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Research Article
Deleterious Effects of Perinatal Exposure to Isotretinoin Drug on the Offspring of Pregnant Mice

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Abstract

Background and Objective: Isotretinoin cytotoxicity showed to produce more than 35% of congenital defects during pregnancy. Thus, the aim of this study was to evaluate cellular and congenital malformations induced by isotretinoin treatments of female mice during pregnancy. **Materials and Methods:** A total of 30 female pregnant albino mice were recruited in this study. The animals were classified into 2 groups; the control group (n = 15) and isotretinoin treated group (n = 15). In all groups, pregnant mothers were injected intraperitoneally either with isotretinoin (1 mg kg^{-1}) or with olive oil (0.5 mL) during the stage of organ formation on day 10 of pregnancy. After 20 days of pregnancy, morphometric, morphological, biochemical and histological techniques were evaluated to estimate abnormalities in uterus, ovary, cellular damages in the liver and the kidney of pregnant mothers and their new offspring's. **Results:** In this study, isotretinoin at a dose of 1 mg kg^{-1} b.wt., significantly produced severe cytotoxicity on liver and kidney tissues of pregnant mothers and their new progeny and significantly performed congenital malformations in the development of offspring. Histological, cellular and biochemical examinations signified that isotretinoin cytotoxicity proceeds via cellular apoptotic mechanisms. In mice group treated with isotretinoin, significant fibrosis, apoptosis and both liver and kidney dysfunction were reported in pregnant mothers and their new progeny. **Conclusion:** The significant liver and kidney dysfunction with congenital malformations in the development of the new offspring's were reported in pregnant mice treated with isotretinoin at a dose of 1 mg kg^{-1} b.wt. The data signified that isotretinoin cytotoxicity proceeds via cellular apoptotic mechanisms.

Key words: Isotretinoin, apoptotic mechanisms, morphometric analysis, hepato-renal toxicity, congenital malformations, cellular damages, kidney dysfunction

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Isotretinoin is one of vitamin A derivatives which has different names as xeractan, accutane, roaccutane and absorbica, respectively. The drug showed significant potentiality in the treatment of acne nodules and its inflammatory lesions[1-3]. In addition to that, isotretinoin showed significant activity against active tumors. It works to bind and activate the nuclear receptors of the acid retinoic (RARs) and activation of these receptors act as catalysts that promote the differentiation of cells and programmed death thus minimizing tumor activity, as well as promoting immune responses and anti-inflammatory agents[3]. Although, isotretinoin has potent effects against acne, several studies recommended its association with many side effects associated with its use such as; increased risk of skin infections, bowel inflammation and functional liver disorders[3-7]. Several studies showed that the use of isotretinoin during pregnancy led to abnormalities in the fetal nervous system and brain abnormalities[8-11]. The toxic effects of isotretinoin proceed via reduction of AMH serum levels and decline ovarian reserve[5,9-11]. Also, isotretinoin was shown to toxically effects on liver and kidney functions with cellular and tissue damage whereas both an increase in liver enzymes and renal dysfunctions were recorded previously[12,13].

Previously, uses of isotretinoin for long times with higher doses showed to produce autoimmune hepatitis with a significant increase in liver and lipid function tests in patients, notably a rise in plasma triglyceride concentrations. Symptoms of nausea, indigestion and abdominal pain were also observed, whereas constipation and anal bleeding were the most common symptoms[1,8,14,15].

In experimental animals, isotretinoin toxicity showed to effect on glutathione content, enzymatic antioxidant and catalase activity in the liver tissues with higher abnormalities in liver function[4]. Initial laboratory results revealed severe renal insufficiency, liver damage and abnormal lipid profile following administration of roaccutane[16,17]. Also, severe abnormalities documented in neonates whose mothers treated with isotretinoin during pregnancy[18]. For the importance of isotretinoin in treating skin disease especially against acne and that right recommended doses and time prescribed may help in avoiding its side effects. Thus, the aim of this study was to evaluate side effects of isotretinoin at a dose of 1 mg kg^{-1} b.wt., which may rarely studied on the embryos and mothers especially congenital malformations, liver and kidney damages. In addition, the proposed cytotoxic mechanism of isotretinoin in producing cellular and congenital malformations were significantly evaluated.

MATERIALS AND METHODS

Materials: In this study, 30 female albino mice housed in healthy atmospheric conditions, normal feeding, drinking and medical care based on the guidelines of the experimental animal care, College of Science, King Saud University, Riyadh, Saudi Arabia. The Ethics Committee of the Experimental Animal Care Society at King Saud University approved the experimental procedures (Permit Number: PT 1252). To obtain healthy environments, the animals distributed in special cages equipped with drinking water in their ventilated rooms subject to the appropriate natural factors of moisture, light and temperature ranging from 25-35°C.

Feeding: The animals given the appropriate food, which is the animal feed experiment No. P 648 obtained from the General Organization of grain silos and flour mills in Riyadh and it consisted of: Raw protein 20%, Phosphorus 6.0%, Raw fat 5.3%, Vitamin A 20% International U g^{-1}, Ash 6% Vitamin D 2.2% International U g^{-1}, Calcium 1% Vitamin E 70% International U g^{-1}, Salt 5.0%.

In addition to the rare mineral elements: cobalt, copper, iodine, iron, manganese and zinc, water are left as needed. The experimental animals selected at an average age of 12-15 weeks and the average body weight was 60 g.

Isotretinoin (roaccutane) uses: Isotretinoin used in this study purchased in the form of capsules from one of the pharmacies in Riyadh. Each capsule contained active dose of 10 mg/60 kg of human weight. Using an injection needle, isotretinoin in a dose of 1 mg kg^{-1} b.wt., dissolved in saline solution injected into the peritoneal cavity on the 10th day of pregnancy as previously reported by Kim *et al.*[10]. The average therapeutic dose of this drug in human weight is 10 mg/60 kg, thus the mean weight of the mouse used in this study (60 g).

Estimation of oestrous cycle: Mice or rats typically have rapid cycle times of 4-5 days. In addition, the ovaries of cycling rats or mice contain three different sets of corpora lutea at different phases of development[19-21].

The stage of oestrous cycle identified by using the vaginal swab examination if the vaginal smear contains a small number of coronary cells and many of the epithelial cells prepared and there are no white blood cells known as the pre-oestrous cycle stage. The next day, the coronary cells proliferated which known as the second oestrous cycle stage[19-21].

Female mice fertilization: Before the second oestrous stage, each of the female mice placed with a healthy male in the breeding cages throughout the night. In the following morning, fertilization with male sperms performed through the vaginal swab[19-21]. In this method, a fine absorbent containing 9.0 drops of saline solution injected with the vagina and the released vaginal discharge applied on slides to dry. Then, the slides stained with 1% blue dye solution for 2 min and washed again with distilled water to remove excess dye. Finally, dried slides then examined by the microscope to detect the presence of sperm and the beginning of pregnancy was calculated since the emergence of sperm.

In all groups, pregnant mothers anesthetized on the 20th day of pregnancy and dissected and the mother's womb extracted, weighed, examined and photographed before opening for embryo extraction.

Experimental design: In this experiment, pregnant female mice divided into two groups; Group 1 (control group; n = 15); female mice injected interpretationally by 0.5 mL of olive oil; Group 2 (Treatment group, n = 15); female mice received isotretinoin interpretationally with a dose of 1 mg kg^{-1} b.wt. Isotretinoin and olive oil injected interpretationally to mice of control and treated groups on day 10 of pregnancy as previously reported in the literature. Whereas, at day 10 of pregnancy, organs of the fetus are formed in this period (stage of organs formation)[10].

On the 20th day of pregnancy, all pregnant mothers anesthetized and samples from liver, kidney, ovary of the mother and uterus with embryos removed and preserved in solutions buffers until reused in the study. Uterus containing embryos was first examined carefully to identify dead, live or distorted progeny and efficiently photographed and the liver and kidney tissues of embryos were taken for further histological analysis.

Assessments of liver and kidney function: Liver and kidney function tests performed according to routine lab methodology previously reported in the literature[22-24].

Assessment of morphometric analysis: All mothers' groups examined carefully to calculate the number of progeny alive, dead or deformed and calculate the percentage for each stage.

Assessment of morphological features the progeny: The embryos examined after extraction from the mother's womb to study changes in the phenotype and to monitor and photograph all the resulting deformities.

Assessment of histological analysis: All samples of the liver and kidney removed from mothers and embryos and stored in a 10% neutral formalin solution for hematoxylin-eosin histological examinations.

The METAVIR system applied to measure the score and the degree of fibrosis in liver and kidney tissues and no fibrosis was defined as F0, mild fibrosis as F1, moderate fibrosis as F2, severe fibrosis as F3 and cirrhosis as F4. Significant fibrosis were also defined as F2-4. Hepatic inflammatory activity and apoptotic index were also scored as before[25].

Statistical analysis: Statistical analysis was carried out with SPSS (Statistical Package for Social Science) program version 10 for Windows (SPSS Inc., Chicago, IL, USA). All data tabulated as Mean±SD. The statistical differences performed by using one-way analysis of variance (ANOVA) and Student's t-test. The $p<0.05$ considered statistically significant.

RESULTS

Assessments of liver and kidney function: In isotretinoin treated groups, significant increase in the concentrations of creatinine (mg dL^{-1}), blood urea (mg dL^{-1}), SGPT (U L^{-1}), SGOT (U L^{-1}) and lower concentrations of albumin were reported in pregnant mothers and their offspring's compared to those obtained in control group (Table 1). In addition, Total Body Weight (TBW), relative liver weight and relative kidney weights were significantly increased in pregnant mothers and their offspring's of isotretinoin treated groups compared to control ones (Table 1).

Assessments of fibrosis score and apoptosis in liver and kidney tissues: The METAVIR system was applied to measure the score and the degree of fibrosis in liver and kidney tissues. Significant fibrosis (F: 2-3) were significantly reported in pregnant mothers 12 (80%) and the new progeny (offsprings) (73.4 %) of isotretinoin treated mice. Compared to pregnant mothers, isotretinoin treated offsprings showed less or no fibrosis score (n = 3; 20%) compared to pregnant mothers of the same group (n = 4; 26.6%), respectively.

In addition, apoptosis in liver and kidney cells was significantly highly scored in pregnant mothers and the new progeny of isotretinoin treated mice compared to those present in control group (Table 1). The data obtained signified that isotretinoin cytotoxicity proceeds via cellular apoptotic mechanisms.

Table 1: Changes in body weight, kidney weight and the levels of liver and kidney function biomarkers in isotretinoin drug intoxicated experimental rats

	Groups			
	Control		Isotretinoin treated group	
Biological parameters	Mother	Offspring	Mother	Offspring
Final weight (g)				
TBW	169.00±2.3	126.00±1.4	178.00±3.6[a]	138.00±2.8[b]
LW	0.46±0.12	0.26±0.11	0.68±0.19[a]	0.48±0.16[b]
KW	0.59±0.23	0.32± 0.14	0.84±0.42[a]	0.58±0.21[b]
Kidney function				
Creatinine (mg dL^{-1})	0.71±0.24	0.48±0.14	2.9±1.3[a]	2.8±0.19[b]
Urea (mg dL^{-1})	23.60±2.8	19.50±1.5	86.6±10.2[a]	49.5±5.7[b]
Liver function				
SGPT (U L^{-1})	21.6±5.6	18.3±3.5	56.9±8.4[b]	39.8±7.1[b]
SGOT (U L^{-1})	28.2±2.4	25.4±3.6	75.4±5.1[b]	58.7±6.1[b]
Albumin (mg dL^{-1})	4.8±1.2	4.2±1.8	3.1±0.18[b]	3.7±1.8[b]
Fibrosis score: (No. (%))				
No fibrosis (0-1)	-	-	4.0 (26.6%)	3.0 (20%)
Fibrosis (2-3)	-	-	12.0 (80%)[b]	11.0 (73.4%)[b]
Apoptotic index	0.42±0.03	0.22±0.12	2.69±1.4[b]	1.69±0.98[b]

All values represent Mean±SD, [a]p<0.01, [b]p<0.001, Student's t-test, GOT: Glutamic oxaloacetic transaminase, GPT: Glutamic pyruvic transaminase, TBW: Total body weight, LW: Liver weight, KW: Kidney weight

Morphological studies: Pregnant mother scarfully subjected for morphometric studies. Both control and treated groups showed no records of maternal deaths. Morphological examination of the uterus of pregnant female mice in control and isotretinoin treated groups was significantly evaluated on the 20th day of pregnancy. In control group, both uterus and uterine horns showed equal distribution and of embryos showed equal organization with them as shown in Fig. 1a. However, in isotretinoin treated groups; both uterus and uterine horns showed unequal length and the embryos were unequally distributed. One side was shorter than the other which also containing lower numbers of embryos were observed in the womb (Fig. 1b-d).

Regarding to mice fetus, the rates of the progeny present alive were estimated. The ratios of alive embryos to dead ones were calculated. The data showed that embryos of control group of 15 pregnant female mice were all alive with (96%) only 4 % dead offspring's. However alive embryos appeared to be good and vital and there were no mutated embryos. In isotretinoin treated group, in which 15 pregnant females were used, the average live embryo of this group was 66%, the dead fetus was 33% and the mutilated fetus was 38% as shown in Fig. 2.

Morphometric studies: Embryos or fetuses examined for any morphological changes like their mothers by day 20 of gestation. In control group, normal appearance of fetus in the mother's womb was observed before and after the extraction from the womb sheath (Fig. 3a-c). However, significant changes in appearance such as small size, warp in the limbs, skin transparent, bleeding under the skin, shortness and deformation of the limbs, the emergence of the brain and the emergence of the heart outside the body figure were significantly reported in isotretinoin treated group (3 C) compared to normal embryos.

Shapes of mice fetuses by day 20 of gestation were significantly studied following the treatment with isotretinoin. Small size, skin transparency, congestion in blood vessels, deformation in limbs, sub-cutaneous blood clots were the most abnormalities present in embryos (Fig. 4a-c), respectively.

Histological analysis

Histological composition of liver in pregnant mice: Normal liver architecture with uniform hepatocytes appearance in H and E stained sections were reported in liver tissues of control group. Hepatocellular polyp cells showed normal granular cytoplasm containing a uniformly spherical nucleus, which has one or more nuclei (Fig. 5a). The liver tissues of isotretinoin treated mice showed signs of cellular destruction with a focal confluent necrosis, scattered foci of lytic necrosis in H and E sections (Fig. 5b). Isotretinoin elaborates apoptosis and cell death parameters such as; pyknosis and chromatin condensation (Fig. 5c).

Histological structure of the liver in the embryos: Normal intact liver architecture was reported in liver H and E sections tissues of control embryos (Fig. 6a). The data showed normal appearance of hepatocellular polyarthritis with a granular

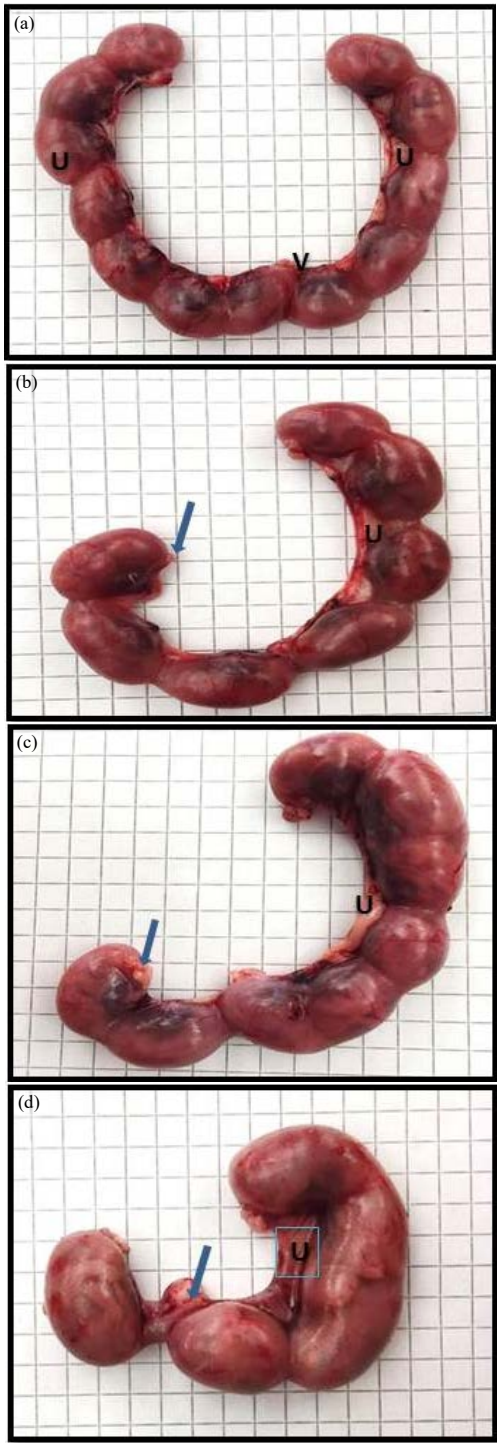

Fig. 1(a-d): Photographs of uterus of pregnant rat, (a) Control uterus at the 20th day of gestation showing normal distribution of fetuses in the two uterine horns and (b-d) Uterus of pregnant rats treated with 1 mg kg^{-1} isotretinoin showing asymmetrical and partial distribution of fetuses
Resorption site (arrow), U: Uterus, V: Vagina

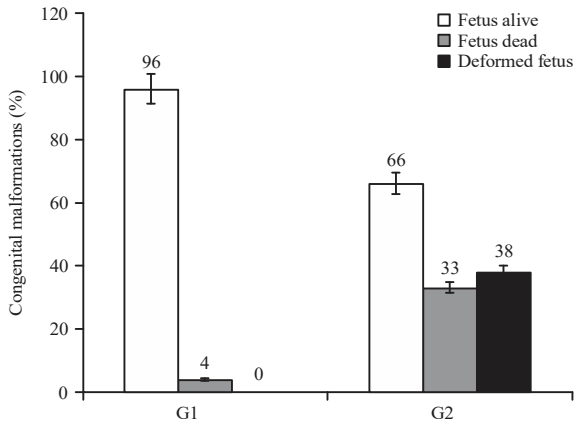

Fig. 2: Congenital malformations (%) in the development of offspring at the 20th day of gestation of pregnant mice treated with isotretinoin
G1: Control group, G2: Isotretinoin treated group

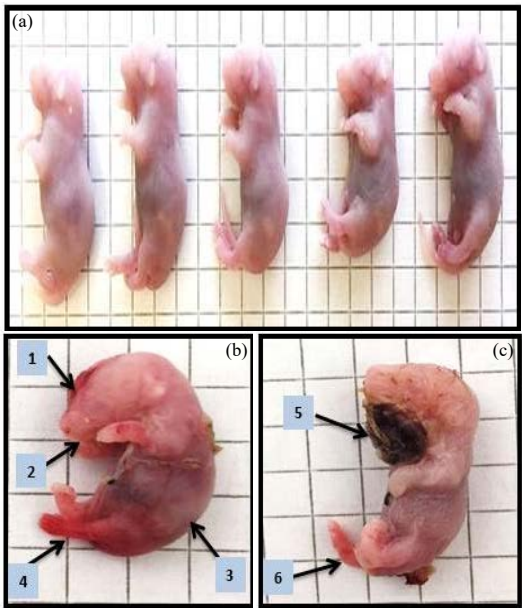

Fig. 3(a-c): Photographs of fetuses at 20th day of gestation, (a) Normal fetus, showing normal growth, (b) Fetus of maternally treated with 1 mg kg^{-1} of isotretinoin, showing emergence of, 1: Brain outside the head, 2: Appearance of the pubic lip, 3: Body curvature, 4: Hematoma at the upper limbs and (c) Fetus of maternally treated, 5,6: Hematoma at the lower jaw and fore limb paralysis

cytoplasm and a uniformly spherical nucleus with one or more nuclei are present. In addition, degeneration in the hepatocytes, congestion of blood vessels, focal replacement

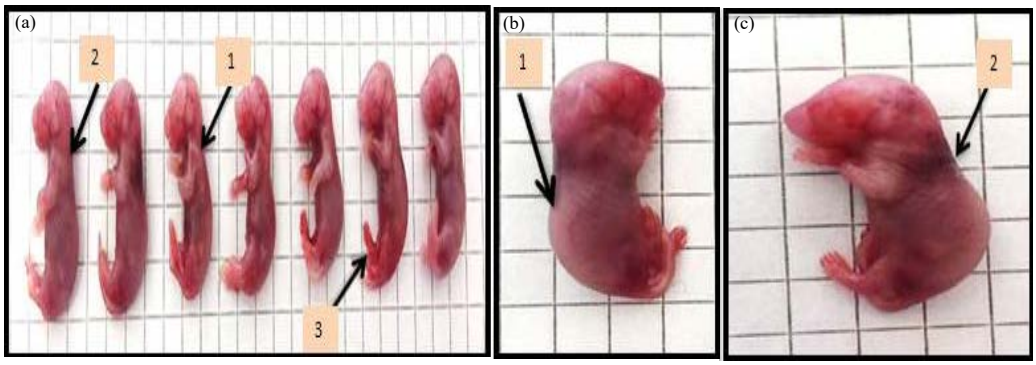

Fig. 4(a-c): Photographs of fetuses at 20th day of gestation, (a) Fetus of maternally treated with 1 mg kg^{-1} of isotretinoin, 1: Showing small size and thin and more transparent skin, 2: Appearance of hematoma under the skin at the upper limbs, 3: Foot loss and fore limb paralysis and (b-c) Fetus of maternally treated showing 1: Body dwarfisms, 2: Subcutaneous blood clots

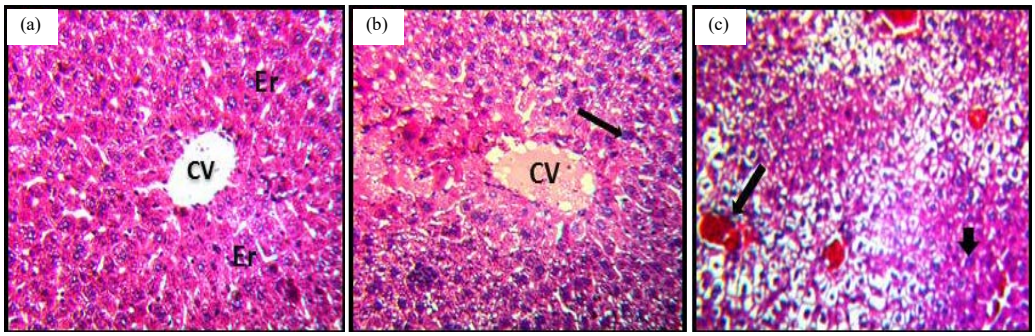

Fig. 5(a-c): Photomicrographs of liver section of pregnant mice at 20th day of gestation, (a) A control section, showing no histopathological alteration. The central vein with its intact endothelial lining (CV), Er: Erythroblasts, (b) Section of liver of mothers maternally treated with 1 mg kg^{-1} isotretinoin, showing congested central vein (CV) and megakaryoblasts (Arrow) and (c) Section of liver of mother maternally treated showing, congested of blood sinusoids (arrow) and pyknotic cells (Head arrow)
H and E stain, Bar: 1 μm

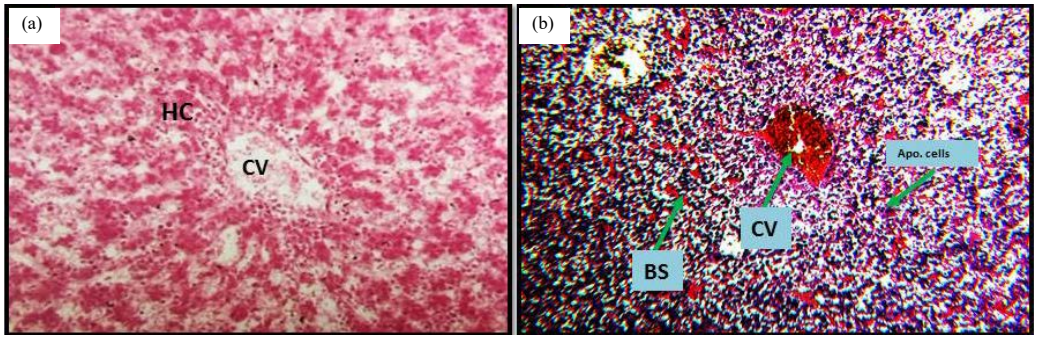

Fig. 6(a-b): Photomicrographs of fetal Liver section at 20th day of gestation, (a) A control section, showing no histopathological alteration. The central vein with its intact endothelial lining (CV). The central vein surrounded by well-organized hepatic cell in a ribbon like structure which enclosing blood sinusoids internally (HC) and (b) Section of fetal liver maternally treated with 1 mg kg^{-1} isotretinoin, showing congested central vein (CV), congested of blood sinusoids (BS) and pyknotic cells (Arrow)
H and E stain, Bar: 1 μm

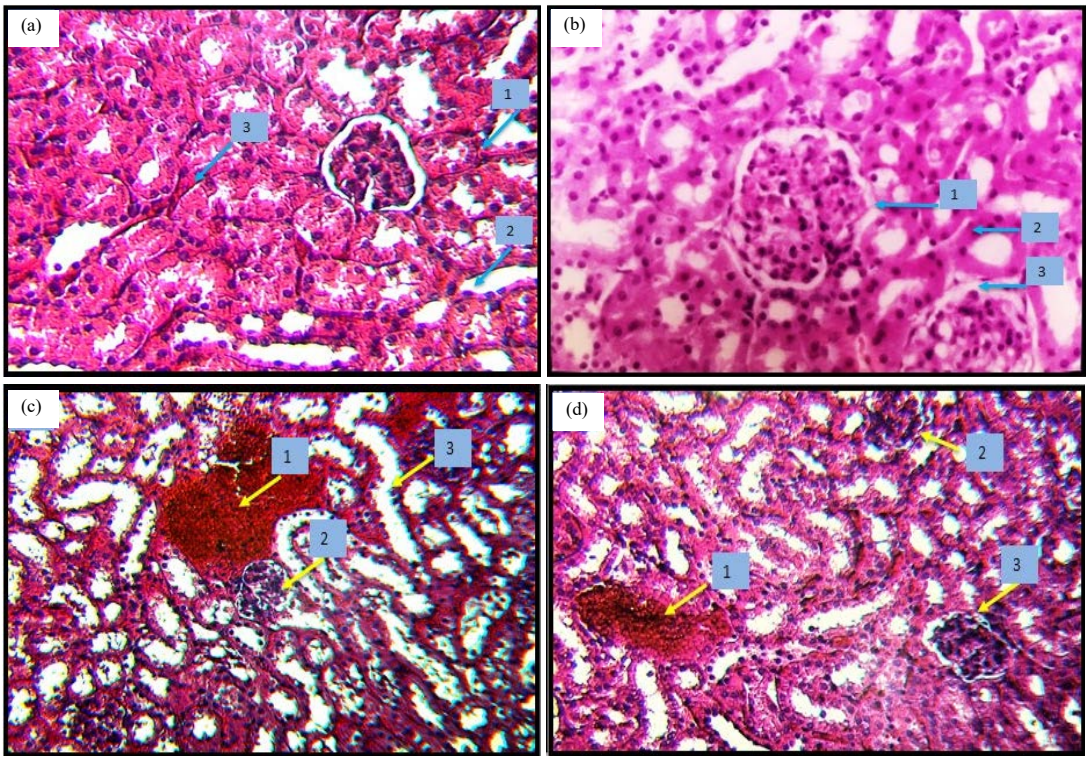

Fig. 7(a-d): Photomicrographs of mother kidney sections, a control section, showing no histopathological alteration, (a) Kidney sections showed normal texture with 1: Normal kidney globules and 2: Close proximal tubules, (b) Normal kidney section with 1: Normal circular nephron and 2, 3: Pre and post convoluted tubules and (b, d) Section of fetal liver maternally treated with 1 mg kg^{-1} isotretinoin, 1: Showing tissue bleeding, 2: Pyknotic cell with the nuclear chromatin condensation and congestion and 3: Narrowing of the glomerular cavity in most parts of the tissue
H and E stain, Bar: 1 μm

of the hepatic parenchyma and liver cell apoptosis was reported in isotretinoin treated group as shown in Fig. 6b.

Histological structure of the kidney in pregnant mice: Kidney tissues form both mothers were evaluated by histological analysis. In control group, kidney tissues of mothers showed normal texture and that the kidney is made up of two cortex cortices and appears granular because it contained renal pellets, renal tubules, medulla, the universal tubules and renal hoops. In kidney, H and E sections showed normal renal cortex, tubules, parenchyma and glomeruli medulla were observed in the kidney of control mice as shown in Fig. 7a, b.

In animals treated with isotretinoin showed congestion of the cortical blood vessels, focal replacement of the renal parenchyma with numerous lymphocytes infiltrates and dilation of glomeruli (Fig. 7c, d).

DISCUSSION

Isotretinoin (roaccutane) is an effective drug and is used primarily in patients with severe cystic acne over the past 30 years and is widely used to treat acne that does not respond to topical treatment whoever, there are many side effects associated with its use[1-7]. In this study, the results showed ideal size of the uterus with normal distribution of the embryos in control pregnant mothers compared to isotretinoin treated group. Whereas, in isotretinoin treated mice, deformation in uterus with abnormal distribution in the embryos were reported. Isotretinoin toxicity produced unequal length in horny uterine with constriction in the uterus and fetal distribution of the embryos compared to control mice. The data obtained confirmed the previously reported toxicity of isotretinoin on organs especially liver, kidney as well as deformations in uterus and new progeny[3-7].

The toxic effect of isotretinoin on the new progeny or embryos was estimated by calculating both alive and dead progeny. The data showed that normal embryos are observed in control pregnant mice mothers, where 15 of the pregnant mice were used, the average live ratio was 96% and lower dead scores (4%) were reported without deformations. In treated groups, isotretinoin in which 15 pregnant females were used, the average live embryos of this group were 66%, the dead fetus 33% and the mutilated fetus 38%. The toxicity elaborated by isotretinoin treatment supported in such a way the previous studies, which confirmed abnormalities in the fetal nervous system and brain abnormalities[8-11]. Isotretinoin showed to produce reduction of AMH serum levels and decline ovarian reserve[5,9-11]. Also, cellular and tissue damage as well as abnormal functions of liver and kidney, whereas both an increase in liver enzymes and renal dysfunctions were recorded previously after intoxication with isotretinoin[12,13]. In addition, in this study, the embryos were examined after extraction from the mother's womb by day 20 of gestation to study the changes in the phenotype. In isotretinoin treated embryos, deformations parameters such small size, warp in the limbs, skin transparent, bleeding under the skin, shortness and deformation of the parties were significantly reported compared to normal health fetus. The current results are consistent with many studies by Rojas *et al.*[8], Henry *et al.*[9], Kim *et al.*[10], Korkmaz *et al.*[11], Roodsari *et al.*[12] and Korolczuk *et al.*[13], which described the newborn with serious and multiple deformities after the use of isotretinoin by the pregnant mother, despite the previous mother's knowledge of the complications of taking the drug during pregnancy. The first postpartum physical examination of the fetus revealed a congenital absence in both eyes, both atria and anal rectification and ring cleft[7]. Also, researchers identified 4 generations of female isotretinoin users (aged 12-48 years) for the period[9] 1996-2011. A total of 59,271 patients received isotretinoin. Out of 118 live births, 11 (9.3%) had congenital malformations. In addition, Shirazi *et al.*[18] noted that severe abnormalities were documented in neonates whose mothers were treated with isotretinoin during pregnancy. Women who have been pregnant after one course of treatment are thought to have a more serious deformity[18,19,22].

Previous studies showed that genetic defects are the main cause of central nervous system malformations, abnormalities in the middle of the hindbrain caused by isotretinoin have occurred during pregnancy[5,7,11,22]. In human studies, 6 pregnant women were exposed after 30 days of gestation to isotretinoin. The toxicity of isotretinoin resulted in the death of a fetus within the uterus in 16 weeks of pregnancy and appeared newborns with major congenital malformations[17,18,21,23].

In this study, histological analysis was significantly evaluated on liver and kidney tissues of pregnant mothers and fetus. The effect of isotretinoin was evident on the hepatic tissues, where the tissue was damaged and the hepatic cells lost their normal appearance with signs of destruction and cellular death. Their cells appeared to be shrunken and dark and there was a marked deterioration in the endothelial lining. In addition, significant histological changes in kidney tissues were reported, such as the decomposition of some cells as well as the intensification of the chromatin in others, shrinkage of renal glomeruli and a narrow appearance in the cavity which finally produces large areas of destruction and visible bleeding. The cellular damage in liver and kidney tissues as well as renal and liver malfunction following isotretinoin toxicity were in line with other previously reported by Ahmad[1], Alzoubi *et al.*[3], Saied and Hamza[15] and Opel *et al.*[16], which supported initiation of fibrogentic, apoptotic and oxidative stress mechanisms whereas significant increases in serum levels of liver and kidney functions along with an increase in cellular lipogenesis and oxidative free radicals with lower cellular antioxidant enzyme's in animals following isotretinoin exposure[15,16].

The data in this study was supported with significant increase in the total body weight and relative weight measures of both liver and kidney of pregnant mothers of mice and their new progeny. The obtained data was agreed with other studies which reported higher toxicity of isotretinoin in human and experimental animals, whereas isotretinoin produced human autoimmune hepatitis[8-10] and severe oxidative stress in liver tissues via affecting on glutathione content, enzymatic antioxidant and catalase activity[4,8,9]. In addition, higher abnormalities in liver function[4]. Initial laboratory results revealed severe renal insufficiency, liver damage and abnormal lipid profile following administration of roaccutane[16,17]. Also, severe abnormalities were documented in neonates whose mothers were treated with isotretinoin during pregnancy[18]. Isotretinoin caused significant biochemical changes in liver function and lipid of patients such as; elevated cholesterol, ALT and AST with high triglycerides, especially with twice daily dose[15-17].

In this study, fibrosis and apoptotic scores were shown to be higher in liver and kidney tissues of pregnant mothers of mice and their new progeny, which treated with isotretinoin. The data obtained signified that isotretinoin

cytotoxicity proceeds via cellular fibrogenesis and apoptotic mechanisms. Isotretinoin toxicity was proposed to initiate or increase in the cellular oxidative damage and consequently the release of active cellular free radicals which significantly associated with liver and kidney damage as well as congenital malformations or death of fetus within uterus of mother's rats[13,14,18,23,24]. This study implicated that exposure to isotretinoin during treatment of acne produces more toxic effects on liver, kidney and leads to congenital malformations during pregnancy. The study also recommended the use alternative substitutes of plant origin rather than isotretinoin to treat acne especially during pregnancy to avoid congenital malformations.

CONCLUSION

This study concluded that isotretinoin cytotoxicity proceeds via cellular apoptotic mechanisms. In this study, isotretinoin at a dose of 1 mg kg^{-1} b.wt., significantly produces severe cytotoxicity on liver and kidney tissues of pregnant mothers and the new progeny and significantly performed congenital malformations in the development of offspring. Histological, cellular and biochemical examinations signify that isotretinoin cytotoxicity proceeds via cellular fibrogenesis and apoptotic mechanisms.

SIGNIFICANCE STATEMENT

This study confirmed that exposure to isotretinoin produces more toxic effects on liver, kidney and leads to congenital malformations during pregnancy. The study also recommended the use alternative substitutes of plant origin rather than isotretinoin to treat acne especially during pregnancy to avoid congenital malformations.

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