Research Article

Study of Teratogenic Effects induced by Verapamil Administration in Pregnant Albino Rats

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Received: 05-07-2019; **Revised:** 24-08-2019; **Accepted:** 02-09-2019.

ABSTRACT

Investigate the safety of the verapamil on the fetal rats and their mothers by teratological and histopathological studies. Pregnant rats (*Rattus norvegicus*) were administrated orally with verapamil (10 mg/kg) during gestation period (5th -19th). Fetuses were removed from the uterus and evaluated for mortality rate, growth parameters, morphological and skeletal malformation as well as histological study. The results revealed that the verapamil induced a significant decrease in the growth parameters, hematoma at different fetal body parts and skeletal abnormalities included ribs anomalies, weak ossification of the skull bones roof and bones formed girdles and limbs. Our histopathological study showed that verapamil cause destruction and alteration in both fetal and maternal liver and placental tissue. It is highly recommended that the hypertension pregnant women avoiding to administrate verapamil during the pregnant period.

Keywords: Teratology, Pregnancy, Verapamil, Hypertension, Rats.

INTRODUCTION

The recent study showed that approximately 2% and 3% of new born in the manifest development suffer the birth defects which categorized in teratogen-induced malformations that percent due to expose the mother to a lot of factors as environmental or iatrogenic during pregnancy.[1]

A lot of problems can face the pregnant women due to the pathological changes that occur during the pregnancy one of this is hypertension which categorized one of the most urgent case that may lead to hard problems, this Elevated Blood Pressure (BP) is due to preeclampsia either alone (pure) or "superimposed" on chronic vascular disease.[2]

Calcium channel blockers prevent calcium from entering cells of the heart and blood vessel walls, resulting in lower blood pressure. Calcium channel blockers, also called calcium antagonists, relax and widen blood vessels by affecting the muscle cells in the arterial walls. Some calcium channel blockers have the added benefit of slowing heart rate, which can further reduce blood pressure, relieve chest pain (angina) and control an irregular heartbeat, for example verapamil.

Verapamil was given a pregnancy Category C rating because of potential problems in animal studies. When given to pregnant rabbits, verapamil increased the risk of miscarriages and decreased fetal growth. However, animals do not always respond to medicines in the same way that humans do, and a healthcare provider may still prescribe verapamil if the benefits outweigh the risks.

In the present study we will investigate the effects of our drug on the pregnancy outcomes, fetal growth parameters, fetal mortality rate, resorption rate, morphological malformations, skeletal anomalies and placental and fetal histology.

MATERIALS AND METHODS

All the experimental protocols and procedures used in this study were approved by the Cairo University, Faculty of Science Institutional Animal Care and Use Committee (IACUC) (Egypt), (CUFS/Comp&Emb/CU/I/F/13/18).

Mature female and male rats (Wistar strain) housed in stainless steel cages and maintained at a temperature of 25±2 ˚C, relative humidity of 50±5% and photoperiod at 12 h dark/12 h light. Animals were fed on commercial standard locally prepared rat pellets and water free from antimicrobials to withdraw any antibacterial residues. Two female rats caged with a male overnight. Mating is confirmed in the next morning by the presence of spermatozoa in the content of the vaginal smear and / or the observation of the copulatory plug in situation; these findings designate first day of presumed gestation.

The tested drug was verapamil hydrochloride (ISOPTIN® AND ISOPTIN SR®). The solutions were prepared by dissolving the tablet in distilled water in such a way that 0.125 mL of solution contains desired concentration, as therapeutic dose 10 mg/kg .[5]

Pregnant rats were weighed and randomly divided into two groups, ten rats each.

Group 1 (10) = Control; Received distilled water by gastric tube.

Group 2 (10) = Administrated orally by 10 mg/ Kg of chose drug from 6th- 19th gestational day (GD).

Morphological examination of the uteri and fetuses:

At GD 20, the dams were weighed, sacrificed and the laparotomy process was performed with exposure of the uterine horns. The uteri from each female were removed, weighted and opened using a scissor and then the fetuses were separated. Numbers of implantation sites (using a magnifying lens) and viable, dead and resorbed fetuses were calculated. The percentages of post-implantation loss, pre- implantation loss and corrected weight gain were calculated.

Corrected weight gain= (WG20-WG5) - gravid uterus

Pre-implantation loss= (number of corpora-number of implantation)/ (number of corpora) X100

Post-implantation loss= (number of implantation- number of living fetuses)/ (number of implantation) X100

The live fetuses were dried on a bloating paper, weighed and examined for external morphological abnormalities. Moreover, the placental weights were recorded.

The examination of the cartilage skeletal elements was done to investigate the skeletal abnormalities.[6]

Histological examination

Samples from placenta, maternal and fetal liver were fixed in 10% neutral buffered formalin for 24 hours at room temperature, then dehydrated in alcohol, cleared in xylol and embedded in paraffin wax. Then sectioned at 5µm thickness and stained with haematoxylin and eosin.[7]

Statistical analysis

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

RESULTS

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 was non-significant change in treated mother weight at the beginning of the experiment (5th GD) and/ or at the end (20th GD) of the treatment also the mother corrected weight gain showed non-significant difference in comparison with control group.

In the current work there was a significant (P < 0.05) decrease in the placenta weight of the treated mother when compared with the control one. The uterus from control group revealed normal distribution of the implanted fetuses between the two horns while the uterus of pregnant rats treated with verapamil showed asymmetrical distribution of fetuses in the two uteri horn (Table 2).

Table 1: Showing effect of verapamil on mother corrected weight gain and placental weight at 20th day of gestation.

Groups	Mother corrected weight gain (g)	Placenta weight (P.WT) (g)
Control	13.15 ± 7.85	0.515 ± 0.08
Treated	9.83 ± 4.01	0.395± 0.022[a]

Values are expressed as Mean ± SEM. a= P < 0.05 compared with control.

Table 2: Showing effect of verapamil on pregnancy outcomes.

Item	Control	Treated
Total pregnant rat	10	11
No. of pregnant rat carrying to term (%)	9(90)	8(72.72)
No. of implantation sites	58	66
Total live fetuses	58	63
No. of dead fetuses	0(0)	0(0)

Values are expressed as Mean ± SEM. a= P < 0.05 compared with control.

Effects on fetuses

Maternal administration of verapamil caused growth retardation represented by a decrease in fetal body weight. There was a significant (P < 0.05) reduction in fetus weight (1.80± 1.48[a]) in treated group when compared with the control group (2.9 ± 0.775) (Table 3).

Table 3: Showing effect of verapamil on fetal growth parameters at the 20th GD.

Groups	Fetus weight (F.WT) g
Control	**2.900 ± 0.775**
Treated	**1.80 ± 1.48[a]**

Values are expressed as Mean ± SEM. a= P ≤ 0.05 compared with control.

Morphological malformations

The fetus from control animals have normal shape, correct weight and length (Fig. 1) and with straight dorsal side in the head region, have closed eyes with upper and lower eyelids and also have fully developed ear pinnae. Both fore and hind limbs are formed of well-developed bones and their extremities had well developed digits. The abdominal region is acquiring a cylindrical shape ending with the tail.

The most observed morphological anomalies in the fetuses from treated group were hematoma (red patches at different parts of bodies), club foot and dropped wrist (Fig. 1&Table 4).

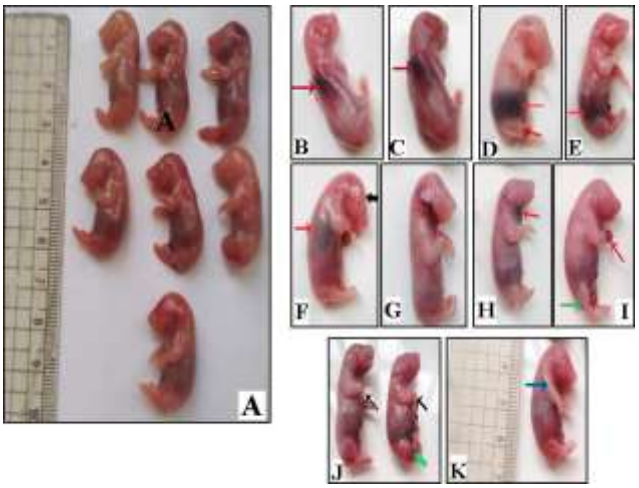

Figure 1: Photographs of fetus at the 20th day of gestation.
Control showing: A) Normal morphology and normal length.
Treated showing:
B-I) Hematoma at different body parts (red arrow).
F) Abnormal head curvature (head arrow).
I&J) deformed hind limb and club foot (green arrow).
J) Dropped wrist (black arrow).
K) Deformed fore limb (blue arrow).

Skeletal anomalies

There were signs of skeletal anomalies in fetal skull from treated pregnant rats which represent as incomplete ossification or absent of ossification of the nasal, frontal, parietal, inter-parietal bones, lack ossified degree in all vertebrae completely un-ossified sternebrae, wavy and bent ribs, costal separation (wide angle between two successive ribs), and weak ossified and un-ossified ribs, incomplete ossification of all bones and lack of ossification of the radius and ulna, un-ossified ilium, ischium, tibia, fibula, phalanges and less ossified femur (Fig. 2) .

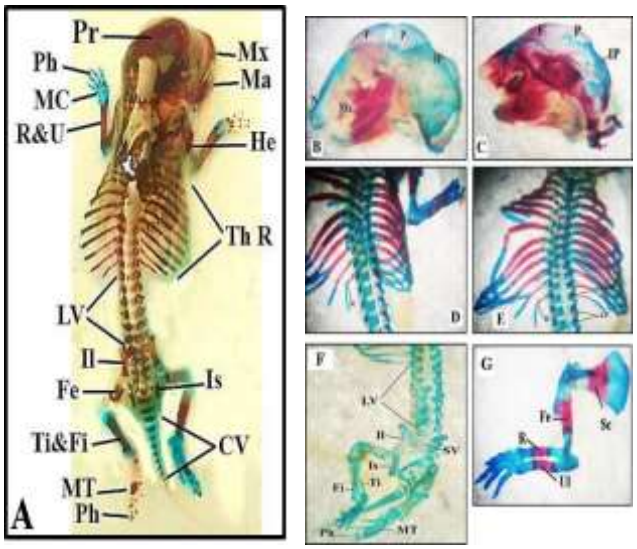

Figure 2: Photomicrographs of the fetal skeleton. Alizarin & Alcian stain.

Control showing: A) Well ossified skeletal system.
Treated showing:
B) Un-ossified cranial bones.
C) Incomplete ossification of all cranial bones.
D&E) Curved and weak ossified last rib (R).
F) Un-ossified ilium, ischium of the pelvic girdle and incomplete ossified tibia, fibula, metatarsal and phalanges of the hind limb.
G) Less ossified scapula, humerus, radius and ulna of the fore limbs and un-ossified metacarpals.

Ce V= cervical vertebrae, CV= caudal vertebrae, Fe= femur, Fi= fibula, Fr= frontal, H= humerus, Ii= ilium, LV= lumbar vertebrae, MC= metacarpals, MT= metatarsus, N= nasal, Pr= parietal, R= radius, Sc= scapula, Th V= thoracic vertebrae, Ti= tibia and U= ulna.

Histopathological observations

Placenta

Rat placenta composed of two zones, the first one called junction zone which consists of mainly outer giant cells that separated exteriorly located maternal deciduas basalis and inner trophospongium with highly packed basophilic spongioblast cells. The second zone named labyrinth zone, which is composed of communicated network of the maternal lacuna and embryonic capillaries. The maternal-embryonic nutritional and gaseous exchange is take place in the labyrinth (Fig. 3). The decidua of the control group was comprised of small flat, oval cells with bundles of fibers and homogenous cytoplasm.

The basal layer of control group consisted mainly of three types of trophoblast cells: giant, basophilic and glycogen cells. The giant cells were big oval cells with pale cytoplasm and pale central nuclei. They were located near the decidual layer. They were found sometimes singly or in groups. The basophilic cells (spongiotrophoblasts) had basophilic cytoplasm and central vesicular nuclei. The glycogen cell, mostly arranged in clusters, had very pale cytoplasm with very fine fibers and central nuclei.

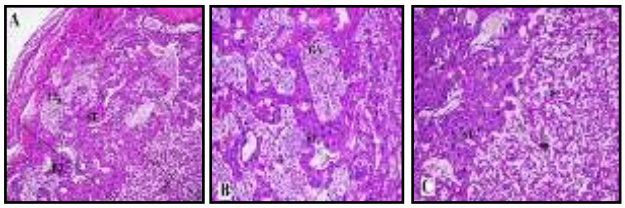

Figure 3: Photomicrographs of a section of placenta on the 20th day of gestation from control pregnant rat. H&E stain.

Showing: A) Placenta with normal thickness & structure, Basal Zone (BZ) with its cells spongiotrophoblasts (ST), giant trophoblast (G) and glycogen cell (Gly) and Labyrinth Zone (LZ) 100X. B) Normal structure and thickness of basal layer with healthy spongiotrophoblasts (ST) and scattered glycogen cell (Gly) 100X. C) Spongiotrophoblasts (ST) and Labyrinth zone; 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 100X.

Light microscopic examination in the placenta of treated rats, placenta structure showing hypoplasia (thinning) of basal layer and metrial layer, cystic degeneration of glycogen cells (cytolysis) and glycogen cells became more numerous and prominent and occupied most of the thickness of the basal layer. Basal zone showed different stages of degenerated spongiotrophoblasts (basophilic cells), in the form of vacuoles in their cytoplasm and their nuclei also appeared degenerated, decreased in size and some of them were pyknotic. Many of them underwent cytolysis and degeneration and were replaced by empty spaces creating big cysts. There was more deposition of fibrinoid material within the cell cluster and trophoblasts giant cells & giant cells were frequent and increased in size. Some cells showed signs of degeneration, smaller darker nuclei, deposition of dark acidophilic substance in the periphery of the cells.

Other cells showed signs of death, markedly dark cytoplasm and small dark (pyknotic) peripheral nuclei (Fig. 4). The labyrinth layer of placenta revealed irregular dilation of maternal sinusoid with deposition of fibrin and hemociderin pigment in trophoblastic septa, a reduction in thickness and destruction of trophoblastic septa and also showed necrotic trophoblasts cells.

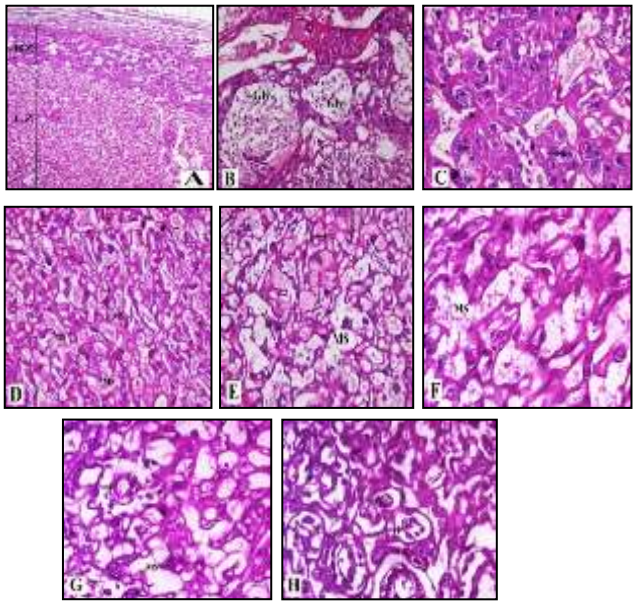

Figure 4: Photomicrographs of a section of placenta on the 20th day of gestation from treated mother. H&E stain.

Showing: A) Hypoplasia of basal layer (BZ) and labyrinth layer 100X. B) Cystic degeneration of glycogen cell (cytolysis) 400X. C) Degeneration of spongiotrophoblasts (ST), pyknotic changes (arrow), fragmentation of nucleus of spongiotrophoblasts (Bold arrow) and hemorrhagic site (head arrow) 400X. D-F) Labyrinth zone increase in thickness of trophoblastic septa with deposition of fibrin (T) and irregular dilatation of maternal sinusoids (MS) 400X. G&H) Labyrinth layer with poor developed blood vessel with degenerative epithelial cells (arrow) 400X.

Maternal Liver

Hepatocytes are the main functional cells of the liver and represent approximately 80% of the total liver mass. The later cells are polygonal in shape and their sides can be in contact either with sinusoids (sinusoidal face) or neighboring hepatocytes. The blood sinusoids are irregularly dilated vessels, lined with a discontinuous layer of endothelial cells. Each hepatic cell has a central nucleus with a distinct nuclear membrane and one or more prominent nucleoli (Fig.5).

The hepatic tissue showed histopathological alterations including both degeneration and necrosis of the hepatocytes. Degeneration was represented by the presence of empty space in the cytoplasm. The hepatocytes were markedly hypertrophied and possessed coarsely granulated cytoplasm. The nuclei of some degenerated cells showed variations in size, shape and chromatin content. Sinusoids were markedly dilated, congested and proliferation of vonkuffer cells. Portal area appeared with dilation of bile duct in the portal area, hyperplesia of its epithelial lining and newly formed bile ductile, with infiltration of the portal area by mononuculear infilammatory cells (Fig. 5).

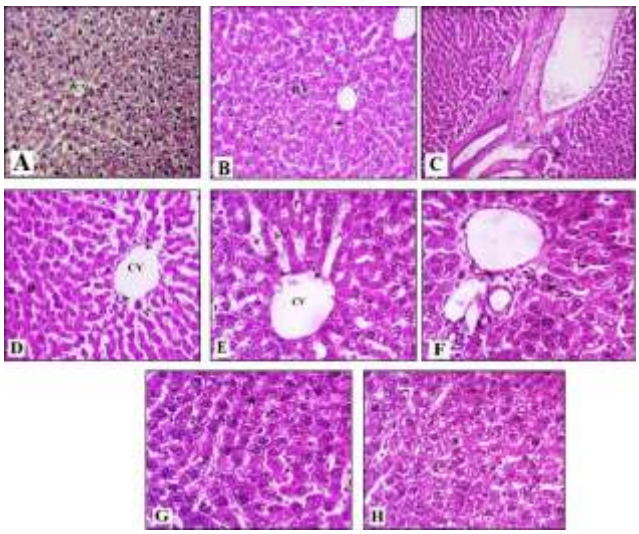

Figure 5: Photomicrographs of a section of maternal liver on the 20th day of gestation H&E stain.

Control showing: A) Normal hepatic tissue structure; normal central vein (CV), hepatocytes (H) and sinusoid (Si) 100X.

Treated showing: B) Vascular degeneration of hepatocytes (arrow) and disarrangement of hepatic port with necrosis hepatic cell (bold arrow) 100X. C) Portal area with connective tissue proliferation and infiltration mononuclear cell (head of arrow) 100X. D&E) Injured hepatocytes (H), dilated sinusoids (S) and rupture of endothelial cells (head arrow) lining central vein 400X. F-H) Hepatocytes with cytoplasmic vacuoles (H), necrotic changes (*) and thick endothelial cells lining portal vein invaded by monocytes (arrow) 400X.

Fetal liver

Fetal liver histology is different from adult liver, the fetal liver at 20th day of pregnancy is covered with a very thin capsule consisting of a single elongated mesothelial cells. The hepatic lobules are composed of irregular branched and interconnected hepatic strands of 1-3 cells thick radiating from the central vein to the lobule periphery. As the liver in the late embryonic stages acts as a hemopoietic organ, a considerable number of different stages of erythroblasts and few megakaryocytes are found in between the hepatic cells (Fig.6).

Examination of the liver sections of fetuses belonging to treated pregnant rats, showed marked loss of the lobular architecture and disorganization of the hepatic strands. Histopathological alterations including both fatty degeneration and necrosis were observed. These degenerated hepatocytes possessed pyknotized, karyorrhexed or karyolysed nuclei. Blood sinusoids were markedly dilated with marked aggregation of erythroblasts, notice increase numbers of megakaryocytes cells (Figure 6).

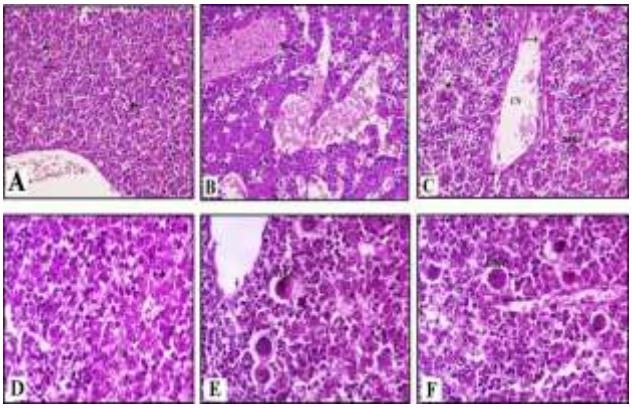

Figure 6: Photomicrographs of a section of fetal liver on the 20th day of gestation. H&E stain.

Control showing: A) Normal architecture of the liver tissue with intact hepatocytes (head of arrow) and diffuse population of hematopoietic cells at different stages, erythroblasts (bold arrow) and megakaryocytes (arrow) 100X.

Treated showing: B&C) Congested central vein (arrow) with congested and dilated sinusoids (S) 100X. D-F) Central vein (CV) with degeneration in endothelial lining (arrow) scattered hemorrhagic spots (bold arrow), necrotic areas (*), unhealthy hepatocytes, dilated sinusoids (S), hepatic cells with fragmented nuclei (arrow) and increase in megakaryocytes number (MKC).

DISCUSSION

Hypertensive disorders of pregnancy are one of the major causes of maternal morbidity and mortality leading to 10–15% of maternal deaths, especially in developing world.[8]

Our study was designed to evaluate the teratogenic effect of veramapil on the growth and development of the fetuses of albino rats. The treated rats showed no external signs of toxicity. No mortality cases were recorded; all the treated dams were survived to the end of study but showed a significant loss in their weight compared with the control mother. The reduction in maternal body weight may be due to reduced food intake, or as a result of developmental toxicity of the used drug as displayed by reduced weight of gravid uterus due to reduced number of alive fetuses and mean fetal weight and increased incidence of early and later resorption, or may be due to metabolic disorders in maternal body.[9]

The previous study [10] revealed significant decreases in body weight of dams in the treated group throughout the gestation period than those in the control rats. In contrast, there were no differences in the placental weights between the two strains.

In the previous study by [11] stated that all females survived to scheduled study termination on GD 20. There were no abnormal clinical signs in all groups. There were no significant differences in the maternal body weight gain in any of the treatment groups at any time during the study. There were, however, significant reductions in the final absolute maternal body weight in dams treated with ivermectin plus verapamil compared to all other groups.

Our study showed that treated rats showed asymmetrical distribution of fetuses in the two uteri horns and reduced number of fetuses, uterine horns showed clearly visible embryonic resorption sites and also showed pinpoint hemorrhagic implantation sites. Increase of resorption sites resulted in a significant reduction of uterine weight compared to the control group at the mentioned dose. Oral administration of the therapeutic doses of veramapil to female rat from 5th day up to 19th days of gestation induced growth retardation represented by a decrease in fetal body weight and body length.

Tuchman-Duplessis[12] attributed the decrease in the percent of viable fetuses and their weights to the accumulation of the drugs in the fetal body than the maternal body. Such accumulation could be enhanced by the very simplified structure of rats' placenta which allowed the passage of drugs from their circulation and concentrated in fetal tissues or act as inhibitors of membrane enzymes involved in embryonic nutrition. It is a fact that the fetal body weights reflect the fetal development and neonatal mortality coupled with the concept that many chemicals may destroy cellular active DNA and so reduced biosynthesis of essential components, like protein and energy source (ATP and NAD/NADP) and consequently the fetal growth.[13] Furthermore, Collins[14] reported that the mechanisms of action of most teratogens occurred through interference with nucleic acid replication/transcription, or RNA translation, deficiency of energy supply for metabolism of the organism by restricting the availability of substrates either directly or through the presence of analogs or antagonist of vitamins, essential amino acids and others.

The placental weight of all treated pregnant rats group was significantly decreased as compared to control. It can be said that, the previous prevailing phenomenal of teratogenicity may attributed to toxicity of verapamil due to accumulation of this drug in certain organs, placental barriers, endometrial layer of uteri as well as placental dysfunction.

In the previous study[15] evaluated maternal and neonatal outcomes of pregnancy in women with cardiovascular disease who were treated with an α/β-adrenergic blocker (carvedilol) or a β-adrenergic blocker (propranolol, metoprolol, atenolol, or bisoprolol), and we identified 3 important clinical issues. First, as a group, the use of β-adrenergic blockers was associated with an elevated risk of fetal growth restriction (FGR), whereas the use of an α/β-adrenergic blocker (carvedilol) was not. Second, in the β group, a longer treatment duration increased the probability of giving birth to an infant with FGR. And third, among β-adrenergic blockers, propranolol and atenolol were associated with the highest incidences of FGR. This highlights the fact that different β-blocking drugs can have a different effect on FGR. Particularly, in 5 patients on bisoprolol, no instances of FGR were noted, while FGR was found in 36%, 17%, and 33% of women treated with propranolol, metoprolol, and atenolol, respectively.

Since earlier, calcium channel blockers are widely prescribed for therapeutic purposes and some have been recommended for use during pregnancy for defined indications; e.g. verapamil for treating fetal paroxysmal tachycardia[16] and during tocolytic therapy.[19] These therapeutic uses of calcium channel blockers are recommended at late pregnancy after organogenesis has already been completed.[20] Calcium channel blockers have been shown in animal experiments to induce teratogenic effects and to increase the incidence of embryolethality in mammalian animals.[21] In addition, verapamil causes malformations, edemas and reduced heart rate in the embryos, larvae and adult fish .[24] Verapamil inhibits glucose uptake in insulin-sensitive tissues such as adipocyte skeletal myocytes and cardiac myocytes.[27] Verapamil also inhibits the glucose transport activity of GLUT 1 in a dose-dependent manner[30]. These effects of verapamil are consistent with the retinopathy induced by verapamil treatment in pregnant animals of this study.[31]

Stein et al., 1990[20] worked on rat embryos (9.5-day-old) were cultured for 48 h in the presence of nifedipine (NIF), nimodipine (NIM), nitrendipine (NIT), gallopamil HCl (GAL), verapamil HC1 (VER) and diltiazem HC1 (DIL). The effects on growth and morphogenetic differentiation in vitro were monitored. The abnormalities concerned yolk sac circulation and morphology, as well as heartbeat, the morphology of the heart, head, neural tube, or forelimbs, and the shape of the embryo. The abnormal embryos were also growth retarded (decrease in protein content and crown-rump length). Interference with calcium channel functions seems to represent an interesting model for studying a special kind of abnormal prenatal development, especially the differentiation of certain mesenchymal structures.

Published data on the embryotoxic potential of calcium channel blockers in vivo are only available for NIF and DIL. Besides an increased rate of embryomortality, NIF induced teratogenic effects in the cardiovascular system,[32] defects of the finger skeleton, i.e. hyperphalangy, [33]short limbs, oligodactyly, short tail, and hematomas.[34] Some of these defects could be induced by single oral administrations of 20-150 mg NIF/kg body wt on 1 day between days 10 and 16 of pregnancy. For NIF additional data from the drug manufacturer in the form of a report to the regulatory agencies (information given to physicians), point to a teratogenic potential of this substance and it is mentioned that disturbances in postnatal development occur at daily doses of 30 mg NIF/kg body wt or higher.

Reproductive toxicity has furthermore been studied with DIL in mice, rats and rabbits,[35] using i.p. injections. While treatment of mice during days 7-12 of pregnancy induced embryo mortality only (with concentrations of or exceeding 12.5 mg DIL/kg body wt) various teratogenic effects were observed subsequent to single doses of 25 or 50 mg DIL/kg body wt given on days 9 or 13. In rats (treated during days 9- 14 of pregnancy) 80 mg DIL/kg body wt was found to be teratogenic, as was the case following a single dose on day 13 or 14; the resorption rate was clearly increased at this dose. Studies with rabbits revealed an increased resorption rate (70%) at 12.5 mg DIL/kg body wt (when applied on days 7-16) and an apparent increase in abnormalities in the few (approximately 20) surviving fetuses.

In our study skeletal defects of fetuses have been observed. These defects included incomplete ossification of some skull bones, irregular shape ribs and incomplete ossification of ribs. Delaying of the ossification and severs skeletal anomalies may be

due to mesenchymal condensation during embryonic development, or may be due to resorption of cartilage, during embryonic development, which precedes endochondral ossification.

In the previous study by (El-Ashmawy et al., 2010)[11] showed that, there was a significant increase in the incidence of visceral and skeletal variations in the group treated with verapamil plus ivermectin in comparison with other groups. The most prominent skeletal malformations observed consisted of delayed ossifications of skull, vertebrae and sternebrae and absence or fused ribs. Other visceral and skeletal variations observed throughout the experimental groups were generally of low incidence and not significantly different between the control and treated groups. Rat fetuses from dams treated with ivermectin plus verapamil showing incomplete ossifications of vertebrae, ribs and skull.

In the current study showed notice hypoplasia of metrial gland cystic degeneration of glycogen cells, basal zone with different stages of degenerated sponagiotrophoblasts and trophoblasts giant cells, the labyrinth layer of placenta notice irregular dilation of maternal sinusoid with deposition of fibrin and hemociderin pigment in trophoblastic septa, severe irregular dilation of maternal sinsusoids, a reduction in thickness and destruction of trophoblastic septa, degeneration and necrosis of trophoblasts cells.

In the previous study[36-38] the placenta of the treated rats showed similar architecture to that of the control. With slightly high dose of Trichloroacetic acid the cytotrophoblasts around the embryonic capillaries were undergoing degeneration. The trophoblasts in the trabeculae showed necrotic foci as well as degeneration of the trophoblastic barrier, which resulted in the admixture of the embryonic erythroblast with the maternal erythrocytes.

The current study showed vascular degeneration of hepatocyte and disarrangement of hepatic cords with some necrosed hepatic cells, atrophied hepatocytes with dilation of sinusoid and proliferation of vonkuffer cells and infiltration of the portal area by mononuclear inflammatory cells.

The fetal liver assumes the primary role of blood cell development at mid- and late-gestation.[39] Hepatocytes from the control group showed prominent nuclei and well demarcated cell boundaries as compared to malformed hepatocytes with ill-defined margins, nuclear fragmentation and condensation in the treated group, the changes in the hepatocyte probably resulted from the cellular damage produced by verapamil during the process of development.

The current study showed dilated and congested central vein and sinusoids, patches of hemorrhage in the hepatic parenchyma, loosely arranged and degenerated hepatocytes with mononuclear cells infiltration, notice increase numbers of megakaryocytes cells.

The shady swelling may be displayed because of aggravations of membranes function that lead to enormous deluge of water and Na+ because of veramapil impacts. Cell swelling may be joined by spillage of lysosomal hydrolytic enzymes that causes cytoplasmic degeneration and macromolecular overcrowded.[40]

The presence of inflammatory cells in the hepatic tissue may recommend that verapamil could cooperate with proteins and enzymes of the hepatic tissue meddling with the antioxidant protection system and causing receptive oxygen species (ROS) formation which may mimic an inflammatory reaction.[41]

Kupffer cells actuation may show that veramapil enact the phagocytic movement of the sinusoidal cells by elevating the Kupffer cells number helping in evacuating the collected verapamil where lysosomes are associated with the intracellular breakdown into small metabolic substances. The Kupffer cells hyperplasia may be associated with the measure of harmful to the hepatic tissue instigated by veramapil intoxication and symbolizes a protective method of detoxification. Kupffer cell hyperplasia is participated in hepatic oxidative stress .[42]

CONCLUSION

It was evident that the use of verapamil in rat females during the gestation caused fetal growth retardation histopathological alternations in main fetal tissues. So, verapamil is considered not being safe to the embryos and it should be used during pregnancy only under careful consideration of the risk benefit.

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Source of Support: Nil, **Conflict of Interest:** None.