The histopathological effect of diclofenac potassium on maternal and fetal tissues

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ABSTRACT

Diclofenac (DCF) is one of the most generally utilized non-steroidal anti-inflammatory therapies during the world for different diseases curing in post pubertal ladies. Be that as it may, constrained data is accessible with respect to its security during pregnancy and teratogenicity. The present study was done to investigate the histopathological impact of diclofenac potassium (DP) on some vital maternal and fetal tissues, placenta and liver during two different periods of gestation. In this study 30 pregnant rats have been used and divided into 3 groups (ten for each one); group 1 (control), group 2 treated with 15.4 mg/kg of diclofenac potassium during organogenesis period, and group 3 subjected during fetal developmental period with the same dosage. At the 20 gestation day the rats were anesthetized and dissected, histopathological studies on placenta, maternal liver, and fetal liver have been done. Our results revealed moderate to severe histopathological alternations in all examined tissues like disorganization of tissue architecture, vacuolation, lymphatic infiltration, apoptosis and more changes. There is a harmful effect of the DP on the placenta tissue and also the liver tissue of the fetus, and on this we recommend that you use the DP with caution during pregnancy at the possible lowest effective dose and for the shortest duration.

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INTRODUCTION

Non-steroidal moderating medications (NSAIDs) are among most regular medications (Pascucci, 2002) that have mitigating, pain relieving and antipyretic impacts (Liu et al., 2005). They have been utilized generally to treat an assortment of ailments and cases like menorrhagia, dysmenorrhea, headaches, joint inflammation and numerous sorts of agony (Siu, 2000; Chan et al., 2002).

Diclofenac Potassium (DP) is one of the most used NSAID that is commonly used in the obstetrics and gynecology area (Aygün et al., 2012; Zengin et al., 2013). NSAID treatment during pregnancy has many negative horrible effects for both the mother and the baby (Aygün et al., 2012). The previous study reported that 21.6% of ladies take drugs during the first trimester of pregnancy; of these drugs, 3% are NSAIDs (Siu, 2000). So, probably DP causes developmental deleterious impact because it passes the placental layer easily in the 1st three months of gestation period in human (Chan et al., 2001; Güven et al., 2013).

Placenta is the correspondence organ that empow-
ers the passage and transport of different materials among mother and her baby control fundamental issues during the fetal development (Serman, 2011). There is no interest in studying the placenta and the effect of some medications on it as a main tissue in safety estimation of the harmful for mother and embryos/fetuses.

The liver is fundamental for endurance since it is basic for the coordination of metabolism in the body, including glucose homeostasis, xenobiotic metabolism and detoxification. The liver is likewise a significant site for steroid hormone production. It constantly gives vitality to the entire body by dealing with the fundamental supply of supplements (Nemeth et al., 2009; Aygün et al., 2012; Gu and Manautou, 2012).

There are several previous studies investigate the teratogenic impact effect of DCF on the fetus during the gestation period. On the other hand, there is no article subjected to the relationship between the maternally DCF administration and the histology of the placenta and hepatic tissue of both mother and fetus so, the aim of this work will study the effect of DP on the latter mentioned tissues during two different stages of pregnancy.

MATERIALS AND METHODS

The present study approved for animals use and for all procedures from the Cairo University, Faculty of Science Institutional Animal Care and Use Committee (IACUC) (Egypt), (CU/I/S /80/17). The current work was used normal adult male and female Wistar rats (Rattus norvegicus) with a weight range from 160 to 180 grams. The animals were purchased from the animal house of the Faculty of Veterinary, Cairo University- Egypt. The animals were housed in ideal conditions (12 h light-dark cycle, and they were fed ad libitum. The recommended maximum dosage for human (150 mg/kg) was modified to suit the weight of rats according to (Reagan-Shaw et al., 2008) so; the dosage used for rats was 15.4 mg/kg.

Group 1 (G1): Control where the pregnant rats received an equivalent volume of distilled water during their gestation period.

Group 2 (G2): Treated where the pregnant rats administrated DP from 5th to 13th day of gestation.

Group 3 (G3): Treated where the pregnant rats subjected to DP from 13th to 19th day of gestation.

Histopathological study (Haematoxylin and Eosin)

Maternal liver and placenta of different groups and liver of fetuses were fixed for histological examination by light microscopy in 10% formal saline for twenty four hours. Serial dilutions of alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained sections were collected on glass slides, deparaffinized, and stained by hematoxylin and eosin for routine examination. Examination was done through the light and electric microscope.

RESULTS

Histopathological reports

Placental tissue

Control group

The placenta is consists of two parts; fetal part and a maternal part (Furukawa et al., 2011). The later part histologically composed of the decidua and metrial gland. The decidua basalis consisted of cellular and fibrous elements. It was separated from the basal zone by unilayer of giant cells. The fetal part composed of the basal zone and labyrinth zone. Referring to (Fonseca et al., 2012) the basal zone is composed of giant trophoblast cells, glycogenic trophoblasts, and a basophilic spongiotrophoblast cells. The labyrinth zone contains giant trophoblast cells, syncytiotrophoblasts, and blood vessels (Figure 1).

Figure 1 shows, (A) normal histology of the basal zone (I), giant cell (Gt), spongiotrophoblasts (St). (B) Labyrinth zone showing trophoblastic trabeculae (T) consisting of trophoblasts and syncytiotrophoblast, 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).

First treated group

In basal zone there is obvious cystic degeneration of glycogen cell islands in addition to enlarged giant
cells with absence of nuclei, it is like as a ghost cell. Irregular shape of giant cells showed presence of nuclear and cytoplasmic vacuoles and presence of hemorrhage. In labyrinth zone there is rupture of endothelial cells that surround blood vessels. The blood vessels are filled with vacuolated cells and vasodilatation of maternal sinusoids (MS). In addition to presence clear reduction in trophoblastic septa, there are many pyknotic cells and as overall there is disorganization of the structure of this zone (Figure 2).

Second treated group

During Light microscopic examination in the placentas of treated mothers the basal zone showed cytolysis of glycogen cells and numerous of pyknotic spongitrophoblast. In labyrinth zone there are clear necrosis and disorganization, vasodilatation of maternal sinusoid with observed increase in thickness of trophoblast septa, degenerated blood vessel and endothelial cells surrounding blood vessels, and presence of dead blood vessels (Figure 3).

Figure 1: Sections of placenta from control pregnant rat.

Figure 2 shows, (A) In basal layer: there is obvious cystic degeneration of glycogen cell islands (star) in addition to enlarged giant cells with absence of nuclei; it is like as a ghost cell (blue arrow). (B) In basal layer: giant cells with shrinked nuclei are reduced and have irregular shape (red arrow), and cytoplasmic vacuoles (blue arrow) and hemorrhage (pink arrow). (C) In labyrinth zone of placenta: there is rupture of endothelial cells that surround blood vessels (fbv) and completely absence of endothelia cells of another blood vessel (blue arrow ) and the blood vessels are filled with vacuolated cells (red arrow). (D) In labyrinth zone: there is vasodilatation of maternal sinusoids (MS), clear reduction in trophoblastic septa (black arrow), pyknotic cells (red arrow) and as overall disorganization of the structure of this zone.

Figure 3 shows, (A) In basal zone: Cytolysis of glycogen cells (Gly C). (B) In the basal zone: there are a lot of pyknotic spongitrophoblast (red arrow). (C) In labyrinth zone: there is clear necrosis and...
disorganization of zone structure vasodilatation of maternal sinusoid (MS) with observed increase in thickness of trophoblastic septa blue arrow) and degenerated blood vessel (black arrow). (D) In the labyrinth zone: there is degeneration of endothelial cells surrounding blood vessels (red arrow) and presence of dead blood vessels (blue arrow).

**Maternal liver tissue**

**Control group**

The fetal hepatic tissue showed the normal structure that detects the hepatic lobules. The hepatocytes were designated as patchy, ramify, and interconnected cords radiating from a central vein. Blood sinusoids were seen in-between these cords. The hepatic cells seemed rounded or polygonal in shape with acidophilic cytoplasm and euchromatic, centrally located, rounded nucleus that had one or more nucleoli (Figure 4).

Figure 4 shows, With no histopathological alteration and it is composed of hexagonal lobules with central veins (CV) and hepatic triads. Hepatocytes (H) are arranged in cords and are separated by sinusoids (Si) containing Kupffer cells (K).

**First treated group**

The Light microscopic examination of maternal liver showed several histopathological changes that summarized as lymphatic infiltration and necrosis. The hepatocytes are swelled with disarray and cloudy view in addition of presence binucleated hepatocyte, fatty degeneration of hepatocytes, obvious widely dilated sinusoids and degenerated epithelial lining of central vein, congested central vein, and detached epithelial layer that lining central vein (Figure 5).

Figure 5 shows, (A)Lymphatic infiltration (Lym f) and necrosis (star). (B) Swelled hepatocytes (red arrow) with disarray and cloudy view, bi-nucleated hepatocyte (blue arrow) with necrotic areas (star). (C) Fatty degeneration of hepatocytes (FD), and bi-nucleated hepatocytes (red arrow). (D) Obvious widely dilated sinusoids (MS) and degenerated epithelial lining of central vein (red arrow). E&F) Congested central vein (star), widely dilated sinusoid (MS) and separation of epithelial layer that lining central vein (Red arrow) and bi-nucleated hepatocyte (blue arrow).
Figure 3: Sections of placenta of pregnant rat from second treated group

Figure 4: A section of liver from control pregnant rat
Second treated group

The Light microscopic examination of the maternal liver revealed many signs of alternations which summarized in presence a lot of necrotic hepatocytes. Obvious widely dilated maternal sinusoid, hemorrhagic area, many necrotic hepatocytes, congested central vein, and much necrotic area were observed. Portal vein infiltrated by chronic inflammatory cells and observed hemorrhagic area (Figure 6).

Figure 6 shows, (A&B) A lot of necrotic hepatocytes (red arrow), with obvious widely dilated sinusoid and vacuolated hepatocyte (star). (C) Nuclear vacuoles (blue arrow), hemorrhagic area (red arrow) and many necrotic hepatocytes. (D) Congested central vein (red arrow), vacuolated hepatocyte (blue arrow) and many necrotic areas were observed (star). (E)Portal vein infiltrated by chronic inflammatory cells (red arrow) and observed hemorrhagic area.

Fetal liver tissue

Control group

The fetal liver formed of hepatic cells arranges in the form of strands and lobules with central vein and separated by interlobular connective tissue.
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- Sections of liver of pregnant rat from second treated group.

- Septa. The hepatocytes are more or less polygonal in shape with distinct boundaries possessing spherical nuclei. The blood sinusoids are irregularly dilated vessels. 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 (Figure 7).

Figure 7 shows, Normal architecture of the liver tissue, the central vein has its intact endothelial lining (CV), Hepatocytes (H), Blood sinusoids (S).

First treated group

Examination of the fetal liver showed obvious changes as compared with that of the control. These changes summarized in repeated appearance of megakaryocytes, presence of pyknoic hepatocyte, occurrence of fatty degeneration in many areas of the section, and presence of degenerated hepato-
cytes (Figure 8).

Figure 8 shows, (A) Repeated appearance of megakaryocytes (black arrow) and presence of pyknoyic hepatocyte (red arrow). (B) Occurrence of fatty degeneration in many areas of the section (FD) and presence of degenerated hepatocytes (red arrow).

**Second treated group**

Examination of the fetal liver revealed many histopathological alternation in comparison with untreated. These alterations summarized in severe fatty degeneration in the hepatocytes, overall the tissue lost their normal structure, and increase number of megakaryocyte (Figure 9).

Figure 9 shows, (A) The hepatocytes exhibit severe fatty degeneration (FD) and overall the tissue lost its normal structure. (B) Repeated appearance of megakaryocyte (black arrow).

**Discussion**

The histopathological investigation of placenta in both treated groups revealed an obvious cystic degeneration of glycogen cell islands, enlarged giant cells with absence of nuclei (ghost cell), presence of cytoplasmic vacuoles, and also presence of hemorrhage in the basal zone. The labyrinth zone showed rupture of endothelial cells that surround blood vessels.

Administration of NSAIDs during the pregnancy period can pass through the placental layers and might be causing incipient organism fetal and neonatal harmful effects, contingent upon the dosage, time of treatment, gestation phase, and time went between maternal NSAID administration and delivery. These effects get from the mechanism of action of NSAIDs and from the physiological changes in drug pharmacokinetics occurring during pregnancy (Siu, 2000).

Liver is the first organ to be exposed to such riskiness via the portal circulation. This study showed abnormal histology in liver of both treated groups when compared with control. The observed abnormalities including lymphatic infiltration, necrotic areas, swelled hepatocytes with disarray and cloudy view, presence of bi-nucleated hepatocyte, fatty...
degeneration of hepatocytes, obvious widely dilated sinusoids, degenerated epithelial lining of central vein, congested central vein, detached epithelial layer that lining central vein, repeated appearance of hemorrhagic area. The necrotic areas investigated in the present study showed disintegrated hepatocytes and cell debris. Infiltration of leucocytes—mainly mononuclear cells (lymphocytes) and hemorrhagic patches were also demonstrated. Necrosis may be occurred due to hypoxia or reduced O$_2$ in the blood.

In line with these findings Charles, (2018) reported that aspirin ingestions at a dose greater than 70 mg/kg revealed no gross changes in the liver appearance but elevate cell basophilia. At a dose of 140 mg/kg, mild hepatotoxic was occurred in the form of sinusoidal congestion. (Mitchell et al., 1993) has before mentioned that aspirin caused considerable effect on the histology of the hepatic tissue. (Rau et al., 1989) reported that aspirin caused liver with degenerative cells and pycknotic changes, presence of cytoplasmic vacuoles, clear sinusoids dilations and hypertrophied hepatocytes. (Charles et al., 2018) reported that aspirin with a dose of 35 mg/kg
did not cause histopathological effects comparable with the control, while 70 mg/kg caused changes in liver histology. With a dose of 140 mg/kg mild sinusoidal congestion and severely increased amount of cell basophilia, were observed.

The increased cell basophilia caused hepatotoxicity by mitochondrial dysfunction and lipid peroxidation mechanism and resulting in significant decrease in intracellular ATP and disrupts free fatty acid accumulation in liver (Sangeetha and Krishnakumari, 2010).

It has been reported by Ishiyama et al that diclofenac induced hepatotoxicity in rats was related with the reactive oxygen species (ROS) generation (Ishiyama et al., 1990). Diclofenac can result in liver damage through various mechanisms such as generation of ROS (Cantoni et al., 2003).

The current study showed obvious changes in fetal
liver as compared to the control. These changes summarized in repeated appearance of megakaryocytes, pyknoic hepatocytes, occurrence of fatty degeneration in many areas of the section, and as overall the tissue appear to loss its normal structure.

There were no previous work studied the effect of NSAIDs on histology of fetal liver, but previous studies in other drug categories reported other abnormalities in fetal liver. Citalopram did not influence the number of megakaryocytes whereas Kupffer cells and lymphocytes increased significantly in the fetuses received citalopram at doses of 10 or 20 mg/kg/day during gestation.

Statistical analysis demonstrated that the increase in number of the Kupffer cells and lymphocytes was dose dependent. Thus, at a dose of 20 mg/kg/day, the cell number increased significantly, whilst at a dose of 10 mg/kg/day it was not (Mohammadi et al., 2013).

The previous studies reported that NSAIDs can cause a premature closure of the fetal ductus arteriosus that leads to pulmonary hypertension, respiratory problems and unwanted impacts on kidney function; this leads to oligohydramnios and neonatal anuria (Ericson and Källén, 2001; Østensen and Skomsvoll, 2004). The mechanism of NSAID induced teratogenicity is unclear (den Veyver and Moise, 1993; Chan et al., 2002). The mechanism of action of NSAIDs is mediated by inhibition of prostaglandin synthesis by cyclo-oxygenase (COX) enzymes (den Veyver and Moise, 1993; Chan et al., 2002). COX-1 and COX-2 are isozymes of the COX enzyme. While COX-1 is constitutively expressed in several tissues, e.g., endothelium, stomach and kidneys (Vane et al., 1998), COX-2 is induced by pro-inflammatory cytokines and endotoxins (Patrono et al., 2005; Cappon et al., 2003). Two vascular effects of prostaglandin inhibitors are constriction of the fetal ductus arteriosus and reduce renal blood flow. These complications have been described for most nonselective COX inhibitors, and they increasingly are reported for selective COX-2 inhibitors (Østensen and Skomsvoll, 2004; Çolakoğlu et al., 2014).

Several medications are oxidized to form radicals and formed extremely reactive oxygen species (ROS) (Halliwell and Gutteridge, 1985; Orhan and Sahin, 2001). DCF produces some metabolic alterations in many tissues or cells by generating free radicals which in turn making oxidative stress (Orhan and Sahin, 2001; Gómez-Lechón et al., 2003). Diclofenac sodium causes apoptosis in hepatocytes by an oxidative stress-induced opening of the mitochondrial permeability transition pore (Gómez-Lechón et al., 2003). The molecular mechanism of DS-induced apoptosis involves the generation of ROS as an initial step (Inoue et al., 2004; Milusheva et al., 2008). It is clear that oxidative stress injure macromolecules as proteins, lipids and nucleic acids that lead to disorganization of the cell membranes as a consequence of apoptotic and/or necrotic events (Cui et al., 2004; Çolakoğlu et al., 2014).

CONCLUSIONS

The diclofenac potassium has a deleterious impact on the vital tissues as placenta which in turn has an effect on the normal and proper development of the fetus. DP effects mediated by its power to generate high level of free radicals and failure the antioxidant system strength. Thus we advise the pregnant woman to avoid DP intake and in the extreme necessity.

Conflict of Interest

None.

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