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Histological Study of Domperidone Effect on Albino Rat Fetuses.

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ABSTRACT

Nausea and vomiting (NVP) were widespread case among the pregnant woman especially in the first trimester (6-12 weeks) of gestation and this case may be lasts till 20 week of gestation or all over the pregnancy period. Treatment of NVP included several routes one of them was antiemetic medication (domperidone). In this study we aimed to investigate the teratogenic effect of domperidone on the embryo. The study was done using pregnant albino rats (*Rattus norvegicus*) which treated during organogenesis period with therapeutic dose of the chosen drug. At 20th gestational day all the animals were sacrificed under anesthesia and fetuses with their placentas were removed from the uterus and evaluated for mortality rate, growth parameters, morphological and skeletal malformation also histological study of placenta, fetal liver, kidney and brain. The results revealed a significant decrease in the all growth parameters, high incidence of resorption in treated animals. Hematomas, open eyelid were noticed in the fetuses of treated group. Skeletal anomalies summarized as less degree of ossification in most bones, costal separation, curved and wavy ribs. Histopathological studies revealed less change in the hepatic cells and shrinkage of glomeruli and degeneration of cells lining renal tubules were observed in the kidney of fetuses of treated groups. Degenerative changes were observed in the brain after drug exposure. Our findings suggest the need for great caution to handle domperidone particularly throughout the pregnancy time.

Keywords: Antiemetic, Domperidone, Pregnancy, Teratogenicity, Fetus, Albino rats, Placenta, Liver, Brain, Kidney.

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INTRODUCTION

During the pregnancy period the prescription of drug is particularly critical. The option to use drug is very limited because of possible bad effect of the drug on the embryo and fetuses. It is very important to us to underget the pharmacology of the drug and to realize also the critical developmental period that affected mostly by the drug during this time the fetus become more susceptible.

A birth defect is a very big trouble which occurs during the fetal development in the mother uterus. The birth defects can be caused by genetics, lifestyle choices and behaviors, exposure to certain medications and chemicals, infections during pregnancy and may be a combination of these factors. However, the exact causes of certain birth defects are often unknown. A teratogen is a substance that interacts with the normal development of the fetus. Medical science cannot always predict how exposure to a teratogenic drug will affect a fetus. The potential for harm depends on several factors including, type of drug, size of the dose, how often it's taken, stage of fetal development (gestational age) at the time of drug exposure, individual response of the fetus to the drug and other factors, such as maternal diet or illness.

Morning sickness, also called nausea and vomiting of pregnancy (NVP), is a symptom of pregnancy that involves nausea or vomiting. Nausea during pregnancy is typically one of the most experienced and complained about symptoms that women report. Up to 70 percent of expectant mothers experience nausea at some point during early pregnancy. Not only is it known to be one of the early signs of pregnancy, but it is a symptom that is common throughout the first trimester, and sometimes even longer.

Management of NVP included many ways as dietary and lifestyle changes, non-pharmacologic and pharmacological Agents. Domperidone is a dopamine antagonist with antiemetic properties. There are no enough data on the safety and teratogenic effect of domperidone on the fetus of animals in the pre-clinical studies. Therefore the present study aimed to evaluate the histopathological effect of domperidone on the main fetal tissue.

MATERIALS AND METHODS

Animals and housing conditions:

The present work was performed on healthful adult male and female rats (*Rattus norvegius*) of weight about 170-180 grams were obtained from the animal house of the Faculty of Veterinary, Cairo university-Egypt. The animals were reserve in suitable cage and maintained in 12 hours light and dark cycle in temperature & humidity controlled environment. The rats were fed with standard food pellet and water ad libitum. Experiment was carried out according to the internationally valid guidelines and the institutional animal ethics committee. Institutional Animal Ethics Committee approval was obtained before the conduct of the study the Cairo University, Faculty of Science Institutional Animal Care and Use Committee (IACUC) (Egypt), (CUFS/Comp&Emb/5/16). After one week of acclimatization two females rats were housed overnight with a mature male rat. The day of sperm detection in the vaginal smear was considered to be day 0 of gestation.

Experimental procedure:

Organogenesis phase was the most critical period during gestation. Female rats were administrated orally (by gavage) once daily in the morning from 5 day to 19 day of gestation. The recommended maximum dose for human is 80 mg/daily. The dose was modified to suit the weight of rats according to [1].

Group 1: Control – The pregnant rats received an equivalent volume of distilled water.
Group 2: The pregnant rats received domperidone (8.2mg/kg body weight dose) during gestational period (from gestation day 5 to 19).

At 20th gestation day all females were weight to calculate the mother~ weight gain then sacrificed by decapitation and their uteri were exposed under dissection. The uteri horns were open and record the position and number of viable, resorbed, or dead fetuses. The placentas were check carefully and their weights were recorded. The fetal weighed and length was score and examination for any external anomalies was performed under a dissecting microscope. Some fetuses were stained by alizarin red for skeletal examination according to

[2]. Cross-sections through the placenta, brain, liver and kidney of fetuses at 20 day of gestation were dissected out; and trimmed of excess fat. Then, they were fixed in 10% buffered formalin and were processed for paraffin sectioning by dehydration in different concentrations of alcohol, cleared with xylol and embedded in paraffin blocks. Sections of about cut 5-7µm thick, stained with haematoxylin and eosin and. All sections were examined using light microscope and photographed. All methods were applied according to Drury and Wallington [3, 4].

Statistical analysis was performed using the T- Test to determine differences between control and treated group, means at significance level of 0.05. Standard errors of treatment means were also estimated. All statistics were carried out using Statistical Package for the Social Sciences (SPSS).

RESULTS

Effects on mothers:

We didn't record any dead case during or at the experimental time and we didn't observe any signs of abortion as vaginal bleeding (Table 1) also there is non-significant (P= 0.059) change in treated mother weight gain at 20th in comparison with control group (Fig. 1). In the current work there was a significant ($P \leq 0.05$) decrease in the placenta weight of the treated mother when compared with the control one (Fig. 1). The uterus from control group revealed normal distribution of the implanted fetuses between the two horns (Fig. 2) while the uterus of pregnant rats treated with domperidone showed asymmetrical distribution of fetuses in the two uteri horn, resorption site (late embryonic resorption) observed and completely early resorbed uterus also revealed that called pinpoint hemorrhagic implantation sites (Fig. 2)and high incident of resorption, pre-implantation loss index and post- implantation loss index was record in the treated group when compared with the control one (Table 1).

Effects on fetuses:

Maternal administration of domperidone caused growth retardation represented by a decrease in fetal body weight and body length (Fig. 1). There was a significant ($P\leq 0.05$) reduction in fetus weight and fetus length in treated group when compared with the control group.

The fetus of control group appeared with normal shape, correct weight and length (Fig. 3). Fetuses from treated group showed number of external malformation that represented as subcutaneous hematoma in many regions as head, face and back, deformed limbs, open eyelid and abnormal back curvature(Fig. 3) but there were no deaths among the fetuses (Table 1).

At the 20th day of gestation, the cleared cartilage and bone preparations of control rat fetuses have designated that all parts of the axial skeleton, skull, vertebrae and ribs as well as appendicular skeleton comprising the fore and hind limbs, pectoral and pelvic girdles, both chondrification and ossification processes have been obviously completed (Fig. 4& Table 2). On the other hand, fetuses maternally treated with 8.2mg/Kg of domperidone showed high percentage of un-ossification in the almost bones also the skull lack the chondrification centers (47.6%) from the examined fetuses and the thoracic ribs revealed curved and wavy shape (47.6%) (Fig. 5& Table 2).

Histopathological studies:

Placenta

Histologically, the placenta is divided into a fetal part and a maternal part [5]. The maternal part consists of the decidua and metrial gland. The decidua basalis was composed of cellular and fibrous elements. It was separated from the basal zone by single layer of giant cells. The fetal part consists of the basal zone and labyrinth zone. According to [6] the basal zone is comprised of three differentiated cells: (1) spongiotrophoblast cells, (2) trophoblastic giant cells and (3) glycogen cells. The labyrinth zone contains the maternal sinusoids and the trophoblastic septa, which are composed of the trilaminar trophoblastic epithelium and fetal capillary (Fig. 6).

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Light microscopic examination in the placenta of treated rats, in the basal zone, increase in number of giant cells with shrinkage, irregular nuclei and vacuolated cytoplasm and also numerous apoptotic spongiotrophoblast cells were scattered, hemorrhagic areas were present in between spongio trophoblast cells of the basal zone. Cystic degeneration of glycogen cells was observed. In the labyrinth zone, degeneration and necrosis of the trophoblasts, a diminution in thickness of the trophoblastic septa and irregular dilation of the maternal blood space and may be filled with cellular debris were scattered. Histological changes in labyrinth area showed decreased trophoblastic septa had lost their cellular architecture which act as a barrier that separates the maternal blood from embryonic capillaries; resulting in admixing of maternal and fetal blood and decreased vessels formation. Degeneration of the fetal blood vessels in the labyrinth zone also revealed (Fig. 6).

Liver

In the fetal liver sections showed normal hepatic structure (Fig. 7). The hepatic cells are large, polygonal in shape and had coarsely granulated cytoplasm. Also represent different types of blood forming cells as lymphocytes and erythroblasts. Liver sections of fetuses belonging to treated pregnant rats, showed more or less normal structure with some variation like dilatation of central veins and detached of endothelial cells that lining the central vein wall and lumen of vein continuous with the sinusoid. Increase in number of megakaryocytes (Fig. 7).

Kidney

Examination of the fetal kidney from control group revealed showed normal histological structures of the glomeruli, and renal tubules in the cortical (Fig.1) and medullary portions (Fig. 8).

Examination of the fetal kidney from treated rats, revealed some pathological changes in glomeruli and some parts of the urinary tubules they were represented by degeneration in the tubular lining epithelium and shrinkage and degeneration of glomeruli within the Bowman's capsule that led to a wide capsular space (Fig.8).

Brain

The brain tissues of fetuses from control pregnant rats showed normal features under microscopic observation (Fig. 9).

Examination of the fetal brain from treated group, revealed several histopathological alterations as presence of dark, dead and pyknotic neurons also degenerated and ghost cell neurons were evident and in some area the cerebral cortex showed disorganization appearance and dilated, enlarged and congested blood vessels were seen (Fig. 9).

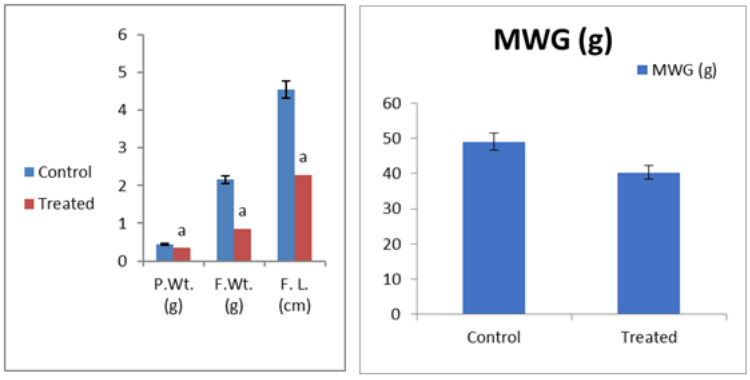

Fig. 1: Histogram showing effect of domperidone on fetus weight (F.Wt.), fetus length (F.L.), placenta weight (P.Wt.) and mother weight gain (MWG) at 20th day of gestation. Values are expressed as Mean ± SEM. The statistical differences were analyzed by independent samples T test. a= P ≤ 0.05 compared with control.

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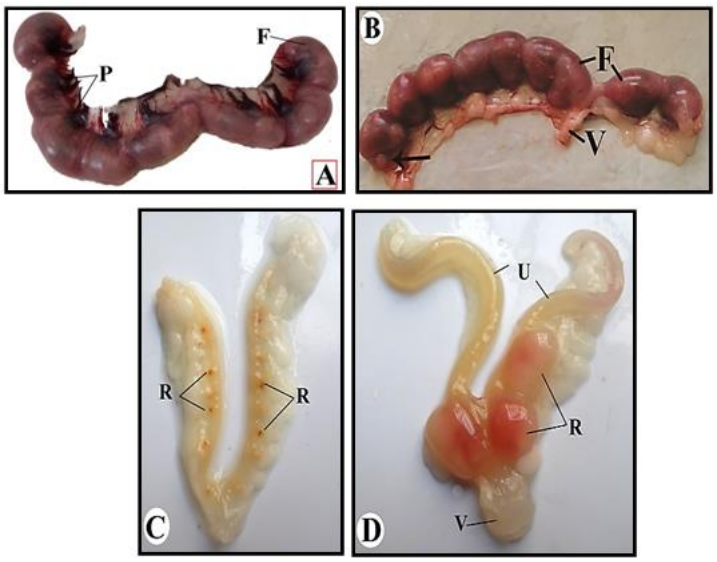

Fig. 2: Photographs of uterus of pregnant rat at the 20th day of gestation.
From control group showing: (A) Normal symmetrical distribution of fetuses (F) in the two uteri horns. From treated group showing: (B) Asymmetrical distribution of fetuses in the two uteri horns with resorbed site (arrow). (C) Uterine horns showing pinpoint hemorrhagic implantation sites (early resorption= R). (D) Uterine horns (U) showing clearly visible late embryonic resorption sites (R). V=Vagina, P=Placenta,

Fig. 3: Photographs of fetus at 20th day of gestation.
Fetus of control mother showing: (A) Normal morphology and normal length.
Fetuses of treated group showing: (B) Fetus with deformed hind limb (arrow). (C) Hematoma at the face (arrow). (D) Fetus with dark spots at head and back (arrow). (E) Fetuses with open eyes (○) and small size.

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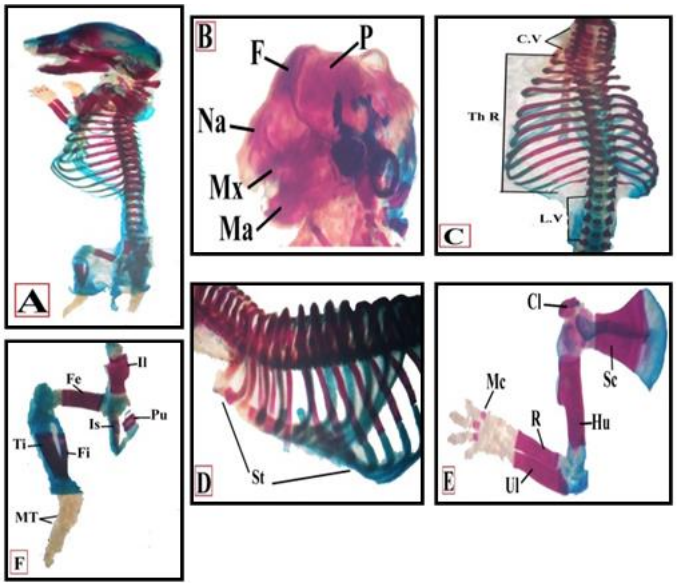

Fig. 4: Photographs of the control fetal skeleton.
Showing: A-E) well ossifies skeletal system. Fr= frontal, Pr= parietal, N= nasal,Mx= maxilla, Ma= mandible, Ce V= cervical vertebrae, Th V= thoracic vertebrae, Th R= thoracic rib, St= sternum, Cl= clavical, Sc= scapula, H= humerus, U= ulna, R= radius, MC= metacarpals, LV= lumbar vertebrae, CV= caudal vertebrae, I= ilium, Fe= femur, Ti= tibia, Fi= fibula and MT= metatarsus.

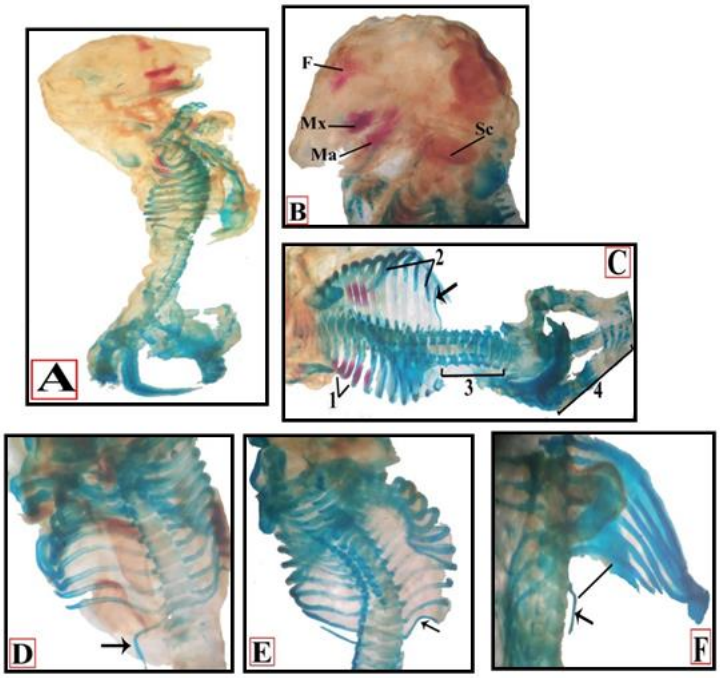

Fig. 5: Photographs of the fetal skeleton from treated group.
Showing: (A) completely unossified skeleton. (B) unossified skull bones and less ossified frontal (f), maxilla (Mx), mandible (Ma) and scapula (Sc). (C) less ossified thoracic ribs (1), unossified ribs (2) lumbar vertebrae (3) pelvic girdle and hind limb (4) and curved rib (arrow). (D-F) curved and wavy ribs (arrow) and costal separation (line).

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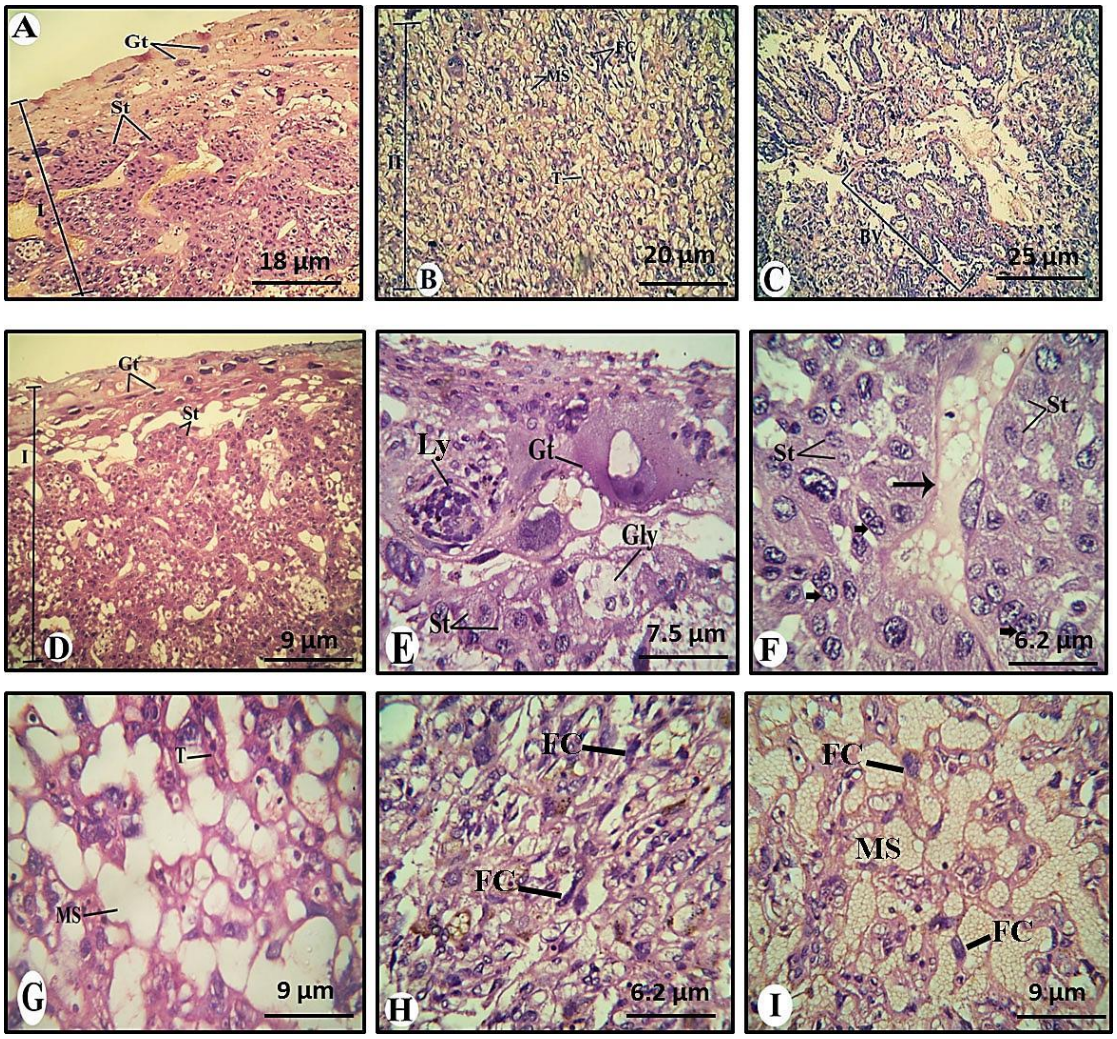

Fig. 6: Photomicrographs of a section of placenta of pregnant rat. H&E stain.
From control mother showing: (A) Basal zone (I); Ga= giant cell, St= spongiotrophoblast. (B) Labyrinth zone (II); showing trophoblastic trabeculae (T) consisting of trophoblasts and syncitiotrophoblast, fetal capillaries (FC) lined by endothelial cells containing fetal erythroblast and maternal sinusoid (MS) containing maternal erythrocytes. (C) Normal appearance of blood vessels lined with epithelial cells (BV).

From treated group showing: (D-I) Giant cells with irregular shape nuclei and cytoplasm, degenerated spongiotrophoblast (ST) and kayolysis (short arrow), hemorrhage (long arrow), cytolysis glycogen cell (Gly) and lymphocytes infiltration, apoptosis, necrosis and degeneration of trophoblasts in the labyrinth zone, a reduction in thickness of trophoblastic septa and irregular dilatation of maternal sinusoids and poor developed of fetal capillary (FC).

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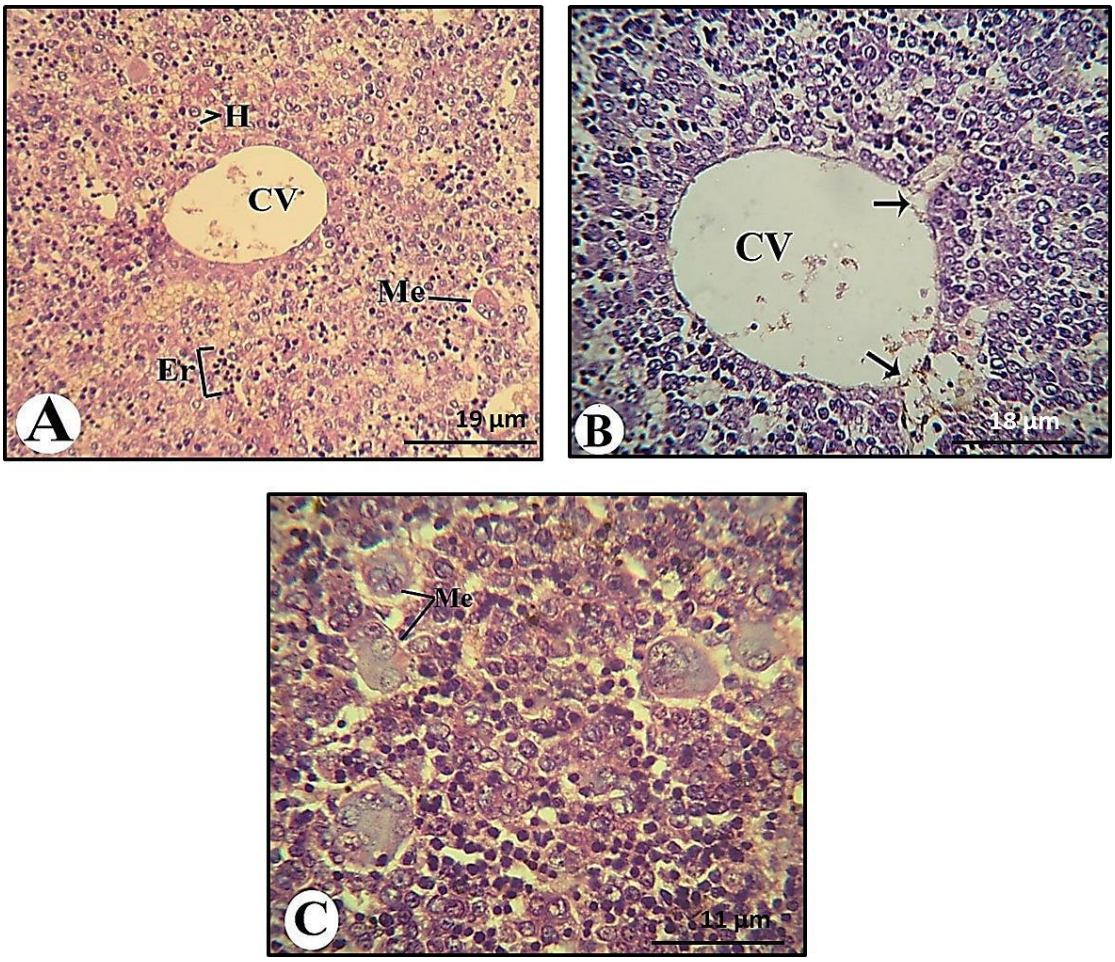

Fig. 7: Photomicrographs of a section of liver of a fetus. H&E stain.
From control mother showing: (A) Normal architecture of the liver tissue. The hepatic lobules that can be only distinguished by their central vein (CV), hepatocytes (H), numerous erythroblasts (Er) and megakaryocyte (Me).
From treated group showing: (B) Dilation of central vein (CV) and rupture of the endothelial cell that lining the wall of central vein (arrow). (C) Increased in number of megakaryocyte (Me).

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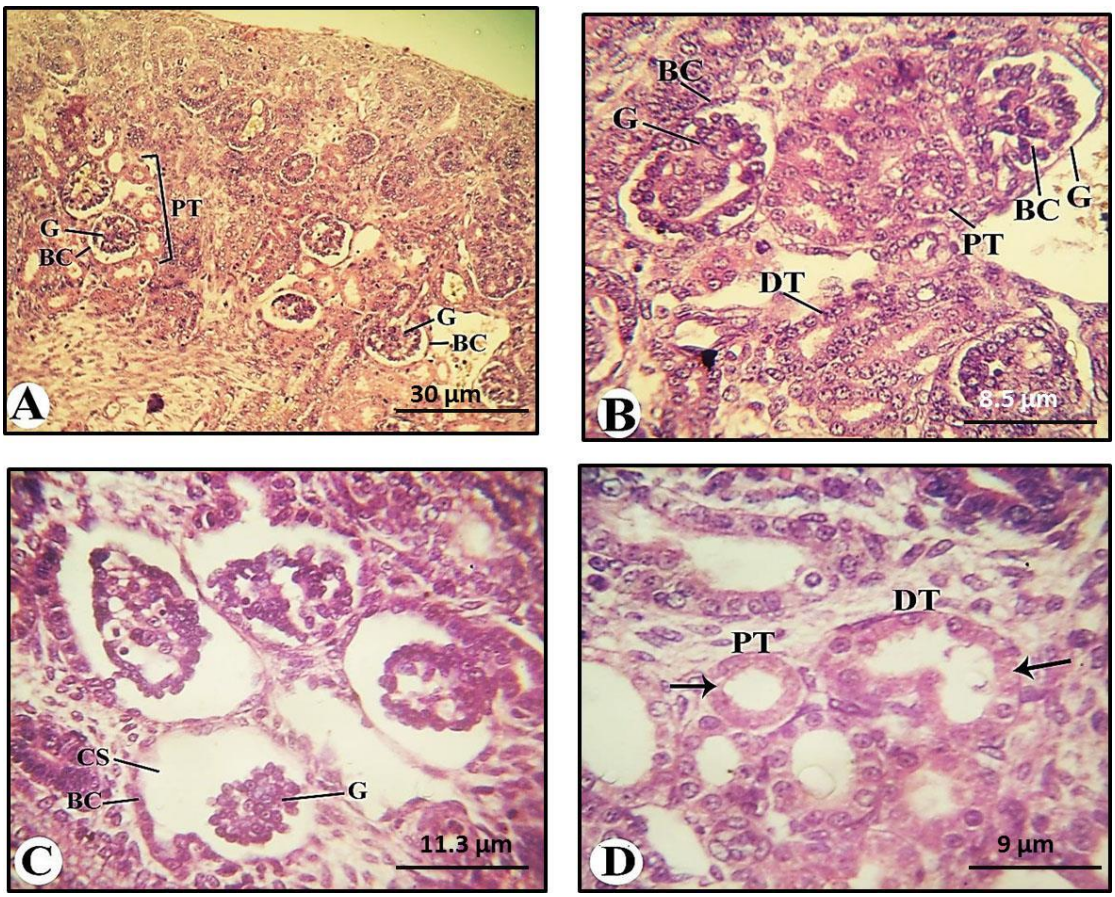

Fig. 8: Photomicrographs of a section of kidney of a fetus. H&E stain.
From control mother showing: (A& B) Apart of the cortical region containing, a glomeruli (G) with optimal size inside Bowman's capsule (BC) and tubules (T). PT=proximal tubule and DT= distal tubule.
From treated group showing: (C) shrinkage glomeruli (G) inside Bowman's capsules (BC) and wide capsular space (CS). (D) Degeneration of epithelium lining renal proximal tubules (PT) and distal tubule (DT).

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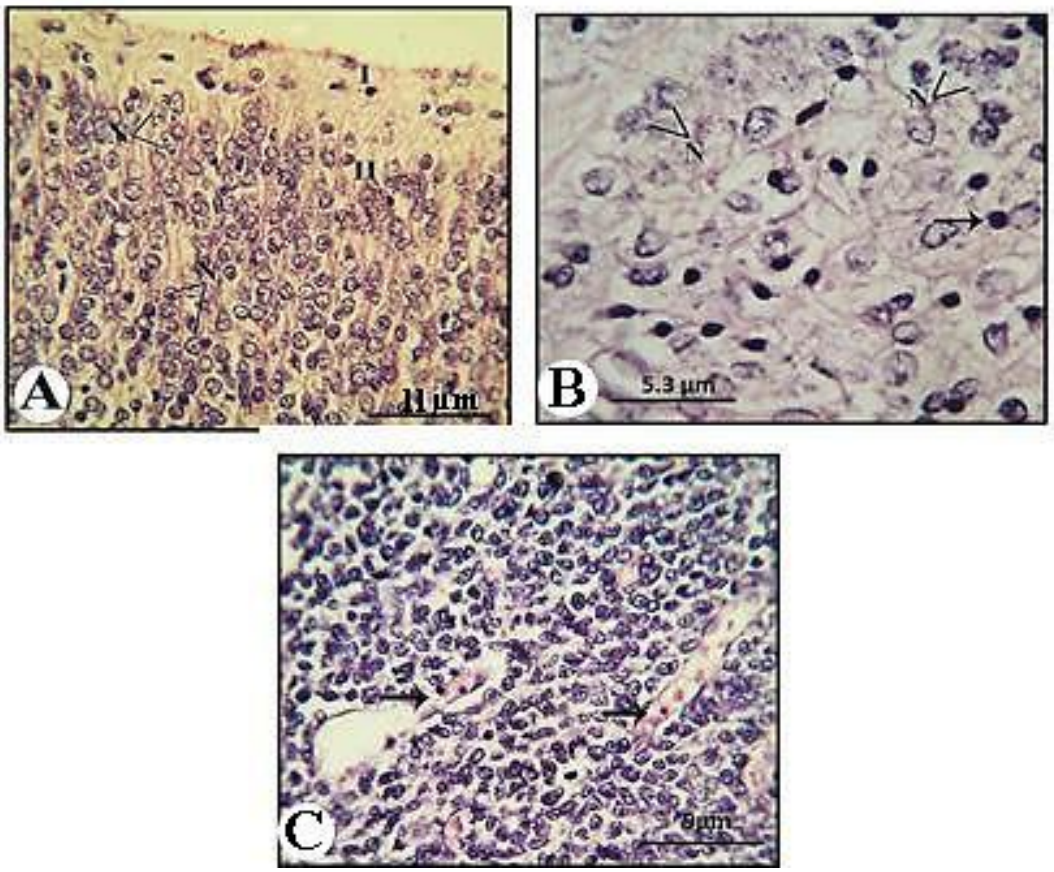

Fig. 9: Photomicrographs of a section of brain of a fetus. H&E stain.
From control mother showing: (A) Normal structure of brain tissue I (marginal layer) and II (cerebral cortex), neuron (N).
From treated group showing: (B) Disorganization of cerebral cortex appearance, pyknotic neuron (arrow) and degenerated nerve cell (N). (C) Dilated and congested blood vessel (arrow).

DISCUSSION

This study indicated that exposure to a drug may be associated with defects in several organ systems.

This work showed a significant reduction in the placenta weight, high incidence of the resotption, fetal growth parameters and high percent of the hematoma appeared on the fetal body as an external malformation. The previous finding may be occurred due to the direct action of the drug or its metabolites on embryos which can transfer via the placental barrier. Also the study revealed a significant decrease in the fetal weight and length. Reduction in the fetal body weight is an indication of growth retardation. The previous studies recorded that acute interruption of blood flow to the uterine horn leaded to growth retardation of the fetuses, in addition the lessening in the uterine vascularization thence lowered blood flow to the uterine horn and this induces fetal-placental growth retardation. Reduction in the fetal weight accompanied by delayed ossification of the fetal skeleton [7].

In the previous study by [8] examined the maternal and developmental effects of Bendectin (0, 200, 500, or 800 mg/kg/day) administered to timed-pregnant CD rats (36–41/group) during organogenesis (gestational days [GD] 6–15). At death (GD 20), all live fetuses were examined for external, visceral, and skeletal abnormalities. Developmental toxicity included reduced prenatal viability (800 mg/kg/day) and reduced fetal body weight/litter (500 and 800 mg/kg/day).No increase in percent malformed live fetuses/litter was observed. Another study by [9] investigates teratogenic risks with ondansetron and found No statistically significantly increased risk for a major malformation was found.

Meclizine and caffeine combination is used for the treatment of morning sickness. Both compounds are teratogenic. In previous study by [10], three doses were taken for the study and the mixture was

administered 8-14 days for embryotoxic study. The study showed significant reduction in fetal body weight, body length and body mass index. There was no increase in external or internal congenital anomalies at both low and medium dose.

In study by [11] used the whole embryo culture model to study the teratogenicity and toxicity of antiemetics and showed that the exposure of the dimenhydrinate, metoclopramide, tri-methobenzamideHCl to the cultured rat embryos decreased overall growth and developmental parameters in vitro in a concentration dependent pattern. It was also found that exposure to the antiemetics causes an increase in overall dysmorphology, including the incidence of haematoma, microcephally, abnormal tail torsion, odema, middle brain deformity, maxillary deformity, open neural tube, abnormal tail torsion and vertebral deformity in a concentration-related manner.

The present study suggested that the oral administration of domperidone to the pregnant rat induced delaying of the ossification of the skull bones (frontal and parietal), ribs, sternum and absence of ossification of metacarpals, metatarsals and phalanges also wavy, curved ribs and costal separation were seen which may be due to the decreased absorption rate of calcium throughout of the intestine and/or injuries induced in liver and kidney of mothers that are essential in the synthesis of vitamin D metabolites, which considered as the important source aiding in calcification of bone [12] our results were similar to the previous work by [8] they observed reduced ossification of metacarpals (800 mg/kg/day), phalanges of the forelimbs (500 and 800 mg/kg/day), and of caudal vertebral centra (all doses).

The placenta is a very important channel for the exchange of materials between the maternal and fetal blood [13]. The placental cells are the major source of the hormones required for the growth and development of the embryo [14, 15]. These hormones ensure pregnancy maintenance and fetal growth and development [16, 6]. The placenta not only provides a link between the circulation of two distinct individuals (maternal and fetal) but also acts as a barrier to protect the fetus from xenobiotics in the maternal blood [17].

The present results revealed pathological changes in the placenta. This is manifested as severe structural changes in rats of treated group (B), thus, may lead to reduced uteroplacental blood flow which appear to be associated with increased risk of poor outcome.

The giant cells possess phagocytic features that act to remove the dead cells and debris from the surrounding tissue [18]. Our study showed that, trophoblast giant cells were present in a huge number with cytoplasmic vacuoles and shrinkage nuclei. Spongiotrophoblasts have some immunological function [18, there were some degenerative changes in the spongiotrophoblasts and hemorrhage area between the later cells and cytolysis of the glycogen cells which normally tore glycogen and act as house of energy.

The current study illustrated necrotic foci of trophoblasts in the labyrinth zone. The labyrinth septa had lost their architecture, with fibrin deposition and decreased vessels formation. Fibrin deposition inhibits maternal perfusion of the placenta, which then causes placental necrosis that is often associated with fetal morbidity and mortality. Abnormality or impairment in the function of the placental tissue may be give arise to insufficient nourishment to the developing fetuses abnormal growth of the fetus will be occur, resulting in fetal blood flow redistribution [19,20].

The results showed that exposure to the therapeutic dose of domperidone induced mild changes in fetal liver. These changes varied from dilatation of central veins and detached of endothelial cells that lining the central vein wall and lumen of vein continuous with the sinusoid. Increase in number of megakaryocytes.

In the present study, the kidney of 20-days-old fetuses maternally treated with domperidone showed few histopathological changes. The cortical region showed shrinkage of the glomerular tufts and increase of the urinary space size, degeneration and vacuolation of the cytoplasm of the cells lining the convoluted tubules.

In this experiment, the histopathological changes in the fetal brain of the treated groups were evident in the early stages of development. As already noted, many nerve cell nuclei were pyknosed, presence of many

intensely stained “dark” neurons and tissue disorganization with signs of degeneration and congested blood vessels.

It was evident that the use of antiemetic (domperidone) in rat females during the “critical period” of gestation caused fetal growth retardation and less or moderate histopathological alternations in main fetal tissues. Therefore, domperidone is considered not being safe to the embryos and it should be used during pregnancy only under careful consideration of the risk benefit and further studies are required to understand the mechanism by which the domperidone affect the fetuses and their tissues.

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