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Therapeutic Potential of Ginger Extract on the Embryotoxicity and Nephrotoxicity Induced by Labetalol in Rat Fetuses

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Abstract

Labetalol is a widely used antihypertensive drug, especially during pregnancy. However, its adverse effects on fetuses are unclear. This study aimed to evaluate the therapeutic role of ginger extract on embryonic and renal toxicity induced by labetalol in 20-day-old rat fetuses. Sixty pregnant rats were divided into four groups. Group I served as the control, group II received 200 mg/kg aqueous extract of ginger orally from the 6th to the 15th day of pregnancy, group III received 300 mg/kg labetalol orally over the same period, and group IV received labetalol and aqueous extract of ginger orally. At the end of the experiment, i.e. on the 20th day, renal specimens from the rat fetuses were processed for light and electron microscopic examination and flow cytometric analysis of the cell cycle and apoptosis. Additionally, embryotoxicity and morphological parameters were assessed. The labetalol group showed a significant increase in embryo resorption and high growth retardation in the 20-day-old fetuses. Fetal kidneys displayed renal corpuscles with shrunken, narrowed urinary spaces, podocyte affection, tubular cell degeneration, hemorrhage, and hyalinization of tubules. Flow cytometric analysis demonstrated G0/G1 phase cell cycle arrest and a significant increase in apoptosis and necrosis. Administration of ginger with labetalol prevented most of these morphological, histological, and molecular changes. Prenatal exposure to labetalol caused embryonic and nephrotoxicity when administrated during the organogenesis phase. Ginger ameliorated these toxicities.

Keywords: Ginger; Labetalol; Embryotoxicity; Nephrotoxicity; Apoptosis; Cell cycle.

1. Introduction

Labetalol is a combined α- and β-adrenoceptor blocker widely used to treat high blood pressure and long-term angina. This includes essential hypertension, hypertensive emergencies, and hypertension during pregnancy (Podymow and August, 2011). It is used alone or in combination to treat high blood pressure, which increases the workload of the heart and the arteries. This can damage blood vessels in the brain, heart, and kidneys, resulting in stroke or heart or kidney failure (Campese and Krol, 2002). Common side effects of labetalol include orthostatic hypotension, dizziness, headache, and nausea (Lee, 2003; Whelan et al., 2020). Serious side effects may include severe bradycardia, hypotension, cardiac impairment, bronchospasm, respiratory distress, hypoglycemia, cardiotoxicity, and hepatotoxicity (Rosenthal and Oparil, 2002; Grassin *et al.*, 2008; Whelan *et al*., 2020; El-Borm *et al*., 2021). Moreover, various β-adrenoceptor blocking agents are associated with perinatal mortality and fetal growth retardation (Petersen *et al*., 2012; Ersbøll *et al.*, 2014).

Ginger is a common natural spice widely used as a powder or a fresh root. It is a subtropical rhizome of the plant *Zingiber officinale* (*Z. officinale)* Roscoe (Zingiberaceae family) (Johari *et al.*, 2013). It contains several compounds, such as gingerol, shogaol, zingiberence, paradol, resin, starch, volatile oil, and vitamins C and A (Dhanik *et al.*, 2017; Kim *et al*., 2022). Ginger has antioxidant, antimicrobial, antiviral, gastroprotective, antidiabetic, antihypertensive, cardioprotective (El-Borm *et al.*, 2021), anticancer, and immunomodulatory effects (Dissanayake *et al.*, 2020).

Many studies advise using ginger as an effective treatment for nausea during the first and second trimesters of pregnancy (Bryer, 2005; Abu Baker, 2013). Moreover, ginger treatment during pregnancy does not increase the risk of congenital malformations, stillbirth, perinatal death, preterm birth, or low birth weight (Weidner and Sigwart, 2000; Heitmann *et al.*, 2013). Furthermore, the therapeutic role of ginger against renal damage has been demonstrated (Tzeng *et al.*, 2013; Gholampour *et al.*, 2017; Pratap *et al.*, 2017; El-Bahr *et al.*, 2022).

In this study, we investigated whether co-administration of an aqueous extract of ginger with labetalol to pregnant rats prevented labetalol-induced embryotoxicity and nephrotoxicity in 20-day-old fetuses. We measured the mother's body weight and percentage of embryonic absorption, fetal morphology, histopathological examination of the fetal kidney using light and transmission electron microscopy, and apoptosis rates and cell cycle analysis using flow cytometry.

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2. Materials and Methods

2.1. Preparation of ginger water extract

Fresh ginger rhizomes (*Z. officinale*) were purchased from a local market at Shebeen El-Koom, Menoufia, Egypt, and were shade-dried and crushed into a powder. 125 g ginger powder was soaked in 1000 ml distilled water for 12 h at room temperature and filtered to obtain an aqueous extract (Kamtchouing *et al.*, 2002). The extract concentration was 24 mg/ml.

2.2. Animals

The principles of animal care and use of laboratory animals guide approved by the Faculty of Science, Menoufia University, Egypt (Approval No. MUFS/F/EM/1/20) and the National Institutes of Health guide for the care and use of laboratory animals (NIH publications No. 8023, received 1978) were carefully followed while conducting this study. Sixty healthy mature virgin females and 30 fertile males of Wistar albino rats (*Rattus norvegicus*) weighing 160 ± 10 g and aged 17 ± 1 weeks were purchased from Helwan Farm, Ministry of Health, Cairo, Egypt. Before the study, the animals were housed in plastic cages under hygienic conditions with good ventilation in the animal house of the Faculty of Science, Menoufia University. They were kept under controlled ambient temperature (25°C ± 2°C) and lighting (12 h light/dark cycle) and were allowed free access to water and food. Two weeks before the experiment, females were checked daily to determine the estrous cycle stage. Only animals in the persistent anestrus (diestrus) stage were included in the experiment. Mating was induced by housing females and males overnight at a ratio of 2:1. The initiation of pregnancy was determined when the vaginal copulatory plug was present, and the vaginal smear was positive.

2.3. Experimental design

The pregnant females were divided equally into four groups (15 in each group) as follows:

- Group I (control group) received 1 ml of distilled water.
- Group II (ginger group) was administrated an aqueous extract of ginger (200 mg/kg) (Abd El-Aty and Morgan, 2011).
- Group III (labetalol group) was administrated labetalol (300 mg/kg). Labipress tablets (each containing labetalol hydrochloride 100 mg) were manufactured by DBK for pharmaceutical industries (Cairo, Egypt). The tablets were ground and dissolved in distilled water (Mahmoud *et al.*, 1993).
- Group IV (labetalol + ginger group) received labetalol (300 mg/kg) followed by ginger (200 mg/kg) 1 h later.

All groups were orally administrated distilled water, labetalol, and/or ginger via an intragastric tube from the 6th to 15th day of gestation (Badawy *et al*., 2019a). By the end of the experiment, i.e. on the 20th day of gestation, the pregnant rats were euthanized and dissected. The whole uterus was removed, weighed, and photographed with a digital camera. Embryos were excised from the uterus, and those that were alive were euthanized and subjected to gross examination to investigate morphological abnormalities under a dissection microscope. The morphological assessment was carried out by counting the number of implants, live, resorbed, and dead fetuses.

2.4. Investigated parameters

2.4.1. Morphological parameters

The crown-rump length (cm) of fetuses and body weight (g) of both the mothers and their fetuses were recorded.

2.4.2. Histopathological investigation

For examination with a light microscope, fetal kidney specimens were fixed in 10% formalin to prepare paraffin blocks. 5-µm sections were cut using a rotary microtome (IHC World, China), stained with hematoxylin and eosin (Suvarna et al., 2018), and photographed with an Olympus microscope (BX41, Japan).

2.4.3. Ultrastructural investigation

For transmission electron microscope (TEM) analysis, fetal kidney specimens were separated, immediately fixed in 2.5% glutaraldehyde, and manipulated according to the method by Kuo (2007) as described in El-Borm *et al.* (2021). Examination and photography were done using the JEOL electron microscope (TEM-1400Plus, Japan), Electron Microscope Unit, Alexandria University.

2.4.4. DNA fragmentation assay

DNA extraction was performed according to the method by El-Garawani and Hassab El-Nabi (2016) and described in detail in Sakr *et al.* (2014).

2.4.5. Cell cycle analysis

Fresh fetal kidney samples were transported in isotonic saline and prepared according to the method of Reichard and Asosingh (2018). The material was washed with isotone Tris EDTA buffer, 3.029 g of 0.1 M Tris (hydroxymethyl aminomethane, 1.022 g of 0.07 M sodium chloride, and 0.47 g of 0.005 M EDTA. These were dissolved in 250 ml distilled water. Then, the pH was adjusted to 7.5 with 1 N HCl. The samples were centrifuged at 1800 rpm for 10 min, and the cells were fixed in approximately 1 ml of ice-cold 96%–100% ethanol (BDH) for each sample.

After at least 12 h of fixation, the samples were centrifuged again, and excessive ethanol was removed. 200 µl of cell suspension in citrate buffer was placed into a 15-ml Falcon tube. Then, propidium iodide (PI) was added. The samples were filtered through a 30-Mm pore diameter nylon mesh filter to eliminate nuclear clumps into another 5-ml tube (12 × 75 mm, cat. no.2058, Falcon). The samples were run in a flow cytometer within 1 h after adding PI. The flow cytometer used in the current study was Accuri C6 (Becton Dickinson, Sunnyvale, CA, USA), equipped with a compact air-cooked low-power 15 mwat argon ion laser beam (488 nm). Data analysis was conducted using the DNA analysis program MODFIT (v.2.0).

2.4.6. Annexin-V/PI dual staining assay

The cell suspensions (1 mL) in phosphate buffer were resuspended in 2ml 1x binding buffer (1ml of 10x buffer+99ml dist. H2O) then 100 µl of cells suspensions were added to 5µl of annexin-V (Cat. No.556547 BD pharmingen FITC apoptosis Kit) then directly 5 µl PI (PE label) was added. The cells were resuspended in 200 µl 1X

binding buffer and immediately analyzed by the flow cytometer Accuri C6 (Becton Dickinson, Sunnyvale, CA, USA).

2.5. Data evaluation and statistical analysis

All data were expressed as mean ± standard error of the mean (SEM). The differences between groups were tested by analysis of variance (one-way ANOVA) using SPSS for Windows v.22 (IBM Corp., Armonk, NY USA), followed by an LSD test for multiple comparisons. $P < 0.05$ was considered statistically significant. The significance of the obtained data was classified into two categories, $P < 0.001$ and $P < 0.05$.

3. Results

3.1. Body weight of mothers

Fig. 1a displays the changes in body weight gain of the mothers. The mothers that received ginger exhibited a gradually progressive increase in body weight, similar to the control group. On the contrary, the body weight of the labetalol group gradually decreased until the 15th day of gestation. The weight slowly decreased until the 18th day, after which the body weight gradually increased slightly until the 20th day. The mothers in the labetalol + ginger group exhibited a gradual increase in body weight but lower than that of the control group.

3.2. Morphology of the uteri

The uteri of the control and ginger groups had a healthy, bright appearance and normal fetal implantation between the two horns. Four pregnant rats from the labetalol group showed some resorbed sites, while two from the same group showed resorption of all fetuses, giving the uterus a dark color. The labetalol + ginger group had an improved number of fetuses and the appearance of the uteri (Fig. 1b).

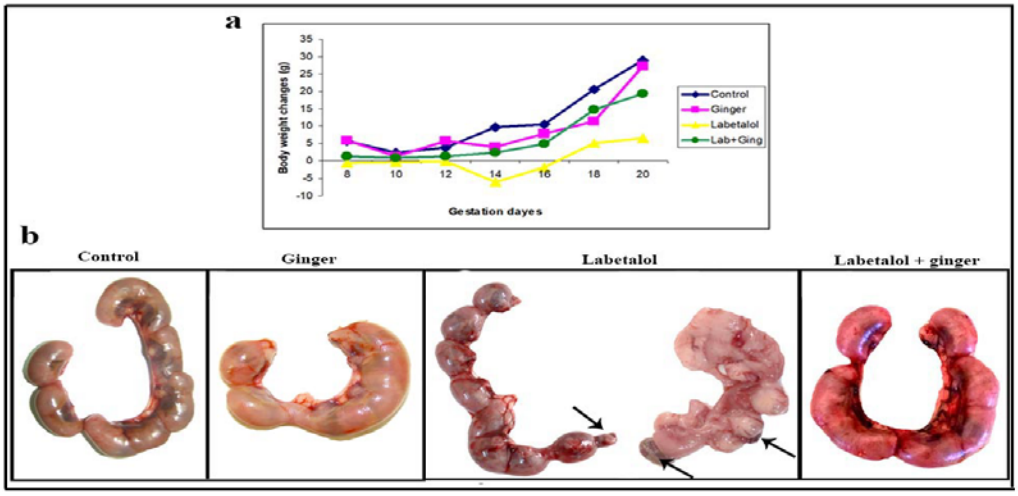

Figure 1: (a) Graph showing changes in the body weight gain of mothers in different groups. **(b)** Photographs showing different uteri of pregnant rats at the 20th day of gestation. Control and ginger groups showed normal appearance and implantation. The Labetalol group showed partial or complete resorption of fetuses (arrow). The Labetalol+ginger group showed normal implantation.

3.3. The average weight of the uteri and the percentage of resorption

As shown in Table 1, there was no significant difference in the uterine weight of pregnant rats from the ginger group (53.5 g) compared with that of the control group (51.95 g). In contrast, the average weight of the uteri from pregnant rats administrated labetalol showed a highly significant decrease (16.83 g) compared to the control group (51.95 g). Meanwhile, the average weight of the uteri of the combined group exhibited a highly significant increase (43.25 g) when compared with that of the labetalol-only group (16.83 g) and a slight decrease compared with that of the control group (51.95 g).

The control and ginger groups showed no embryonic resorption in their uteri, while the uteri with partial or complete resorption in the labetalol group recorded 40%. However, the percentage of uteri with resorption in the combined group decreased to 13.3% compared with that of the labetalol group (Table 1).

Table 1: Average weight of uteri and the percentage of resorption at the 20th day of gestation.

Groups	Total no. of sacrificed rats	Uteri		With resorption C%	Without resorption C%
		Average weight of uteri (g)	C%		
Control	15	51.95±0.025	0 (0%)	0 (0%)	15 (100%)
Ginger	15	53.5±0.013	1.55 (2.9%)	0 (0%)	15 (100%)
Labetalol	15	16.83±0.12***	-35.1 (67.6%)	6 (40%)	9 (60%)
Labetalol+ Ginger	15	43.25±0.065*a	-8.7 (-16.7%)	2 (13.3%)	13 (86.6%)

C%= percentage of change compared with control.
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.

3.4. Body weight and crown-rump length of the fetuses

The body weight and crown-rump length of fetuses from the ginger-treated group showed insignificant changes compared with controls. Compared with controls, there was a highly significant decrease in the weight and length of fetuses from the labetalol group. However, the fetal weight and length of the combined treatment group showed a highly significant increase compared with the labetalol-only group and a slightly significant difference compared with controls (Figs. 2a–c).

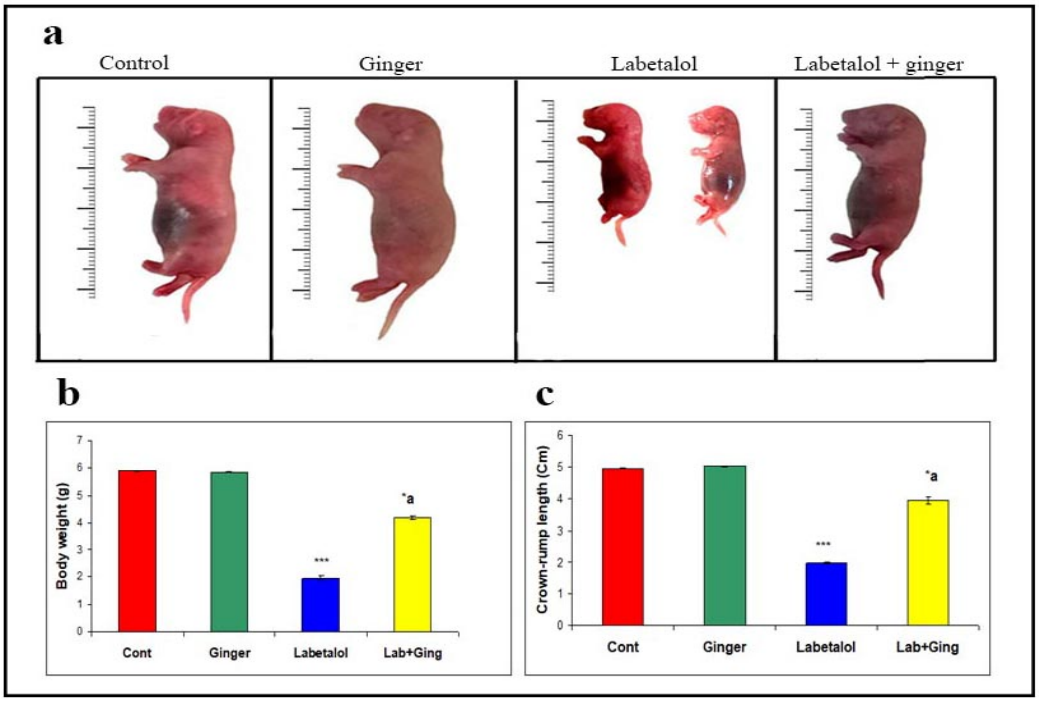

Figure 2: (a) Photographs of fetuses aged 20-day (n=40/group). **(b)** graph of the body weight of the fetuses. **(c)** graph of the crown-rump length of the fetuses in different groups. Asterisks (*** P< 0.001, * P< 0.05) refer to P values compared with the control group. a= highly significant (P<0.001) compared with the labetalol group.

3.5. Histopathological observation

The renal cortex of the control fetuses displayed two zones: the subcapsular and the juxtamedullary zones. The subcapsular zone contained immature renal corpuscles, while the juxtamedullary zone contained mature renal corpuscles and proximal and distal convoluted tubules. Medullary rays were observed as extending between the two zones (Fig. 3a). The ginger group showed a normal structure, like that of the control group (Fig. 3b).

The fetal kidney of the labetalol group exhibited degenerated renal corpuscles, which appeared shrunken with increased preglomerular space. Moreover, the proximal and distal convoluted tubules showed lumen dilation and epithelium vacuolation (Figs. 3c and 3d). Hemorrhage, hyalinization of tubules, hypertrophy of renal glomeruli, and tubular cell degeneration were also seen (Fig. 3e–g). The kidneys of the combined-treatment group showed improvement; the glomeruli and tubules regained their normal appearance, and there were no areas of obvious degeneration (Fig. 3h).

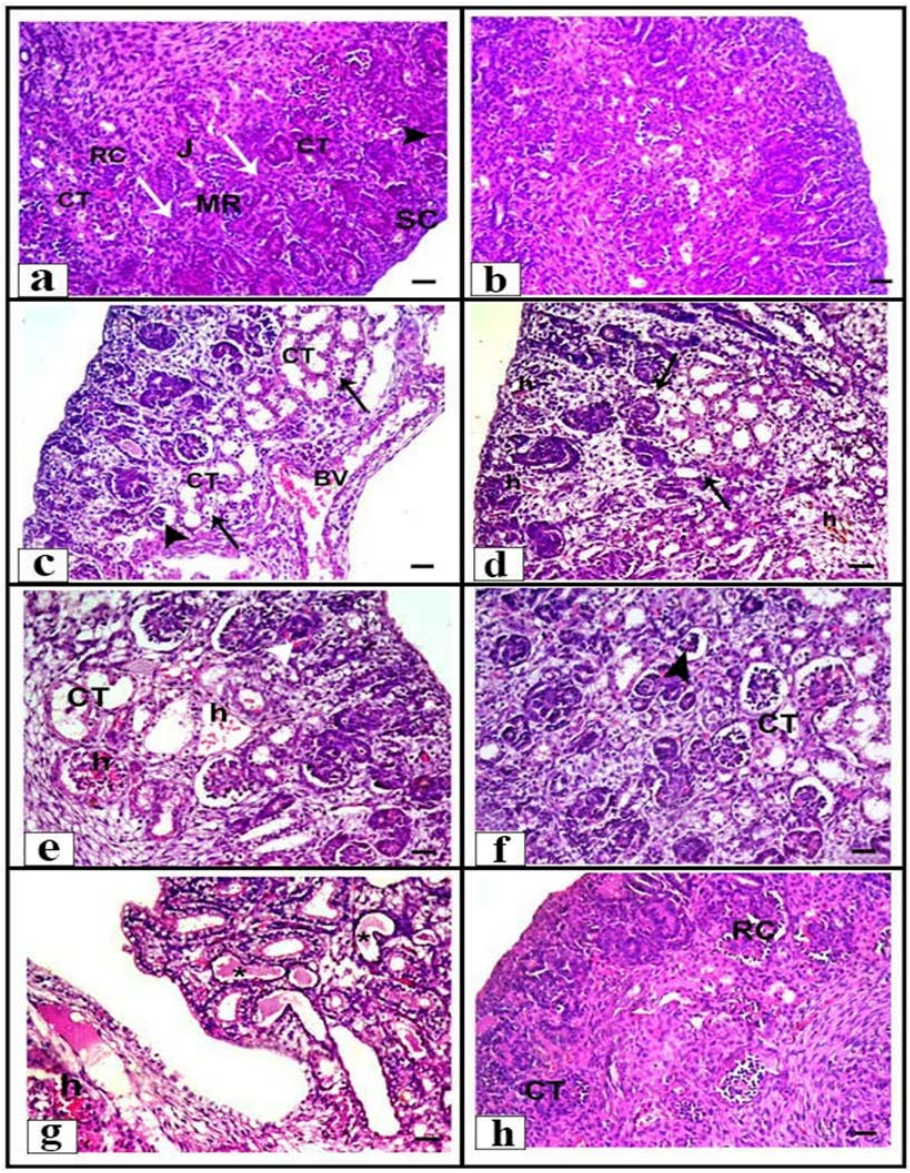

Figure 3: Photomicrographs of transverse histological sections in the 20-day-old fetal kidney. **(a)** control showing subcapsular zone (SC), immature forms of renal corpuscles (Arrowhead), the juxtamedullary (J), mature renal corpuscles (RC), convoluted tubules (CT), medullary rays (MR), nephrogenic mesenchyme (White Arrow), **(b)** ginger showing the normal structure, **(c-g)** Labetalol showing disrupted glomeruli (arrowhead), dilated and congested blood vessel (BV), degenerated convoluted tubules (CT), vacuolation in the epithelium of the convoluted tubules (Arrow), hemorrhage (h), congested renal corpuscles (White Arrowhead), shrinkages in the glomeruli (arrowhead) and tubular hyalinization (Star) **(h)** Labetalol + ginger group showing renal corpuscles (RC) and the convoluted tubules (CT). (H&E) Scale bare = 15µm.

3.6. TEM observation

The renal cortex of control fetuses showed normal glomeruli containing podocytes with many foot processes, a glomerular basement membrane, and a wide urinary space (Fig. 4a). The proximal convoluted tubule cells rested on a thin basal lamina and contained a basal euchromatic nucleus with a prominent nucleolus, an intact nuclear envelope, and a well-formed apical brush border. The cytoplasm was electron-dense with numerous rod-like mitochondria of various sizes between the basal folds (Fig. 4b). The distal convoluted tubules contained large basal euchromatic nuclei, numerous mitochondria, thin basal lamina, and few microvilli at the apical surface (Fig. 4c). The renal cortex of fetuses from the ginger group had the same structure as those of the control group (Fig. 4d–e).

The fetal renal cortex of the labetalol group showed many degenerative changes, including glomerular basement membrane thickening and urinary space narrowing. The podocyte nuclei were electron-dense with an irregular nuclear envelope (Fig. 4f). The proximal tubule cells showed electron-dense and shrunken nuclei, a degenerated brush border in some areas, and swollen mitochondria. The basal membrane enfolding was destroyed (Fig. 4g–h). Other cells appeared vacuolated with a ruptured cell membrane and basal lamina (Fig. 4i). Most nuclei were fragmented with electron-dense shapes (Fig. 4j). The cells lining the distal convoluted tubules exhibited various degrees of nuclear damage. The nuclei were irregular and ruptured in some places with chromatin clumps, while others were fragmented (Fig. 4k).

The convoluted tubule cells from the combined-treatment group showed normal cytoplasm and organelles, an intact brush border, euchromatic nuclei, mitochondria with preserved crista, and a thin basal lamina (Fig. 4l).

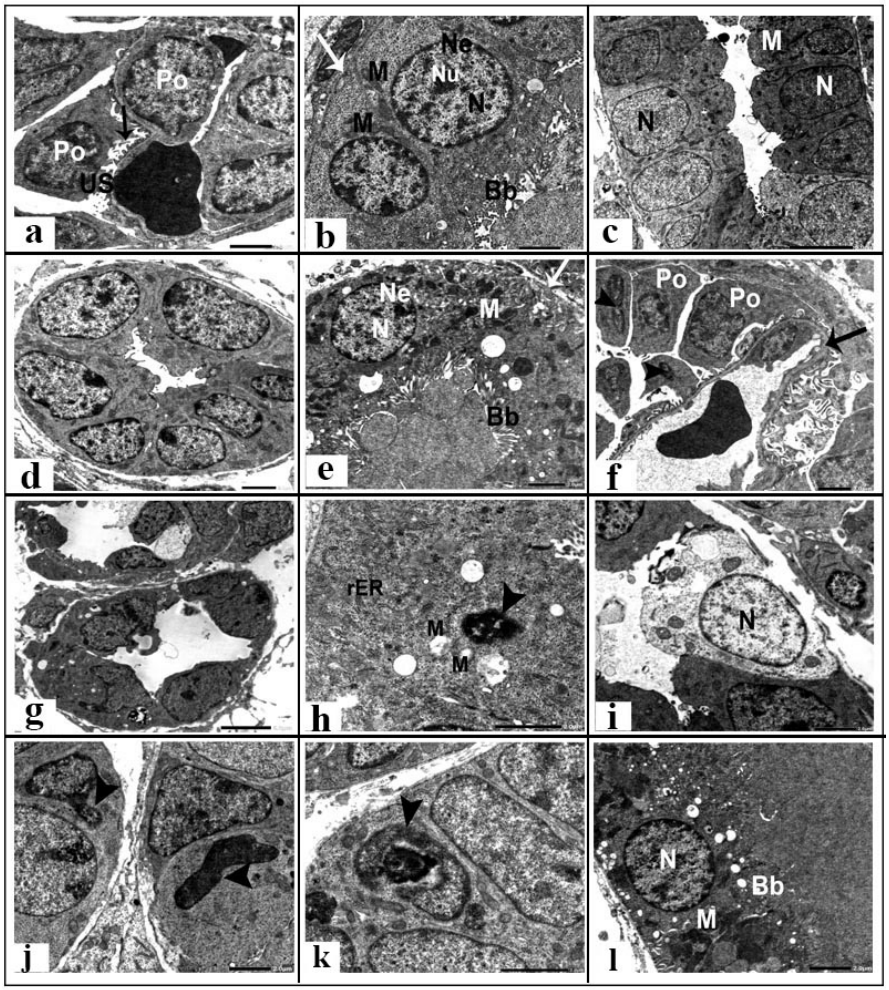

Figure 4: Transmission electron micrographs of fetal renal cells. **(a-c)** control group showing podocytes and their foot processes, thin basement membrane, proximal convoluted tubule cells and distal convoluted tubule cells. **(d-e)** ginger group. **(f-k)** labetalol group showing degenerated podocytes, degenerated proximal convoluted tubules, and degenerated distal convoluted cells. **(l)** labetalol + ginger group. podocytes (Po), basement membrane (Arrow), visible urinary space (US), apical brush border (Bb), euchromatic nucleus (N), nucleolus (Nu), nuclear envelope (Ne), mitochondria (M), rough endoplasmic reticulum (rER), degenerated and pyknotic nuclei (Arrowhead). Scale bare = 2µm for all except (**c** and **g**) =5µm.

3.7. DNA fragmentation

Administration of labetalol caused marked DNA damage in the renal cells of fetuses. In Fig. 5, lane 3 shows the migration of DNA fragments compared with the control and ginger groups (lanes 1 and 2, respectively). Meanwhile, the administration of ginger after labetalol resulted in less DNA fragmentation than in the control and ginger groups (Fig. 5).

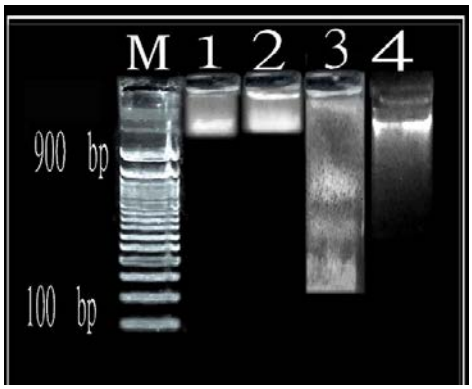

Figure 5: Photomicrograph of an agarose gel showing an evident variation of the DNA fragmentation in the renal tissue extract of 20-days-old rat fetuses treated with labetalol with/without ginger. bp: base pair, M: marker DNA (100 bp DNA Ladder, New England Bio-labs, Ipswich, MA, USA), 1: control, 2: ginger, 3: labetalol, and 4: labetalol + ginger.

3.8. Cell cycle distribution

Flow cytometric analysis of PI-stained renal cells of the labetalol group showed the accumulation of most cells in the G0/G1 phase (80.8%). In comparison, the fraction of cells in the S phase was non-significantly increased (17.6 %) compared with that of the control (14.8%) and ginger groups (17.6) (Table 2 and Fig. 6). The percentage of cells in G2/M revealed a highly significant decrease compared with that of controls (2.7% and 20.8% for the labetalol and control groups, respectively). However, co-administration of ginger with labetalol caused a highly significant reduction in G0/G1 phase cells (69.1%) and a highly significant increase in cells in the G2/M phase (15%) compared with the labetalol group (Fig. 6 and Table 2).

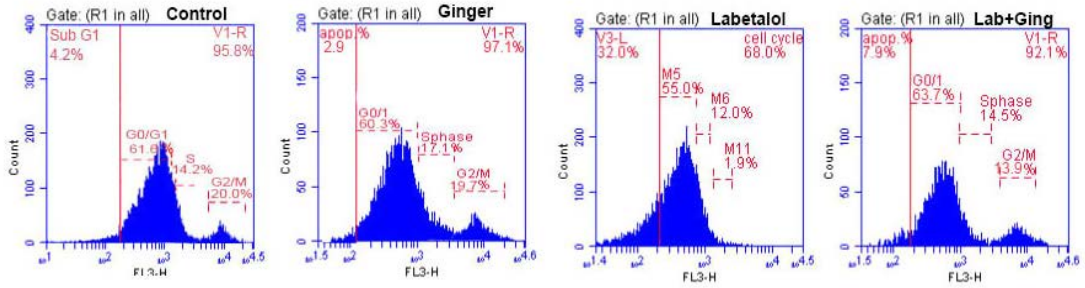

Figure 6: Representative flow cytometry graphs of cell cycle distribution of renal cells.

Table 2: Percentage of total apoptosis and cell cycle distribution in renal cells of 20-day-old fetuses.

Cells% / Groups	% Sub G1 (Apoptosis)	% No. Cells/Cell cycle	% cells analyzed G0/G1	S	G2/M
Control	4.2±0.01	95.8±0.15	64.3±0.08	14.8±0.11	20.8±0.06
Ginger	2.9±0.08	97.1±0.13	62.1±0.21	17.6±0.09	20.2±0.03
Labetalol	32.0±0.01	68.0±0.02	80.8±0.012***	17.6±0.03	2.7±0.138***
Lab+Ginger	7.9±0.05	92.1±0.17	69.1±0.12a	15.7±0.14	15.0±0.043*a

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.

3.9. Dual detection of apoptosis by Annexin-V/PI

Annexin V/PI analysis of fetal renal cells from the ginger group showed that most cells were viable (95.4%). On the other hand, the labetalol group showed a 59.1% reduction in the number of viable cells and a highly significant increase in the apoptosis and necrosis rates (24.4% and 16.5%, respectively) compared with the control group, as shown in Figs. 7a and b. Administration of ginger after labetalol caused a noticeable amelioration (86%, 8.2%, and 5.8% for viable, apoptotic, and necrotic rates, respectively) compared with those of the labetalol group.

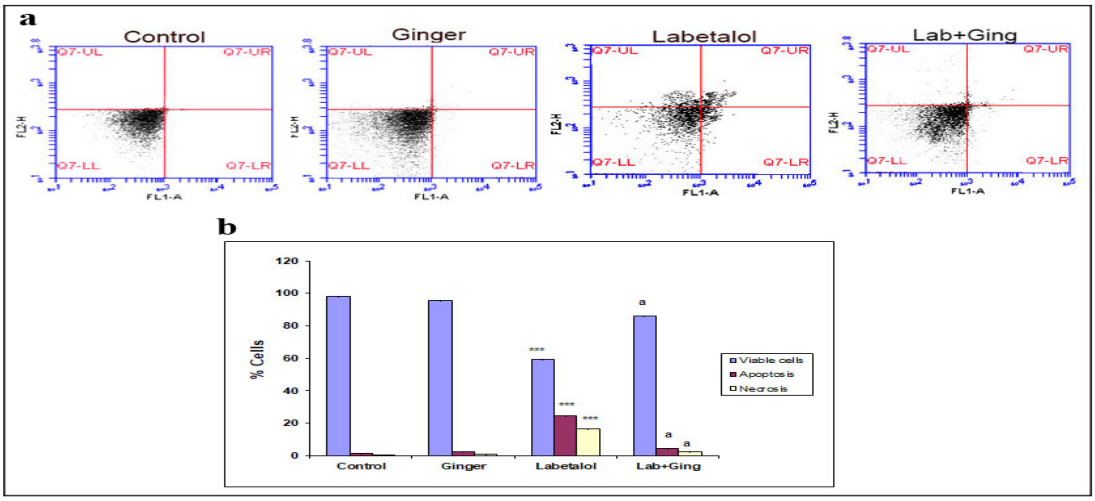

Figure 7: (a) Fluorocytograms of fetal renal cells; (FL1-A) = Annexin V +ve cells and Y-axis (Fl2-H)= PI-labeled cells. The lower left portion (Q7-LL) of the fluorocytogram (-ve for both stains) shows viable cells, whereas the lower right portion (Q7-LR) (+ve for Annexin) shows early apoptotic cells, the upper right portion (Q7-UR) (+ve for both stains) shows late apoptotic cells and the upper left portion (Q7 UL) (+ve for PI) shows necrosis. **(b)** Graph showing the percentage of viable, apoptotic, and necrotic renal populations in experimental groups. Asterisks (*** P< 0.001) refer to P values compared with the control group. a= highly significant (P<0.001) compared with the labetalol group.

4. Discussion

As observed in this study, all females treated with labetalol survived but showed significant weight loss compared with the control group. The reduction in maternal body weight may be due to reduced food intake or the developmental toxicity of the drug as displayed by reduced weight of the gravid uterus due to reduced mean fetal weight and the increased incidence of early and later resorption. Alternatively, this may be due to metabolic disorder in the mothers (Bateman *et al.,* 2012). Our study shows that treated rats displayed an asymmetrical distribution of fetuses in the two uteri horns and a reduced uterine weight. Also, the uterine horns displayed clearly visible embryonic resorption sites. An increase in resorption sites resulted in a significant reduction in uterine weight compared with the control group.

Oral administration of labetalol to female rats from the 6^{th} day until the 15^{th} day of gestation induced growth retardation, represented by a decrease in fetal body weight and length. β-blockers, especially atenolol, have been linked to fetal growth retardation when given early in pregnancy (Lydakis *et al.,* 1999). Other studies showed that β-blocker use during pregnancy increases the risk of fetuses that are small for gestational age, premature birth, perinatal mortality, and neonatal hypoglycemia (Petersen *et al.,* 2012; Ersbøll *et al.,* 2014), but some studies have reached the opposite conclusion (Heida *et al.,* 2012).

In contrast, a study by Rashidi *et al.* (2012) displayed that a carvedilol (5 mg/kg) injection at the 9^{th} to 11^{th} day of gestation in pregnant rats had protective effects on cleft palate induced by caffeine in rat fetuses. Injection of nifedipine and amlodipine at three dose levels each (5, 10, and 20 mg/kg, and 0.5, 1, and 2 mg/kg, respectively) showed no developmental toxicity in rat fetuses (Ramadan and Ashry, 2010; Jaiswal *et al.,* 2019). Also, Broulõk *et al.* (2001), reported that losartan (2 mg/kg b.wt) and enalapril (0.4 mg/kg b.wt) exerted no significant effects on body weight.

Although β-blocker exposure may increase the risk of small for gestational age, stillbirth, and growth retardation, the reasons remain unclear. Most β-blockers are thought to cross the placenta (Lennestål *et al.,* 2009), which is associated with various adverse effects, including intra-uterine growth retardation, neonatal respiratory depression, bradycardia, and hypoglycemia (Bateman *et al.,* 2012; Ishibashi *et al.,* 2017). A mechanism of β-blockers on placental hemodynamics has been proposed, suggesting that the decrease in placental blood flow is due to the selective vasoconstriction of placental vessels caused by the effect of β-blockers, which also leads to the absence of intrinsic sympathomimetic activity (Liu *et al.,* 2019). Other mechanisms might contribute to fetal bradycardia caused by β-blocker exposure, negatively affecting fetal cardiac output and development. The negative inotropic and chronotropic effects of β-blockers can reduce maternal cardiac output, affecting fetal growth (Gelson *et al.,* 2011). This effect could, at least in part, explain fetal growth retardation.

Labetalol induced many histological and ultrastructure changes in the kidney tissue of rat fetuses. Seleem (2016) observed similar effects, finding that verapamil caused atrophy and shrinkage of the renal corpuscles and degeneration of the kidney tubules. Also, Sánchez *et al.* (2008) showed that administration of losartan during pregnancy resulted in severe renal abnormalities in both newborn and one-week-old animals. Moreover, Swelim and Sakr (2004) demonstrated that injection of captopril to pregnant mice resulted in degenerative changes in the renal tissue.

In contrast, a study by Abdelhamid et al. (2019) reported that carvedilol (20 mg/kg), nebivolol (10 mg/kg), and nadolol (50 mg/kg) have a mild protective effect on the histopathological changes in the kidneys of hypertensive rats. Additionally, carvedilol and nebivolol have protective effects against nephropathy and acute renal failure in rats (Akgullu *et al.,* 2015; Atwa *et al.,* 2016). Similarly, captopril (60 mg/kg/day) had ameliorative effects on the renal cortex of chronic hypertensive rats (Rezk and Ibraheim, 2017). Furthermore, captopril had a protective effect against cisplatin nephrotoxicity (Gad *et al.,* 2016), and amlodipine acted against gentamicin-induced nephrotoxicity (Abdel-Rahman and Kandeel, 2012).

Many studies have shown that antihypertensive drugs induce apoptosis and cell cycle arrest (Koyama *et al.,* 2014; Oura *et al.,* 2017). Similarly, we showed that according to flow cytometric analyses, labetalol administration during organogenesis induced cell cycle arrest at the G0/G1 phase and a noticeable increase in apoptosis and necrosis rates in renal cells.

These findings were further corroborated by Attiq *et al.* (2018), who reported that carvedilol and celecoxib had genotoxic and cytotoxic effects on peripheral blood mononuclear cells. Losartan-treated rats at a dose of 15 mg/kg per day showed an increased percentage of cells in the G1 cell cycle phase in vascular smooth muscle cells (Bravo *et al.,* 2001). Also, Salman *et al.* (2011) reported that antihypertensive drugs such as clonidine, methyldopa, amlodipine, ramipril, and rilmenidine have a toxic effect on DNA in rat uterus tissue. This genotoxicity and cytotoxicity may be attributed to the overproduction of free radicles caused by labetalol administration, which impairs the innate antioxidant defense system of cells. This, in turn, induces a different type of DNA damage, cell cycle arrest, and apoptosis (Finkel and Holbroork, 2000).

This study confirms the ameliorative activity of ginger against the toxic developmental effects of labetalol, represented by the increase in maternal weight and fetal weight and length. This agrees with Abd-Allah and Sharaf El-Din (2013), who reported that ginger at a dose of 200 mg/kg had an ameliorative role against a methotrexate-induced reduction in body weight. Similarly, El-Aziz *et al.* (2018) concluded that injection of *Z. officinale* (250 mg/kg) before cadmium chloride resulted in a noticeable improvement in the body weight and gravid uterine weight of pregnant rats. Moreover, Faried *et al.* (2013) indicated the effectiveness of ginger extraction in regaining body weight loss in diabetic rats.

Injection of ginger tea during the organogenesis of rats resulted in no maternal toxicity or morphologic malformations (Wilkinson, 2000). Additionally, Weidner and Sigwart (2000) investigated the effect of *Z. officinale* in different doses (100, 333, and 1000 mg/kg/day) on organogenesis in rats, and no developmental or maternal toxicity was observed. Moreover, Yon *et al.* (2012) reported that [6]-gingerol has a protective effect against ethanol-induced teratogenicity during mouse

embryogenesis *in vitro*. Furthermore, Badawy *et al.* (2019b) demonstrated that the administration of ginger from the 6th to 15th day of gestation in rats reduced the incidence of malformation and growth retardation induced by gabapentin. Also, it increased the mother's body weight and uterine weight.

This study revealed that ginger significantly ameliorated the histological and ultrastructural effects induced by labetalol in fetal renal tissue. Many recent studies confirmed the therapeutic effect of ginger against nephrotoxicity (Dawood *et al.*, 2022; El-Bahr *et al.*, 2022). Ali *et al.* (2020) reported that the ethanolic extract of *Z. officinale* (200 mg orally for 8 weeks) had a protective effect on the renal damage induced by mercuric chloride in rats. Moreover, Badawy *et al.* (2019a) reported that ginger extract at a dose of 200 mg/kg had a protective effect against gabapentin-induced nephrotoxicity in rat fetuses. Similarly, a study by Mohammad *et al. (*2013) indicated that ginger extract at a dose of 2 g/kg/day daily in drinking water for 40 d acted against cadmium bromide-induced nephrotoxicity in adult female albino rats. Abdulhameed *et al*. (2017) found that the ginger plant protects against CCL4-induced kidney damage in mice. Sheriff *et al*. (2018) reported that ginger extract at a dose of 400 mg/kg protects against contrast media-induced nephrotoxicity in rats. Additionally, ginger extract ameliorates renal damage in high-fat diet-induced obesity in rats (Bin-Meferij *et al*., 2019). Moreover, Ali *et al.* (2015) reported that injection of ginger at a dose of 120 mg/kg every other day for 4 weeks in rats resulted in clear improvements in the acute renal damage induced by cisplatin.

Ginger extract can also prevent DNA damage in rats (Makpo *et al*., 2020) and genotoxicity induced by toxicants (Lee *et al.*, 2011; Yang *et al.*, 2011). Moreover, Hosseinzadeh *et al.* (2017) revealed that ginger extract reduces oxidative stress and mitochondrial apoptosis induced by IL-1β chondrocytes. Recently, ginger extract decreased DNA damage, chromosomal abnormalities, and micronucleus formation in both bone marrow cells and sperm caused by radiation in rats (Abd El-Monem and Elwakeel, 2020). The rhizome of *Z. officinale* have high flavonoid level and a high antioxidant activity (Ghasemzadeh *et al.*, 2010). This study revealed that ginger extract ameliorated labetalol-induced genotoxicity. This effect may be mediated either by its direct free radical scavenging activity or the antioxidant defense system.

5. Conclusion

Taken together, maternal administration of labetalol during 6th to 15th of gestation induced embryotoxicity and nephrotoxicity in rat fetuses as indicated by increases in embryo resorption, fetal growth retardation, renal tissue degeneration, G0/G1 phase cell cycle arrest, and a significant increase in both apoptosis and necrosis rates. However, treatment with ginger extract reduced these toxicities. Ginger can be used during pregnancy as a therapeutic agent to alleviate the nephrotoxicity and embryotoxicity induced by labetalol.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

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