilatation of maternal sinusoids with hemorrhage (Furukawa et al., 2008a). Moreover, cystic degeneration of glycogen cells was induced by streptozotocin (Padmanabhan et al., 1988), chlorpromazine (Singh & Padmanabhan, 1980) and 6-mercaptopurine (Furukawa et al., 2008b).

Although placental apoptosis is believed to be a part of normal developmental placental aging (Smith et al., 1997), placental apoptosis is also increased in some drugs and chemicals administration (Furukawa et al., 2011). Trophoblast apoptosis led to a lack in the cell populations required for later normal histogenesis, resulting in a small placenta. It has been reported that glucocorticoids (Waddell et al., 2000), lipopolysaccharide (Ejima et al., 2000), T-2 Toxin (Doi et al., 2008), anoxia, and some anticancer drugs caused placental apoptosis (Katayama et al., 2002).

It has been known that overproduction of reactive oxygen species (ROS) plays an important part in the development of many chronic diseases (Poprac et al., 2017). Medicinal plants are rich in polyphenolic compounds and flavonoids which act as protective agents against chemical drugs due to their antioxidant characteristics (Ji et al., 2017). Based on scientific finding, dried ginger exhibited the strongest antioxidant activity, because the number of phenolic compounds was higher than that of fresh ginger. In addition, many investigations have demonstrated that ginger possesses other biological activities, including anti-inflammatory and cardiovascular protective activities (Mao et al., 2019).

The present study showed that administration of water extract of ginger to pregnant rats improves labetalol-induced pathological changes in the heart. The effectiveness of ginger in prevention of cardiac damage was studied by many investigators. A study by Humaish (2019) had been found that alcoholic extract of ginger without or with omega-3 improved doxorubicin-induced cardiac toxicity. Also, ethanolic extract of ginger (100, 200 and 400 mg/kg) pretreatment with isoproterenol-treated rats exhibits cardio-protective property in rats (Amran et al., 2015; Ansari et al., 2006). Elgendy (2016) found that ginger extract at a dose 400 mg/kg/day has moderate protective effect against isoproterenol-induced myocardial infarction and better protective effect when used with stem cells. Moreover, it has been found that ethanolic ginger extract at doses 0.5 g/kg/day or 1 g/kg/

day (Attyah & Ismail, 2012) and 600 mg/kg/day (Abas, 2017) protects the heart against the toxicity induced by cisplatin in albino rats. Ajibade et al. (2013) reported that water extract of ginger at doses 1 g/kg/day and 2 g/kg/day can improve the histological changes induced by Monosodium Glutamate in the cardiac tissue. It has been reported that 6-gingerol pretreatment decreased the damage in the cardiac tissue induced by doxorubicin administration in rats (El-Bakly et al., 2012). In addition, Subbaiah et al. (2017) concluded that ethanolic ginger extract at a dose 200 mg/kg could protect against alcohol-induced myocardial damage in albino rats. Also, Beklar et al. (2018) reported that 100 mg/kg/day of ginger extract improved the histopathological damages induced by diazinon in the heart tissue of the female Wistar rats.

In the present study, caspase-3 reaction showed that the apoptotic cells in the group administrated with ginger after labetalol were lower than the labetalol group. This is in agreement with Abas (2017) who reported that ginger extract reduced the elevation in caspase-3 level in the heart of cisplatin treated rats. Also, a study by El-Bakly et al. (2012) showed that pretreatment with 6-gingerol significantly reduced the cardiac caspase-3 in doxorubicin treated rats. Moreover, it has been reported that the hydroalcoholic ginger extract reduced the caspase-3 immunoreactivity induced by diazinon in rat liver (Beklar et al., 2020).

The ultrastructural focal damage in cardiomyocytes observed in labetalol group of this study was evidently ameliorated with ginger treatment. Our findings are supported by previous studies, who reported that ginger ameliorated the ultrastructure of cardiomyocytes in alcohol treated rats. The ginger property was evidenced by reducing the architectural damage, recovery of the nucleus, regeneration of myofibrils, and recovery of the intercalating disc (El-Borm et al., 2021; Subbaiah et al., 2017). Moreover, El-Hawwary and Omar (2019) reported that ginger administration at a dose 500 mg/kg/day led to marked improvement in the cardiac ultrastructure. Also, pretreatment with ginger at a dose 200 mg/kg/day partially prevented cyclophosphamide-induced cardiotoxicity in rats (Shalaby et al., 2019).

The weight, histology and ultrastructure of the placenta of the female rats administrated with labetalol of this study was evidently ameliorated with ginger treatment. This is in agreement with Abd El-Aziz et al. (2018) who reported that ginger administration at a dose 250 mg/kg b.wt. ameliorates the placental weight in cadmium induced toxicity in pregnant rats. Moreover, many medicinal plants used traditionally for treating wounds and retained placenta in animals (Luseba et al., 2007). Rezai et al. (2016) found that galbanum essential oil can protect placenta tissue against toxic effects of cyclophosphamide. Also, Kim et al. (2012) reported that administration of pine bark extract inhibits the toxic effects of cyclophosphamide on the rat placenta due to its potent antioxidant activities. In addition, extract of the plant *Chrysanthemum fantanesii* with vitamin E and C protects against valproate-induced embryo and placental toxicity in pregnant mice (Amrani et al., 2012).

Conclusions

It is concluded that labetalol administration into pregnant rats at the dose of 300 mg/kg caused cardio and placental toxic effects. However, ginger can mitigate these toxic effects when administrated at a dose of 200 mg/kg with labetalol. On the other hand, worldwide use of labetalol requires more accurate studies on the effects of this drug on the architecture of certain targeted organs of the body during pregnancy.

Abbreviations

Cy: Cytotrophoblasts; D: Decidua basalis; E: Endothelium; FC: Fetal capillary; Ga: Giant cells; Gly: Glycogen granules; Glyc: Glycogen cells; h: Hemorrhage; ID: Intercalated discs; L: Labyrinthine zone; M: Mitochondria; MS: Maternal sinusoids (MS); N: Nucleus; Ne: Nuclear envelope; Nu: Nucleolus; RBCs: Red blood cells; St: Spongiotrophoblasts; T: Trophospongium zone; V: Vacuolation; Z: Z-lines.

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Authors' contributions

EH contributed to the design and carried out the animal experiment, materials preparation data analysis, prepared figures, wrote and revised the manuscript. AM also prepared the materials, contributed to data analysis and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data generated during this study are included in this published article. Please contact authors for data.

Declarations

Ethics approval and consent to participate.

Principles of animal care and use were carefully followed during conducting the present study according to the guide for the care and use of laboratory animals approved by Faculty of Science, Menoufia University, Egypt (Approval No. MNSE2215), and according to the National Institutes of Health guide for the care and use of laboratory animals (NIH publications No. 8023, received 1978).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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