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Modulatory effect of ginger on skeletal malformations, cell cycle, apoptosis and structural changes in the liver of rat fetuses prenatally exposed to labetalol

Hend T. El-Borm[1*], Marwa S. Gobara[2] and Gamal M. Badawy[3]

Abstract

Background Drug-induced liver damage with clinical symptoms has been related to labetalol in a number of instances. In addition to having a wide range of anti-inflammatory and antioxidant qualities, ginger also includes biotrace that are crucial in the fight against disease and skeletal deformity. In this study, we hypothesized that prenatal supplementation of ginger (200 mg/kg) attenuates skeletal malformation and hepatotoxicity mediated by labetalol during the organogenesis period. The tested dams were divided into four groups: control, ginger (200 mg/kg), labetalol (300 mg/kg) and combined group (labetalol and ginger at the same doses).

Results The labetalol group showed various skeletal abnormalities represented by mandibular hypoplasia, costal separation and retardation in the ossification. Histological and ultrastructural examination of the fetal liver tissue revealed multiple pathological changes. DNA damage, G0/G1 cell cycle arrest and a high percentage of apoptosis were also detected in the fetal hepatocytes from labetalol groups through gel electrophoresis and flow cytometry using PI and annexin V/PI methods, respectively. Administration of ginger after labetalol caused an evident decrease in these skeletal malformations, structural changes, DNA damage, apoptosis and G0/G1 cell cycle arrest.

Conclusions It can be concluded that ginger has great potential in attenuating the skeletal malformation, structural changes and cyto-genotoxicity of fetal hepatocytes upon prenatal exposure to labetalol.

Keywords Labetalol, Ginger, Skeletal malformation, Fetal liver, Cell cycle, Apoptosis

1 Background

Labetalol is a nonselective and selective β-adrenergic antagonist that is frequently employed in the management of hypertension. Mixed α_1, β-antagonists therapy is believed to be associated with orthostatic hypotension, nausea, diarrhea, bronchospasm, dyspnea and cold extremities [1]. It has been reported that labetalol was associated with hepatocellular injury and drug hepatotoxicity is the most common cause of acute liver failure. [2, 3]. Up to 80% of patients receiving labetalol therapy have mild-to-moderate increases in serum aminotransferase levels, compared to much greater levels with other blockers. The liver damage caused by labetalol develops 4 to 16 weeks into treatment, and the pattern of elevated blood enzymes is often hepatocellular with an acute hepatitis-like onset and duration. The majority of cases resolve quickly once labetalol is stopped, although there have been a few cases of acute liver failure, death or the

*Correspondence:
Hend T. El-Borm
Hend.elbarm@science.menofia.edu.eg; Hendtarek98@yahoo.com
[1] Embryology, Zoology Department, Faculty of Science, Menoufia University, Menoufia, Egypt
[2] Zoology Department, Faculty of Science, Menoufia University, Menoufia, Egypt
[3] Experimental Embryology, Zoology Department, Faculty of Science, Menoufia University, Menoufia, Egypt

need for an urgent liver transplant related to labetalol use [4].

Numerous compounds, including gingerol, shogaol, zingiberence, paradol, resin, starch, volatile oil and vitamins C and A, are present in ginger [5, 6]. It has a long history of use in traditional medicine for ailments like migraines, toothaches and colds, as well as for improving blood circulation in the limbs and lowering blood cholesterol [7–9]. It is also used for its antioxidant, antimicrobial, antiviral, gastroprotective, antidiabetic, antihypertensive, cardioprotective, anticancer and immunomodulatory effects [5, 7, 10–12]. Additional components of ginger, such as acid resins, vitamin C compounds, gingerol, vitamins B3 and B6, volatile oils and biotrace, are crucial for the development of bones [13]. Ginger's 6-gingerol, which stimulates osteoblast development, has been linked to a positive impact on bone production, according to Fan et al. [14].

Preclinical studies carried out on laboratory animals have also shown that ginger possesses hepatoprotective effects, on agents like alcohol [15, 16], Country liquor [17], heavy metals [18, 19], paraben [20] and bromobenzene [21]. Moreover, ginger has a protective effect against Acetaminophen (paracetamol) [22–24] and CCl_4-induced hepatotoxicity [25, 26]. Moreover, many observations clearly indicated that ginger is effective in the inhibition of cyto-genotoxicity caused by metiram [27], methotrexate [28], carbimazole [29] and benzopyrene [30] in different experimental animals. The current study's goal was to determine whether prenatal administration of ginger aqueous extract could improve fetal skeletal abnormalities, histopathological, ultrastructural and cyto-genotoxic changes in hepatic cells of 20-day-old rat fetuses related to labetalol use during the organogenesis period.

2 Methods

2.1 Experimental materials

Ginger (*Zingiber officinale*) powder was extracted according to Kamtchouing et al. [31] as previously described in El-Borm et al. [32].

Labipress tablets contains labetalol hydrochloride 100 mg, were manufactured by DBK, Cairo, Egypt. At a dose of 300 mg/kg (which is equivalent to the recommended human dose) [33], tablets were crushed and diluted in distilled water.

2.2 Animals

Virgin females and fertile males of Wistar albino rats (*Rattus norvegicus*), weighted 160 ± 10 g and aged 17 ± 1 weeks, were purchased from Helwan Farm, Ministry of Health, Cairo, Egypt. Prior to the study, the animals were kept in a quiet and nonstressful environment for 3 weeks in the Faculty of Science, Menoufia University, under controlled conditions of ambient temperature (25 ± 2°C) and lighting (12 h light/dark cycles) and were allowed free access to water and food. Females were tested every day for two weeks before the study start to identify their estrous cycle stage. The experiment was only conducted on animals that were in the diestrus stage. By housing males and females in a 2:1 ratio, mating was induced overnight. When the vaginal copulatory plug was present, and the vaginal smear was positive, the pregnancy's start date was determined [34].

2.3 Experimental design

The pregnant females were divided equally (15/group) into 4 groups. All groups were orally administrated distilled water, labetalol and/or ginger via intragastric tube once daily at the same time over the 10 days of the organogenesis period (from 6 to 15th gestational days) as follows:

- The control group was given 1 ml of distilled water.
- The ginger group was given 1 ml of 200 mg/kg of ginger extract [35].
- The labetalol group was given 1 ml of 300 mg/kg of labetalol.
- The labetalol + ginger group was given 1 ml of 300 mg/kg of labetalol followed by 1 ml of 200 mg/kg of ginger an hour later.

The dams were euthanized and underwent cesarean section operations on the 20th day of gestation.

2.4 Fetal endoskeleton examination

According to Badawy et al. [36], fetuses were preserved in 10% formalin, eviscerated and stained using the double staining transparency technique for cartilage and bone by Alcian blue–Alizarin red S stains. In order to analyze the stained skeleton for any anomalies, they were stored in pure glycerol and photographed using a digital camera (Sony, Germany).

2.5 Histopathological examination

Fetal liver from all four groups was immediately fixed using 10% neutral formalin for 24 h, washed under running tap water, transferred to 70% ethanol and then dehydrated in an ascending series of ethanol. Preparation of paraffin sections and histological staining was performed with Ehrlich's hematoxylin and counterstained with aqueous eosin according to Suvarna et al. [37]. Histological sections were subjected to microscopical examination, and selected sections were photographed using an Olympus microscope (BX41, Japan).

2.6 Transmission electron microscope examination

Liver specimens were immediately fixed in 2.5% glutaraldehyde, washed in phosphate buffer, postfixed in 1% osmium tetra-oxide, washed in phosphate buffer multiple times and dehydrated in ascending grades of ethanol. Following that, the samples were cleared in of propylene oxide solution, infiltrated with propylene oxide and epon (1:1) and embedded in epoxy resins. After producing semithin sections, ultrathin (50 nm) sections were cut, mounted and stained with uranyl acetate and lead citrate [38]. The JEOL electron microscope (TEM-1400Plus, Japan), Electron Microscope Unit, Alexandria University, was used for examination and photography.

2.7 Assessment of DNA fragmentation by gel electrophoresis

The technique for extracting nucleic acids was first introduced by Aljanabi and Martinez [39] and later modified by El-Garawani and Hassab El-Nabi [40]. Fetal livers were lysed by lysing buffer, saturated NaCl was added and centrifuged, DNA was precipitated by cold isopropanol and centrifuged, and pellets were washed with 70% ethyl alcohol and then centrifuged again. Then the pellets were redissolved in Tris–EDTA buffer (10 Mm Tris–HCL, 1 Mm EDTA, pH 8). The resuspended DNA was incubated in a loading mix (Rnase + loading buffer + microliter ethidium bromide) and then loaded into gel wells. Using a trans-illuminator and 312 nm UV light, DNA was observed, and the gel was captured on camera.

2.8 Cell cycle analysis by flow cytometry

According to Reichard and Asosingh [41], fresh fetal liver samples were homogenized, suspended in PBS and centrifuged and the cell was preserved in ice-cold 96–100% ethanol. 200 µl of cell suspension was combined with a solution of propidium iodide (PI) and processed in the flow cytometer at 488 nm (BD Accuri™ C6 (Becton Dickinson, Sunnyvale, CA, USA) according to the manufacturer's instructions.

2.9 Annexin V/PI dual staining assay

1 ml of phosphate buffer-suspended cell was resuspended in 2 ml of 0.1 × binding buffer (1 ml of 10 × buffer with 99 ml dist. water), 100 µl of the cell suspensions was then added to 5 µl of annexin V (Cat. No. 556547 BD pharmingen FITC apoptosis Kit), and finally, 5 µl PI was added. The cells were resuspended in 200 µl of 1X binding buffer before being promptly examined by the BD Accuri™ C6 flow cytometer from Becton Dickinson in Sunnyvale, California, USA, which features a small, air-cooked laser beam with a 15-mW output (488 nm). Viable cells (unlabeled), early apoptotic cells (bound to annexin V only), necrotic cells (stained with PI) and late apoptotic/necrotic cells (both bound annexin V and PI) were four distinct types of cells that could be easily separated. The fluorescent cell percentage in each quadrant was calculated after the fluorescence distribution was exhibited as a two-color dot plot analysis.

2.10 Statistical analysis

The statistical analysis was performed using a statistical package of social science (IBM SPSS) software for windows, version 22 (IBM Corp., Armonk, NY USA). For multiple comparisons, a one-way ANOVA analysis was conducted, followed by an LSD test.

3 Results

3.1 Endoskeleton examination

The endoskeleton of the fetuses of the ginger group exhibited normal structure. The skull, vertebral column, sternum, ribs and limbs showed normal skeletal structure and had the same degree of ossification (Fig. 1a) as that of the control group (Fig. 1b).

The labetalol group's fetuses exhibited a variety of skeletal abnormalities. The most noticeable endo-skeletal anomalies were mandibular hypoplasia and significant ossification delay. The frontal bones showed no evidence of ossification. Additionally, there were some fetuses whose cartilage drafts had not yet developed. The mandibles, maxillae, occipital bones and parietal bones lacked ossification. The vertebral column was stained blue indicating its cartilage nature. Spinous processes, intervertebral disks and caudal vertebrae in the distal third of the body were all cartilaginous in composition. Most fetuses had irregular ribs separation and wavy ribs. The ribs, which were laterally divided, were not joined to the costal arch. All fetuses were found to have deteriorated costal ossification. The hummers, radius and ulna of the forelimbs did not ossify in the majority of fetuses. Additionally, there were no ossifications found in the bones of the hindlimbs. A few fetuses had small regions of ossification in the femur, hummers, radius and ulna of the right forelimb. Additionally, both the right and left hindlimbs' ilia showed small areas of ossification (Fig. 1c).

Administration of labetalol followed by ginger caused an evident decrease in the above-mentioned skeletal malformations. In terms of the ossification, the whole endoskeleton of the fetuses showed overall significant amelioration. The partially ossified bones of the skull were the nasal, frontal and parietal bones while the un-ossified bones were interparietal and supraoccipital. Partially ossification at various levels was observed in the vertebral column. The intensity of the ossification gradually decreased from the thoracic vertebrae through the caudal direction. The scapula, clavicle,

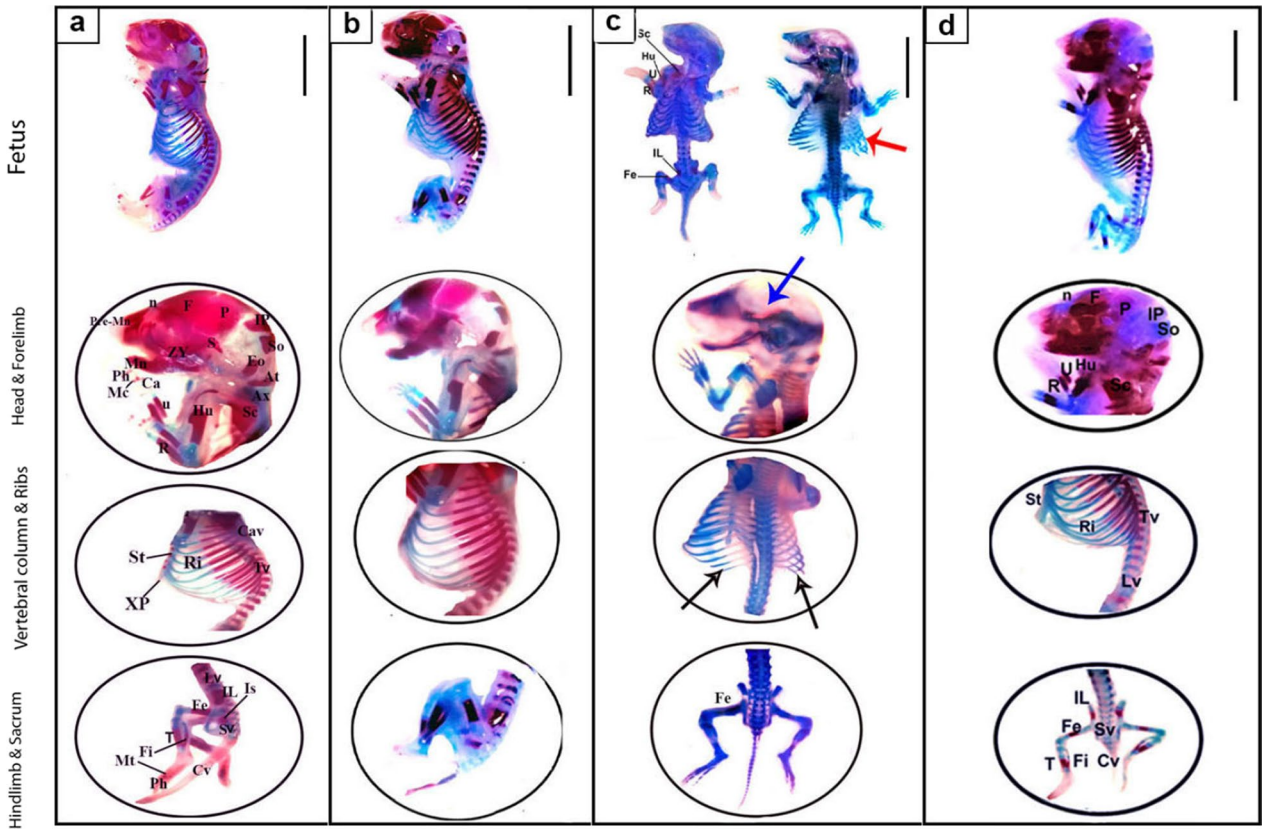


Fig. 1 Photographs of the double-stained endoskeleton of fetuses and their body parts from different groups. **a** Control group; normal ossification of the cranial, forelimbs and hindlimbs bones, complete ossification of the vertebral column and normal appearance of ribs. **b** Ginger group; normal ossification of the endoskeleton. **c** Labetalol group; severe lack of ossification in the whole skeleton, loss of some cranial bones (blue arrow), wavy ribs (red arrow) and costal separation anomaly (black arrows). Also, some fetuses showed pinpoint ossification areas in the hummers, radius and ulna of the right forelimb and in the femur and ileum of the right and left hind limbs. **d** Labetalol + ginger group; overall amelioration in the ossification of the endoskeleton but some bones of the skull, vertebral column and limbs with incomplete, slight or lacking ossification can be seen. Atlas (At), axis (Ax), carpus (Ca), caudal vertebrae (Cav), cervical vertebrae (Cv), exoccipital (Eo), frontal (F), femur (Fe), fibula (Fi), humerus (Hu), ilium (IL), interparietal (Ip), ischium (Is), lumbar vertebrae (Lv), metacarpus (Mc), mandible (Mn), metatarsus (Mt), nasal (n), parietal (P), phalanges (Ph), pre-maxilla (Pre-M), radius (R), ribs (Ri), squamosal (S), scapula (Sc), supraoccipital (So), sternebrae (St), sacral vertebrae (Sv), tibia (T), thoracic vertebrae (Tv), ulna (U), xiphoid process (XP), zygomatic (Zy) (Alizarin red S and Alcian blue)

radius and ulna of the forelimb were well ossified. No ossification was determined in any of the metacarpals and phalanges. The ilium, body of the femur, tibia and fibula of the hind limb were partially ossified while the ischium and pubic bones were un-ossified. The metatarsals and phalanges of these fetuses were also un-ossified (Fig. 1d).

3.2 Histopathological examination

The fetal liver of the control group showed normal hepatocytes arranged around the central vein. They had eosinophilic cytoplasm, spherical nuclei and polyhedral shapes. Hepatic sinusoids divided the hepatocyte plates. Additionally, a few megakaryocytes were observed (Fig. 2a). The fetal liver tissue maternally administrated

(See figure on next page.)

Fig. 2 Photomicrographs of transverse sections in 20th-day fetal liver (stained with hematoxylin and eosin). **a** Control group; normal hepatocytes (H) arranged around the central vein (CV) and alternating with blood sinusoids (Si), and megakaryocytes (Mg). **b** Ginger group. **c**–**g** Labetalol group; congested central vein (CV), leukocytic infiltration (Li), dilated portal vein (PV) numerous leukocytes (LI), degenerative hepatocytes with vacuolated cytoplasm (arrowhead) and bile ductulus (BD), severe hemorrhage (h) between the parenchyma, dilated and elongated central vein (CV), numerous megakaryocytes (Mg), proliferated bile ductulus (BD) around the portal vein (PV) and vacuolated hepatocytes (V). **d** Labetalol + ginger group; somewhat normal hepatocytes (H), central vein (CV), sinusoids (Si) and few megakaryocytes (Mg). Scale bare = 15 µm

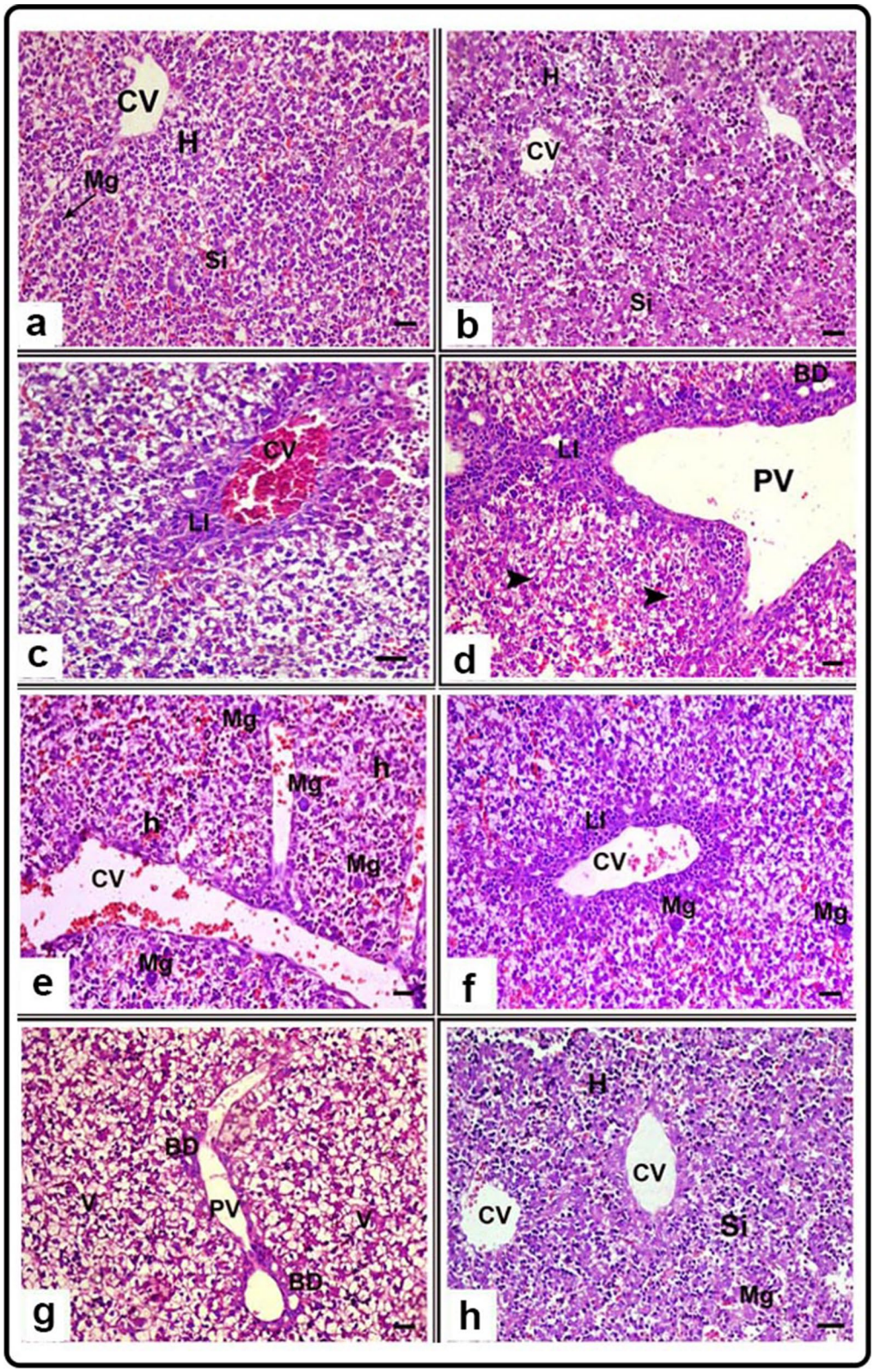


Fig. 2 (See legend on previous page.)

with ginger displayed normal architecture similar to the control group (Fig. 2b).

Severe congestion and dilation in the central veins and leukocytic infiltration were observed in the fetal liver of the labetalol group. The liver cells lose their normal architecture with disruption of the hepatic cords. Pyknotic nuclei and severe vacuolar degeneration of the hepatocytes along the central vein were also seen (Fig. 2c, d). Elongation and congestion of the portal vein, infiltration of mononuclear inflammatory cells and proliferation of the bile ducts were all visible in the portal region. Also, there was an increase in the number of megakaryocytes and hemorrhage (Fig. 2d–g). The liver tissue of the labetalol + ginger group showed evident improvement in the structure of the hepatic strands, hepatocytes appeared nearly normal in size and shape, less vacuolated cytoplasm and the central vein appeared nearly normal in size (Fig. 2h).

3.3 Ultrastructural examination

Electron micrographs of the hepatocytes of the control fetuses showed large, spherical, centrally located nuclei with a regular nuclear envelope, large electron-lucent euchromatin and scattered heterochromatin. The cytoplasm showed a granular appearance with numerous rounded and elongated mitochondrial profiles with an electron-dense matrix. Also, there were profiles of rough endoplasmic reticulum between the mitochondria and glycogen granules. The hepatocytes contained free ribosomes in the cytoplasm (Fig. 3a). Examination of hepatocytes from the ginger group exhibited ideal hepatocytes (Fig. 3b).

The nuclei of some hepatocytes from the labetalol group appeared pyknotic and shrunken with marginal condensation of their heterochromatin with large electron-dense nucleoli. The cytoplasm exhibited swollen and fragmented rough endoplasmic reticulum, compacted small-sized mitochondria with electron-dense matrix and destructed cristae (Fig. 3c, d). Most of the hepatocytes exhibited rarified cytoplasm containing lysosomes of different sizes and homogenous electron-dense microbodies. Wide intercellular spaces were observed among the parenchymal cells. Moreover, hemorrhage, leukocytic infiltration and ruptured cell membrane were obviously seen (Fig. 3e–g). In the labetalol and ginger group, most hepatocytes showed marked improvements at the ultrastructural level (Fig. 3h).

3.4 DNA fragmentation by gel electrophoresis

Figure 4 reveals that the extracted total genomic DNA of the hepatic tissue of control and ginger groups was intact as seen in lanes 1 and 2, with no apparent damage in DNA. The fetal hepatocytes of the labetalol group showed a marked increase in DNA damage by the migration of DNA fragments, as seen in lane 3. The hepatocytes of the combined group (Lane 4) displayed marked improvement in the DNA breaks compared with the control group.

3.5 Cell cycle distribution

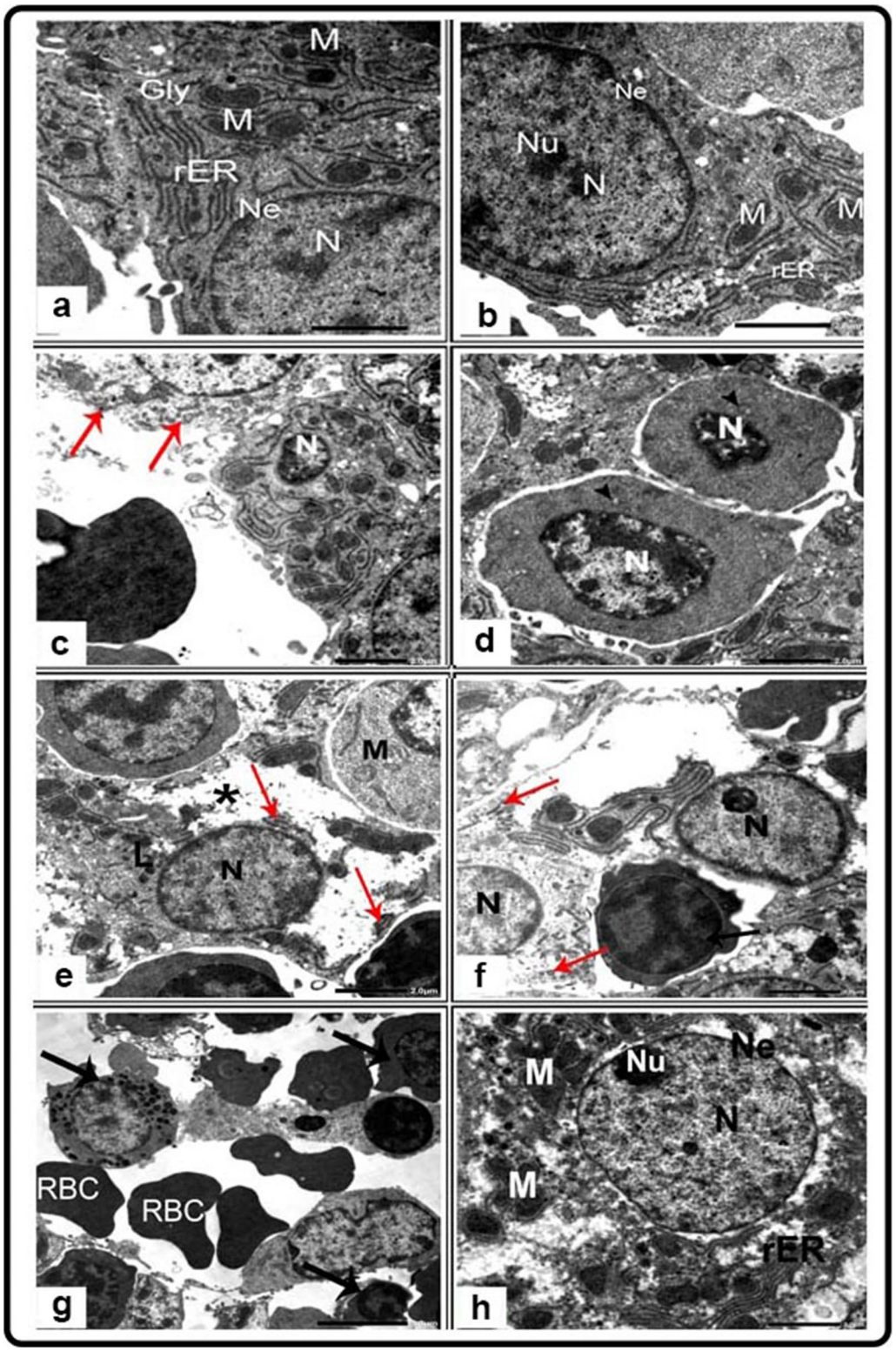
Following labetalol administration, fetal hepatocyte cell cycle distribution analysis revealed that more cells accumulated in the G0/G1 phase (97.2%) as compared to the control (57.7%) and ginger (57.8%) groups. Additionally, the proportion of cells in the S and G2/M phases was significantly reduced to 0.8 and 0.2% compared to the control group (16.9 and 25.3%, respectively). Comparing the combined group to the labetalol group, the G0/G1 phase exhibited a highly significant drop (67.6%) and a clear increase in the S and G2/M phases (15.8 and 16.5%), as shown in Table 1 and Fig. 5.

3.6 Dual detection of apoptosis by annexin V/PI

The percentages of various hepatic cell populations are shown in Fig. 6. Most of the cells in the control and ginger groups were viable (92.8 and 94.23% for the two groups, respectively). The hepatic cells of the labetalol group showed a clear decline in the percentage of viable cells (55.01%) and a highly significant increase in the rates of apoptosis and necrosis (23.79 and 21.2, respectively) compared with the control group (2.42 and 1.77, respectively). Contrarily, the combined group demonstrated a highly significant increase in the percentage of viable cells (86.4%) compared to the labetalol group (55.01%) and a highly significant decrease (8.1 and 5.5, respectively) in the apoptotic and necrotic rates compared to the labetalol group (Fig. 6).

(See figure on next page.)

Fig. 3 Transmission electron micrographs of the fetal hepatocytes. **a** Control, **b** Ginger groups; euchromatic nucleus (N), regular well-identified nuclear envelope (Ne), prominent nucleolus (Nu), granular cytoplasm, glycogen granules (Gly), rough endoplasmic reticulum (rER) and numerous mitochondria (M). **c–g** Labetalol group; shrunken electron-dense nuclei (N), fragmented and swollen rough endoplasmic reticulum (red arrow), ruptured cell membrane and degenerated mitochondria (arrowhead). Destructed hepatocytes contain shrunken electron-dense nuclei (N), abnormal nuclear heterochromatin condensation, lysosomes (L), rarified cytoplasm (star), different inflammatory cells (black arrow) and red blood cells (RBC) among hepatocytes. **h** Labetalol + ginger group showing a hepatocyte with euchromatic nucleus (N), regular nuclear envelope (Ne), nucleolus (Nu), rough endoplasmic reticulum (rER), mitochondria (M) and some areas of an electron-lucent cytoplasm. Scale bar = 2 µm for all except (g) = 5 µm



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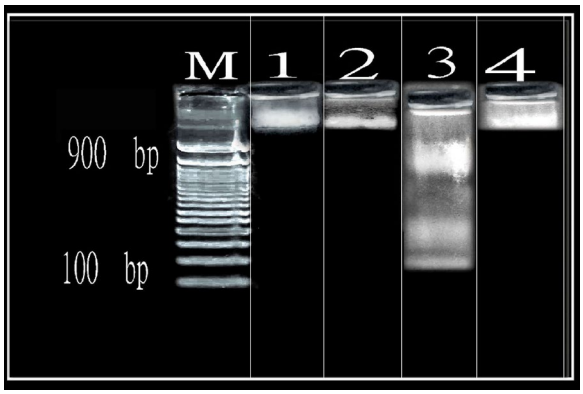

Fig. 4 Photomicrograph of an agarose gel showing an evident variation of the DNA fragmentation in the hepatic tissue extract of 20-day-old rat fetuses treated with labetalol with/without ginger. bp: base pair, M: marker DNA (100 bp DNA Ladder, New England Bio-labs, Ipswich, MA, USA), 1: control, 2: ginger,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

4 Discussion

In the current study, skeletal anomalies such as mandibular hypoplasia, wavy ribs and costal separation were caused by prenatal exposure to labetalol at a dose of 300 mg/kg during organogenesis. The ossification of the entire fetal endoskeleton was also clearly delayed. This is consistent with a number of studies that found that injecting various antihypertensive medications into pregnant rats resulted in skeletal deformities and ossification retardation [42–44]. In contrast, Jaiswal et al. [45] reported that injection of nifedipine and amlodipine did not produce any skeletal or visceral defects in rat pups.

According to the present data, prenatal exposure to labetalol caused obvious pathological effects in the fetal liver. Similarly, the hepatotoxicity of labetalol has been documented in multiple reports [4, 46–49]. Numerous pathological changes in the liver tissue were brought on by β-adrenergic receptors, such as metaprolol, including severe hydropic degeneration, perivenular sinusoidal dilatation and moderate-to-severe inflammation [50]. Additionally, Ibrahim et al. [51] reported that the α- and β-blockers carvedilol caused congestion in both central and portal veins, associated with diffuse Kupffer cell proliferation and inflammatory cell infiltration in the portal area in albino rats.

The actual mechanism of labetalol hepatotoxicity is unknown; however, it may be caused via a number of intracellular processes, including the mitochondrial cytochrome P450 enzymatic system. Patients with isoenzyme differences or mutations in this enzyme gene may be more susceptible to its hepatotoxic effects or the metabolic idiosyncratic disposition of the agent, which is known to be extensively metabolized in the liver. A reaction that is autoimmune and inflammatory may be other processes [4, 48].

According to this study, labetalol was shown to cause DNA damage, apoptosis and G0/G1 cell cycle arrest in the fetal hepatic cells. Many antihypertensive drugs have been documented to cause genotoxicity and cell cycle arrest, including carvedilol which induces cell cycle arrest in G0/G1 and increased the apoptotic rates in rat C6 glioma cells [52] and has genotoxic and cytotoxic effects on the peripheral blood mononuclear cells [53]. Moreover, the antihypertensive telmisartan was shown to contribute to the induction of G0 /G1 cell cycle arrest of hepatocellular carcinoma cells [54]. Also, it has been reported that atenolol, a β-blocker antihypertensive drug, exerts genotoxicity in human lymphocytes [55]. This evident genotoxicity and cytotoxicity may be attributed to the overproduction of free radicles caused by labetalol administration which impairs the innate antioxidant defense system of the cells. This caused a different type of DNA damage, cell cycle arrest and apoptosis [56].

Plant-derived phytochemicals are used to treat a wide range of diseases. Several studies indicate that many plant extracts have different activities, such as antimicrobial, antioxidant and anticancer. The present

Table 1 Percentage of total cell cycle distribution in hepatic cells of 20-day-old fetuses

Groups	%	%	% cells analyzed in Gate R		
	Sub G1	No. Cells/Cell cycle	G0/G1	S	G2/M
Control	3.7 ± 0.030	96.3 ± 0.012	57.7 ± 0.06	16.9 ± 0.05	25.3 ± 0.07
Ginger	2.4 ± 0.060	97.6 ± 0.01	57.8 ± 0.015	17.4 ± 0.01	24.6 ± 0.011
Labetalol	38.9 ± 0.012	61.1 ± 0.14	97.2 ± 0.12***	0.8 ± 0.012***	0.2 ± 0.035***
Lab + Ging	8.4 ± 0.010	91.6 ± 0.03	67.6 ± 0.15[a]	15.8 ± 0.031[a]	16.5 ± 0.021*[a]

Data are shown as the mean ± SEM

The *P* value in comparison with the control group is indicated by an asterisk (***$P < 0.001$, *$P < 0.05$)

(a) is highly significantly ($P < 0.001$) different from the labetalol group

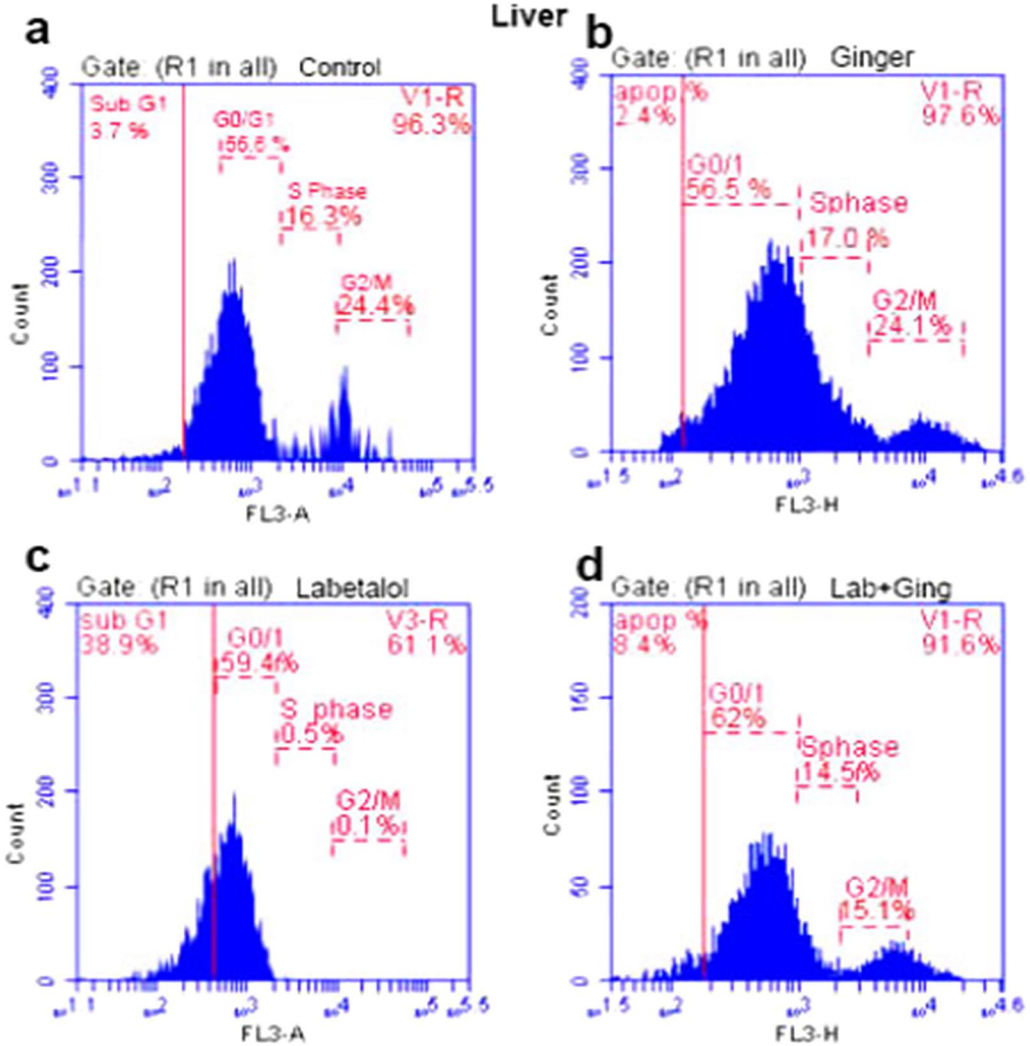

Fig. 5 Diagrams depicting the cell cycle distribution of hepatic cells from flow cytometry. **a** Control; **b** ginger; **c** labetalol; **d** labetalol + ginger

study demonstrated that ginger extract caused an evident decrease in the skeletal malformations induced by labetalol. Ginger has been reported to be effective against bone-related disorders such as osteoarthritis [57, 58], bone microarchitectures and osteoporotic changes in femur diaphysis and metaphysis caused by cadmium chloride [59] and skeletal malformations induced by gabapentin in rat fetuses [60]. The polyphenolic compounds of ginger have been shown to inhibit osteoclastogenesis induced by receptor activators of nuclear factor-kB (NF-kB) ligand (RANKL) [61]. Additionally, 10-gingerol activates the bone morphogenetic protein signaling pathway in zebrafish, according to Chen et al. [62]. Moreover, Fan et al. [14] concluded that 6-gingerol has a beneficial effect on bone formation as a therapeutic agent for treating bone disorders, as it stimulated the differentiation of osteoblast-like MG-63 cells and ameliorated TNF-a-suppressed osteoblast differentiation.

The present study showed that ginger significantly ameliorated the histopathological and ultrastructure changes induced by labetalol in the fetal liver tissue. This finding agrees with the recent findings which showed the ameliorative role of ginger on liver tissue; Abd-Elrhman et al. [63] indicated that ginger nanoparticles (50 mg/kg) protected the architecture of hepatic tissue from damage by CCl_4. Also, ginger extract (125 mg/kg) ameliorates the histological changes associated with fatty liver in Albino rats [64, 65]. Similarly, Badawy et al. [66] indicated that ginger extract (200 mg/kg) ameliorates the histopathological and ultrastructure changes in the hepatocytes of the fetuses maternally injected with the antiepileptic drug gabapentin. Also, Badr et al. [67] showed that ginger extracts at a dose of 120 mg/kg caused some

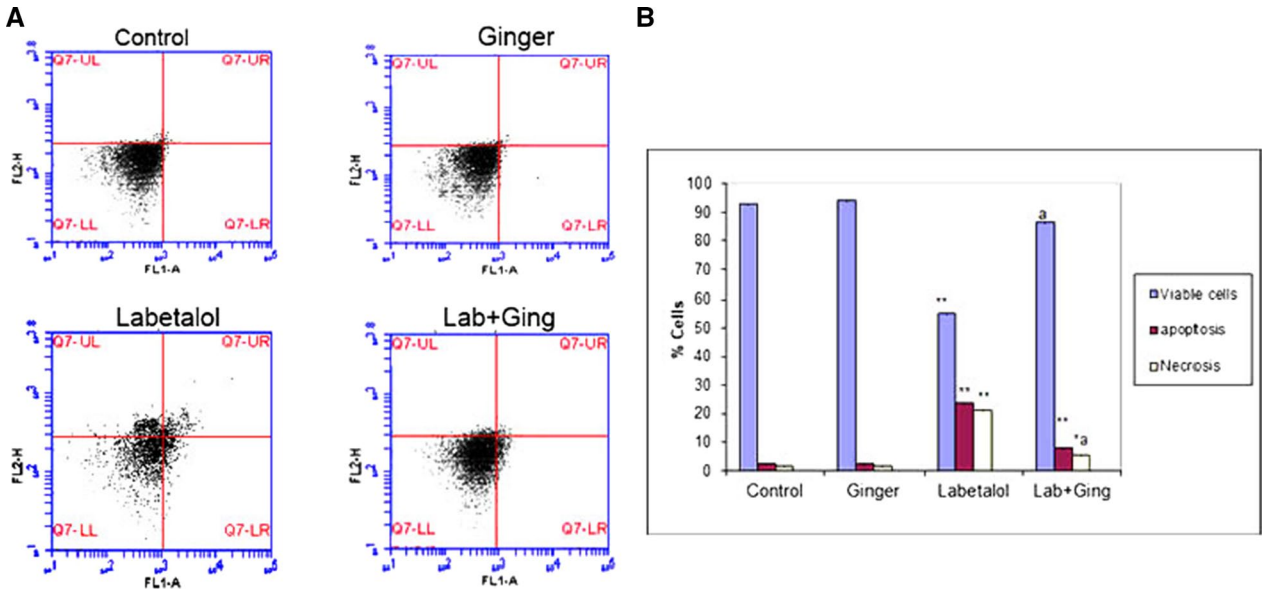

Fig. 6 Fluorocytograms of fetal hepatocytes **a** control; **b** ginger; **c** labetalol; **d** labetalol + ginger (FL1-A) = annexin V-positive cells and Y-axis (Fl2-H) = PI-labeled cells. The lower left portion (Q7-LL) of the fluorocytogram (negative for both stains) shows viable cells, whereas the lower right portion (Q7-LR) (positive for annexin) shows early apoptotic cells, the upper right portion (Q7-UR) (positive for both stains) shows late apoptotic cells and the upper left portion (Q7 UL) (positive for PI) shows necrosis. **e** Graph showing the percentage of viable, apoptotic and necrotic hepatic 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

ameliorations in the liver structure after solid tumor formation in mice. Gholampour et al. [68] reported that ginger hydroalcoholic extract exerts a protective effect against ferrous sulfate-induced pathological changes in both rat liver and kidney.

Supporting the present findings, it has been found that aqueous ginger extract (250 mg/kg) caused an improvement in the ultrastructure of the hepatocytes of diclofenac [69] and adriamycin [70]-treated rats. Also, Mahmoud and Hegazy [71] showed the ameliorative effects of the antioxidant supplement, ginger tablets on hepatic ultrastructural alterations in old rats. Overall, ginger may exhibit a protective effect against hepatotoxicity due to its antiapoptotic, antioxidant and anti-inflammatory properties.

Moreover, ginger has beneficial effects on the cell cycle, as it significantly decreases the proportion of arrested cells in the G0/G1 phase and raises the proportion of S and G2/M cells. Additionally, ginger prenatal exposure significantly reduced DNA fragmentation, apoptosis and necrosis rates while raising the percentage of viable cells and reducing apoptosis and necrosis rates in the hepatic cells. The present data are matched with many studies which reported that ginger protects against DNA damage in albino rats due to its ability in scavenging oxygen radicals [30, 72–74]. Furthermore, ginger has been observed to decrease apoptosis caused by gentamicin [8] and metiram [27]. Moreover, it could decrease DNA damage and apoptosis in liver cells by decreasing the oxidative stress caused by lead in rats [19].

5 Conclusion

From the present study, it has been concluded that ginger extract has great importance in the treatment of skeletal malformation, hepatotoxicity and genotoxicity induced by labetalol in rat fetuses. Ginger extract restored most of the pathological changes in the fetal hepatocytes and reduced the fetal skeletal malformation and apoptosis, DNA damage and G0/G1 arrest in the fetal hepatocytes. Further research is needed to find out the exact mechanism of ginger against diseases.

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Author contributions
H.T. and M.G. worked on practical part. H.T. made an interpretation of the results, and H.T., G.B. and M.G. wrote the main manuscript text. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
The study was conducted according to the guidelines of the ARRIVE and the institutional guideline and was approved by the Institutional Animal Care and Use Committee at the Faculty of Science, Menoufia University, Egypt (Permit Number MUFS/F/EM/1/20).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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