Research Article

Assessment of the Effect of Non-steroidal Anti-inflammatory Drug (Diclofenac Potassium) on the Pregnant Rats and their Fetuses

Heba Ali Abd El-Rhaman[1*], Zeinab Abdel-Hameid Mohamed[1], Abdel Rahman Sayed Sultan[2], Abdel-Rahman Tawfik[1]
Department of Zoology, Faculty of Science, Cairo University, Egypt.
Department of Zoology, Faculty of Science, Asuit/Al-Azhar University, Egypt.
***Corresponding author's E-mail:** ha793481@gmail.com

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ABSTRACT

Diclofenac potassium is one of the most common non-steroidal anti-inflammatory drugs that used during pregnancy period for pain, inflammation, fever, dysmenorrheal, and menorrhagia. Their mechanism of action is through the inhibition of the biosynthesis of prostaglandins. The previous studies showed that diclofenac potassium has teratogenic effect. However, the mechanism of its teratogenic effect is not yet understood. Here, we investigated for the first time, the effects of diclofenac potassium on the fetal organs and placenta as attempt to find the mechanism of its teratogenicity through inhibition of placental development. The pregnant rats have been divided into 3 groups; (G1) control, (G2) administrated orally with 15.4 mg/kg diclofenac potassium from 5th to 13th gestation day, and (G3) treated with the same dose from 13th to 19th GD. The pregnant rats were sacrificed at the 20th GD. The uteri were isolated and the fetuses have been exposed to morphological examination and skeletal staining. Moreover, biochemical studies on placenta, maternal liver, and fetal liver have been done. We recorded high embryonic resorption rate, subcutaneous hematoma in different parts of fetuses of the treated animals and skeletal abnormalities that mostly observed in the ossification of skull, ribs, and vertebrae. Moreover, wavy ribs were detected. Biochemical studies indicated a significant alteration in SOD, GSH, Catalase, and MDA levels. Diclofenac potassium should be avoided during pregnancy and given in just if its benefits outweigh the maternal and fetal risks, at the possible lowest effective dose and for the shortest duration.

Keywords: Non-steroidal anti-inflammatory, Pregnancy, Rats, Fetuses.

INTRODUCTION

The science that study the defects occur at the birth or at different stages of pregnancy is known as "Teratology". Once the developing embryo subjected to the teratogen (any toxic agent) will give respond vary from malformation or death. The degree of that response is dependent on list of exposure conditions[1]. The factors that caused congenital malformations are termed the "teratogenic factors". These factors may be physical agents as radiation, chemical agents as drugs, and maternal problems as diabetic pregnant and viral infection.

Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of oxygen reactive species (ROS) as unstable molecules in cells and tissues and the ability of a biological system to detoxify these reactive products. ROS are normally generated as by-products of oxygen metabolism, environmental stressors (ionizing radiations, UV, pollutants, and heavy metals), and xenobiotics (anti-blastic drugs). The most important free radicals that cause oxidative stress are superoxide (O^{-2}), hydroxyl radical (^{-}OH), and hydrogen peroxide (H_2O_2). When the generation of reactive oxygen species (ROS) exceeds, these unstable molecules interact with biologic macromolecules such as lipids, proteins, and DNA to cause structural changes as well as functional abnormalities [2].

One of the most common NSAIDs drugs is Diclofenac potassium (DCF) that can be used at the pregnancy period for treatment of inflammation, fever, pain, dysmenorrheal, and menorrhagia. The embryotoxicity due to the use of NSAIDs in experimental animals comprise many abnormalities like spina bifida [3] and cleftpalate[4]. Despite a wide range of usage, there is limited literature regarding the mechanism by which DCF exerts teratogenicity during the critical period of organogenesis. This study will perform to investigate the possible mechanism by which DCF can cause hazard and lethal effects on the pregnant rats and their offspring during second (organogenesis period) and third trimester (fetal developmental period).

MATERIALS AND METHODS

Animals

The approval for the use of animals and for the procedures required for the experiments was obtained by the Cairo University, Faculty of Science Institutional Animal Care and Use Committee (IACUC) (Egypt), (CU/I/S /80/17). The current work was performed on normal adult male and female rats (*Rattus norvegius*) of weight about 160-180 grams. They 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 and humidity controlled environment. The

rats were fed with standard food pellet and water ad libitum.

Mating procedure

After one week of acclimatization, two female rats were left overnight with a mature male rat. Every morning, a vaginal smear was taken from each female and exposed to microscopic detection for the evaluation of the presence of spermatozoa which is an indicator that matting between male and female rats has done. This was recorded as the zero day of gestation[5].

Experimental procedures

The pregnant rats were divided into three groups each of 10 rats. Rats were orally given Catafly (diclofenac potassium 1.8 mg/ml) that purchased from Novartis Pharmaceuticals. The recommended maximum dosage for human (150 mg/kg) was modified to suit the weight of rats according to [6] so, the dosage used for rats was 15.4 mg/kg.

Group 1 (G1): The pregnant rats received an equivalent volume of distilled water during their gestation period. They considered as a control.

Group 2 (G2): The pregnant rats treated with 15.4 mg/kg of diclofenac potassium from 5th to 13th day of gestation (GD).

Group 3 (G3): The pregnant rats treated with 15.4 mg/kg of diclofenac potassium from 13th to 19th day of gestation.

Morphological examination

At 20th day of gestation all females were sacrificed by decapitation and dissected to expose and remove their uteri. Eachuterus was weighted and opened to record the position, number of viable, resorbed, or dead fetuses. The placental weight was recorded. The fetuses were weighed and have been carefully examined under a dissecting microscope for any external anomalies.

The post-implantation loss index ({(Number of implantation sites - Number of live fetuses)/ (Number of implantation sites)} x100), and pre-implantation loss index ({(Number of corpus luteal – Number of implantation sites) / (Number of corpus luteal)} x100) were calculated.

Skeletal examination

After fetal morphological examination and extracting all necessary organs that will be examined, the fetuses were prepared for skeletal examination. Fetuses of all groups were fixed in 95% ethyl alcohol for 10 days. The skin, viscera, and adipose tissue were removed. The specimens were cleared in acetone solution for 7–10 days to remove fats. Fetuses were stained with Alcian Blue and Alizarin Red S stains for 4 days at 40º C then washed carefully in running tap water for 2 hours and transferred into an aqueous solution of 1% KOH for clearing until the complete visibility of skeleton through the surrounding tissues. Cleared fetuses were placed successively into 50%, 80%, and 100% glycerin solution for 7 days each step. The cartilage parts appear blue while the bones appear redin color. The stained skeletons were examined under dissecting microscope and all abnormalities were photographed[7].

Biochemical studies

Autopsy samples were taken from the liver and placenta of mother while fetal liver from the three different groups were stored at -40°C for oxidative stress investigation. Piece of each tissue were weighted and homogenized in 10 mmol/L phosphate buffer saline (PBS) as 10 % (W/V) at pH 7.4. The homogenates were centrifuged and the supernatants were taken for the estimation of reduced glutathione (GSH)[8], catalase (CAT)[9], superoxide dismutase (SOD)[10], and malondialdehyde (MDA)[11].

Statistical analysis

A computer program, the statistical package for social science (SPSS 17.0), was used for statistical analysis. One way analysis of Variance (ANOVA) followed by Tukey's multiple comparison post hoc analysis to determine the differences between groups. The data were expressed as means ± standard error (SE). Differences between the groups were considered as statistically significant when $p < 0.05$.

RESULTS

Pregnancy outcomes

The uterus of the control pregnant rats showed normal distribution of the implanted fetuses between the two horns. In G2, complete early and late resorption in some cases was observed. The uterine horns in G3 showed clearly visible embryonic resorption sites (Fig.1).

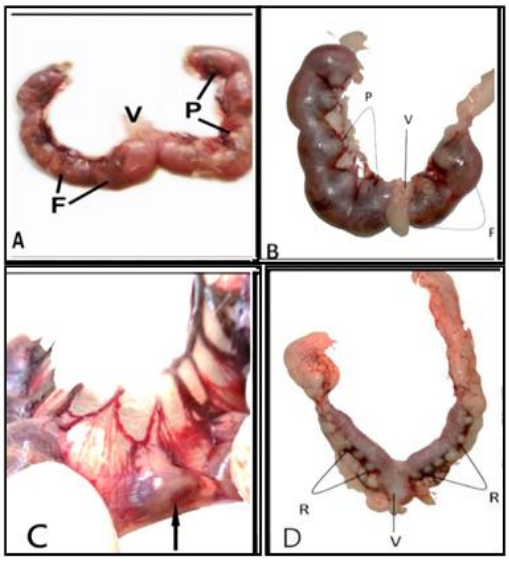

Figure 1: Uterus at the 20th day of gestation.

A. Control uterus with normal distribution of the implanted fetuses between the two horns.

B. Asymmetrical distribution of fetuses in the two uteri horns of G2.

C. The uterus of G2 appeared with late resorbed site(arrow).

D. The uterine horns of G3 showed clearly visible embryonic resorption.

(F=Fetus, V= Vagina, P= Placenta).

The average weight of placenta of all treated pregnant rats groups both of G1 & G2 was significantly (P< 0.05) decreased as compared to control (Table. 1). There was decreased in number of implantation site. Both treated groups showed a significant increase in the post-implantation loss index (%) as shown in Table 1.

In control group the fetuses with a normal body weight and length. On the other hand, the treated groups (G2 & G3) revealed that the diclofenac potassium caused a significant (P< 0.05) reduction in fetal body weight and length (growth retardation) when compared with the control group (Fig. 2 and Table 1). In fetal body, there were repeated appearance of hematoma (hematoma is red or dark patches) in different parts of fetal body (head, neck, abdomen, fore limb and back) (Fig. 3 and Table 1).

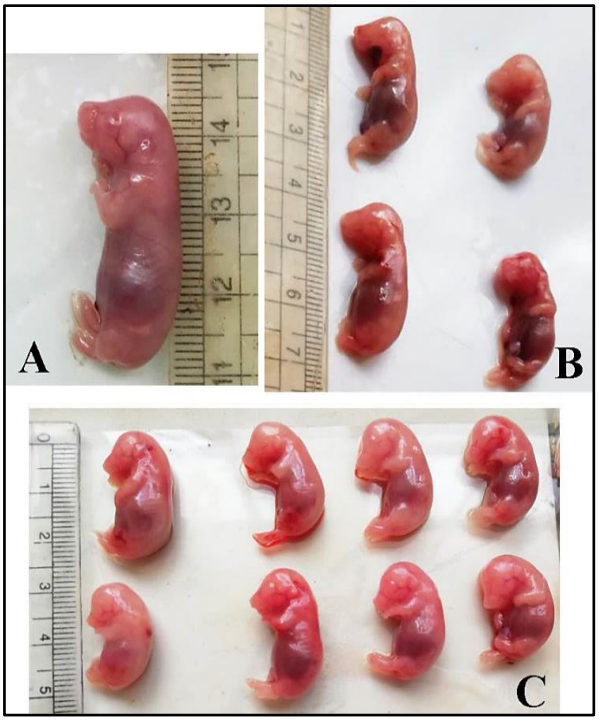

Figure 2: A Photograph offet uses at the 20th day of gestation showing external morphology.

A. Control showing fetus with correct shape and length.

B. B &C. Fetuses of G2 & G3, respectively with obvious growth retardation in body weight and body length.

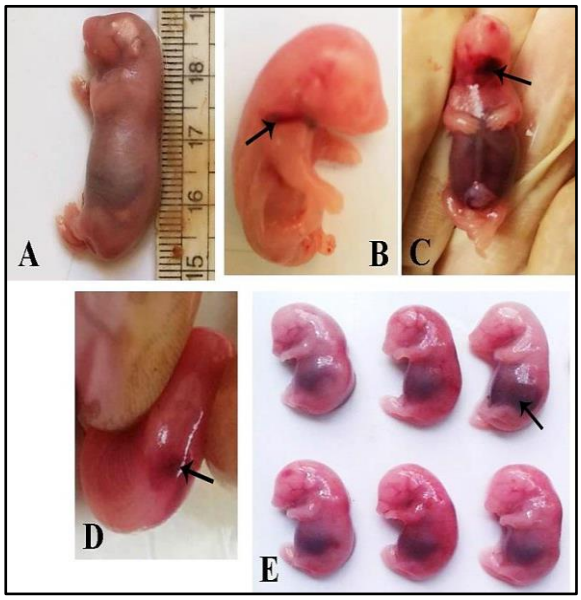

Figure 3: A Photograph of fetuses at the 20th day of gestation showing external abnormalities.

A. Control showing normal fetus appearance.

B&C. Fetuses of G2 showing hematoma at fore limb and sub mandible.

D&E. Fetuses of G3 showing hematoma at back and viscera.

Table 1: The pregnant outcome in different groups

Groups	Control	Treated	Treated
Items	G1	G2	G3
Total number of pregnant	10	6	7
Number of implantation sites	76	39	55
Number of a live fetuses	7.500±.73	7.40 ±0.58	7.40±1.05
Post-implantation loss index %	1.25±1.25	2.13± 7.51[a]	22.22 ±8.60[a]
Uterine weight (g)	38.85±1.30	27.13±0.297[a]	25.95±1.43[a]
Placenta weight (g)	0.545±0.0086	0.449±0.008[a]	0.417±0.0104[a]
Fetal weight (g)	2.97 ± 0.094	2.14±0.051[a]	1.64±0.94[a]
Hematoma number	1.70±0.37	5.40±0.50[a]	4.90±0.96[a]

Each value represents the mean and SME. One-way analysis of variance (ANOVA) followed by Turkey's honestly significant difference (HSD) test, the $p < 0.05$ level was set as statistically significant different; (a) significant compared to control.

Skeletal anomalies

At the 20th day of gestation, chondrification and ossification processes of the axial and appendicular

skeleton in the control rat fetuses have been obviously completed. The maternally treated groups G2 and G3 revealed incomplete ossification and un-ossification of different parts of fetal skeleton as skull (Fig. 4), skeletal anomalies mostly appeared at the vertebral column (Fig. 5), wavy, curved, un-ossified, and incomplete ribs (Fig.6). Sternum (Fig. 7), fore limb (Fig.8), and hind limb (Fig.9)were recorded.

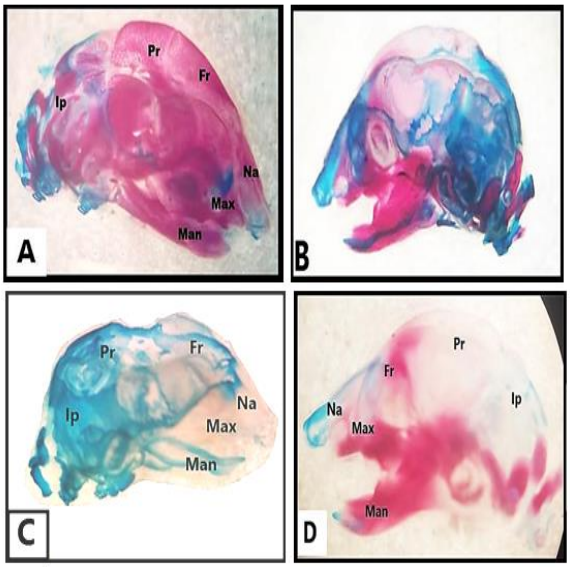

Figure 4: Photographs of the cranial skeleton of fetuses at 20 th of gestation.

A. Control fetuses showing: complete ossification of the cranial bones .

Treated groups showing:

B. Incomplete ossification of cranial bones

C. Un- ossified cranial bones

D. Incomplete ossification of all cranial bones .

Na= nasal, Mx= maxilla, Ma= mandible, Fr= frontal, P= parietal and IP= inter parieta .

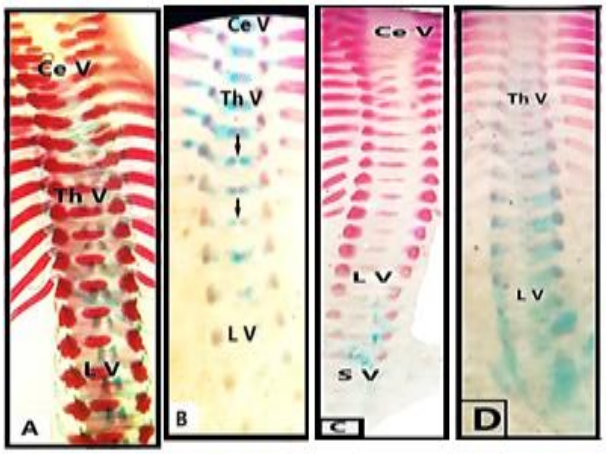

Figure 5: Photographs of the vertebral column of fetuses at 20th of gestation.

A. Control fetuses showing complete ossification of all vertebrae .

Treated groups showing:

B&C. Un-ossified centers of cervical, thoracic and lumber vertebrae and dumbbell shape vertebrae (arrow).

D. Fine ossified centers of thoracic vertebrae and un-ossified lumber vertebrae.

Ce= cervical vertebrae, Th V= thoracic vertebrae, L V= lumbar vertebrae, S V= sacral vertebrae and Cu V= caudal vertebrae

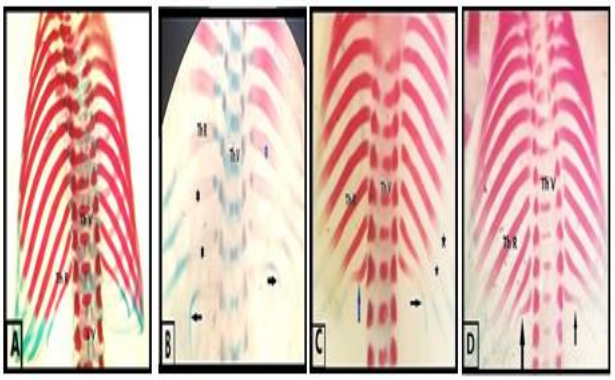

Figure 6: Photographs of the ribs of fetuses at 20th of gestation.

A. Control fetuses showing complete ossification and normal shape of ribs .

Treated groups showing:

B. Incomplete ossification (blue star) curved ribs (arrow) and un-ossified thoracic rib (black star) .

C. Incomplete ossification (star) curved ribs (blue arrow) and wavy rib (blue arrow)

D. less ossified and wavy rib (black arrow) Th V= thoracic vertebrae and Th R= thoracic ribs .

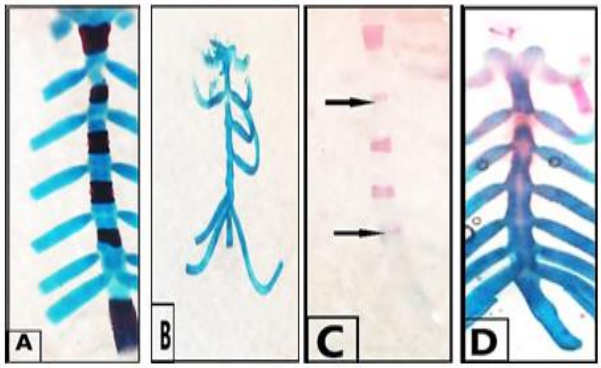

Figure 7: Photographs of the sternum of fetuses at 20th of gestation.

A. Control fetuses showing complete ossification of sternbrae bones.

Treated groups showing:

B. Completely un-ossified sternbrae bones.

C. The second and last sternbrae are un-ossified (arrow).

D. un-ossified sternbrae bones.

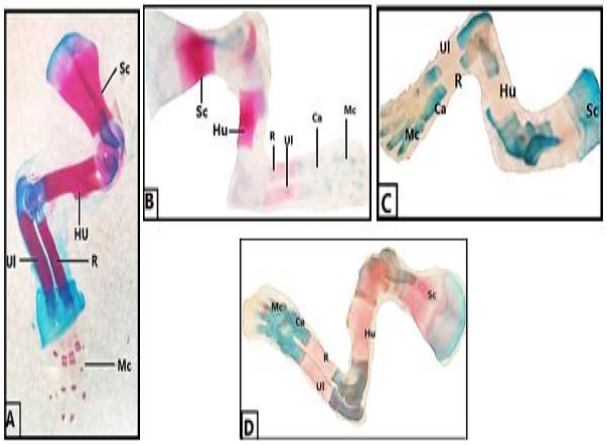

Figure 8: Photographs of the pectoral girdle and fore limb of fetuses at 20th of gestation.

A. Control fetuses showing complete ossification of all bones.

Treated groups showing:

B. Incomplete ossification of radius & ulna bones and un-ossified metacarpals.

C. un-ossification of all bones

D. Incomplete ossification of bones and un-ossified metacarpals.

Sc= scapula, Hu=humerus, R= radius, Ul=ulna, Ca= carpals, MC= metacarpals

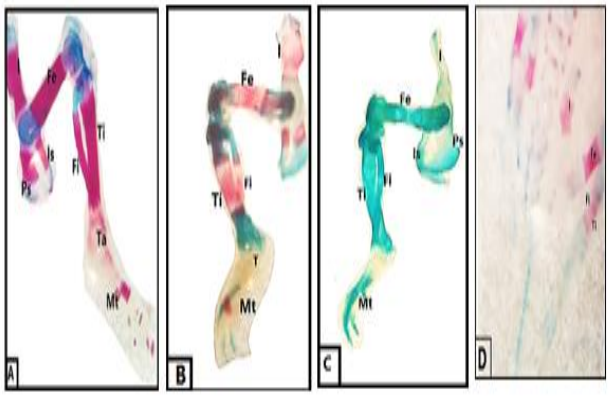

Figure 9: Photographs of the pelvic girdle and hind limb of fetuses at 20th of gestation.

A. Control fetuses showing complete ossification of all bones.

Treated groups showing:

B. Incomplete ossification of all bones.

C. Un-ossification of all bones.

D. Less ossified bones and un-ossified metatarsus

I= ilium, IS= ischium, P=pubis, Fe= femur, Fi= fibila, Ti= tibia, Ta= tarsals. MT= metatarsals and Ph= phalang.

Biochemical assays

The current study demonstrates the change of antioxidant defense system by administration of 15.4 mg/kg Diclofenac potassium to pregnant female rats.

Reduced glutathione (GSH)

Placenta tissue

G2showed non-significant increase in GSH level while G3showed a significant decrease in level of GSH as compared to the untreated Table (2).

Maternal liver

Possessed a significant increase ($P < 0.05$)in G2 and non-significant decrease in G3in comparison with the control group Table (2).

Fetal liver

G2showed non-significant increase and G3 showed a significant increase ($P < 0.05$) as compared to the control Table (2).

Table 2: GSH level (u/g) in placenta, maternal and fetal liver of pregnant rats treated with 15.4 mg/kg of diclofenac potassium through two different gestation periods.

Tissues	Placenta	Maternal liver	Fetal liver
Groups			
G1	0.534±0.054	0.373 ± 0.0379	0.586±0.044
G2	0.334±0.080	0.201 ± 0.019[a]	0.413±0.094
G3	0.106±0.006[a]	0.250 ± 0.062	0.202±0.038[a]

Each value represents the mean and SME. One-way analysis of variance (ANOVA) followed by Turkey's honestly significant difference (HSD) test, the $p < 0.05$ level was set as statistically significant different; (a) significant compared to control.

Catalase

Placenta

G 2 revealed a significant increase ($P < 0.05$) whileG3 showed a significant decrease when compared with the control value Table (3).

Maternal liver

Catalase enzyme level was decreased significantly ($P < 0.05$) in G2 andG3ascompared to the untreated Table (3).

Fetal liver: Oral administration of diclofenac potassium resulted in a significant reduction ($P < 0.05$) of catalase content in G2and G3in comparison with the control Table (3).

Table 3: Catalase content (u/g)in placenta, maternal and fetal liver of pregnant rats treated with 15.4 mg/kg of diclofenac potassium through two different gestation periods.

Tissues	Placenta	Maternal liver	Fetal liver
Groups			
G1	0.052± 0.0025	0.174 ± 0.0108	0.1469 ± 0.012
G2	0.029 ± 0.004 [a]	0.036 ± 0.005[a]	0.030 ± 0.002 [a]
G3	0.021± 0.004 [a]	0.038 ± 0.006 [a]	0.045 ± 0.003 [a]

Each value represents the mean and SME. One-way analysis of variance (ANOVA) followed by Turkey's honestly significant difference (HSD) test, the $p < 0.05$ level was set as statistically significant different; (a) significant compared to control.

Superoxide dismutase (SOD)

Placenta

Administration of diclofenac showed a significant reduction ($P < 0.05$) in the SOD activity in G2 and G3 when compared to the control Table (4).

Maternal liver

The diclofenac potassium administration resulted in a significant decrease ($P < 0.05$) in SOD activity in G2 and G3 as compared to the control Table (4).

Fetal liver

The treatment with 1.68 mg/kg of diclofenac potassium showed a significant reduction ($P < 0.05$) in SOD activity in G2and G3when compared to the control Table (4).

Table 4: SOD level (u/g) in placenta, maternal and fetal liver of pregnant rats treated with 15.4 mg/kg of diclofenac potassium through two different gestation periods.

Tissues	Placenta	Maternal liver	Fetal liver
Groups			
G1	33.87 ± 2.19	48.88 ± 21.41	30.1 ± 2.77
G2	16.1 ±0.935[a]	7.2 ± 0.699[a]	8.2 ± 0.734[a]
G3	6.8 ± 0.717 [a]	4.6 ±0.455[a]	7.6 ± 0.931[a]

Each value represents the mean and SME. One-way analysis of variance (ANOVA) followed by Turkey's honestly significant difference (HSD) test, the $p < 0.05$ level was set as statistically significant different; (a) significant compared to control.

Malondialdehyde (MDA)

Placenta: Pregnant rats treated with diclofenac potassium showed a significant elevation of MDA level ($P < 0.05$) in G2whileshowed non-significant elevation in G3when compared to the control Table (5).

Maternal liver

The treatment of diclofenac potassium caused non-significant elevations in MDA level of G2and G3 when compared to the control Table 5.

Fetal liver

The MDA level was elevated non-significantly in G2) and G3in comparison with the untreated Table (5).

Table 5: MDA level (u/g) in placenta, maternal and fetal liver of pregnant rats treated with 15.4 mg/kg of diclofenac potassium through two different gestation periods.

Tissues	Placenta	Maternal liver	Fetal liver
Groups			
G1	0.621 ± 0.057	1.17 ± 0.356	0.315 ± 0.066
G2	3.088± 0.283[a]	1.78 ± 0.149[a]	1.98 ± 0.250[a]
G3	2.014 ± 0.323[a]	2.06±0.32[a]	2.69 ± 0.346[a]

Each value represents the mean and SME. One-way analysis of variance (ANOVA) followed by Turkey's honestly significant difference (HSD) test, the $p < 0.05$ level was set as statistically significant different; (a) significant compared to control.

DISCUSSION

The present study is carried out to evaluate the teratogenic potential of the non-steroidal anti-inflammatory drug (NSAIDs), diclofenac potassium that orally administrated with dosage of 1.68 mg/kg to pregnant rats during two different periods of gestation. To evaluate the teratogenic effect of the drug, morphological anomalies, skeletal abnormalities and oxidative stress markers were examined.

In the current study, the administrated of diclofenac potassium during two different periods of gestation showed slight decrease in the number of viable feti per female rat and increase the number of resorption and fetal loss.

The study carried by[12]revealed highly significant decreases in the number of viable fetuses per female rat after orally administrated with diclofenac potassium with a dose of 6.75 and 13.5 mg/kg from the 1st to the 4th day of pregnancy and from 6th to 15th day of pregnancy. Our results also agreed with the study done by[13] who administered Wistar-KY rats with aspirin in dosage 62.5, 125,187.5 or 250 mg/kg suspended with 0.5% CMC-Na. The previous study proved that diclofenac administration to rats inhibited the ongoing process of implantation and placentation. It might be attributed to direct toxic effect of drug on fetal cell as it easily pass through placenta due to its low molecular weight (less than 1000 dalton)[13].

The previous work showed that the live birth rate was significantly lowered at 187.5 mg/kg of diclofenac than that in control[14].They cultured rat blastocysts in

diclofenac in vitro and implanted it to host mother on day 5 as pseudo pregnancy. It was clear that diclofenac had a profound effect preventing implantation as 72% of control embryo developed compared to only 35-41% after the treatment with diclofenac. Since prostaglandin is produced by the blastocyst and appeared to be necessary and an important factor during the process of placentation and implantation which continues up to day 12 in the rat, it is probable that the prostaglandin was inhibited by diclofenac potassium which in turn decreased the implantation.

The current study showed a significant decrease in the placenta and fetal body weight in both treated groups. It was agree with [15] who reported that when pregnant mice treated with 1.5 and 3mg/kg body weight of diclofenac sodium for 6 days (during pre-implantation and implantation stages) and for 8 days (during organogenesis period) caused growth retardation in maternally treated fetuses. Such retarding effect on fetal growth during gestation is consistent with the reported data that the general development of mice fetuses was delayed post injection with a 10-fold therapeutic dose of diclofenac sodium on days 16-18 of pregnancy[16]. A positive association between use of NSAIDs during pregnancy and growth retardation along with stunting in size was also observed[17]. Cyclooxygenase (COX) inhibitors such as ibuprofen and tolmetin, types of drugs that are used to treat inflammation, were toxic to pregnant rats in the highest doses. They caused significantly greater incidence of intrauterine growth retardation (IUGR) and developmental variations [18; 19].

The growth retardation of maternally treated fetuses observed in the present investigation, is may be due to an impairment of blood flow to the placenta and reduced uterine blood flow by the effect of the drugs thus leading to reduced transport of nutrients and oxygen from the mother to the fetal circulation.

The present work revealed a broad variety of incompletely ossified, un-ossified skull bones (mostly nasal, frontal, parietal and inter-parietal), abnormal vertebrae, appearance of supernumerary defect of ribs, reduced number of ossified sternbrae, fine ossification of the sternum, fine ossified, un-ossified bones of fore and hind limbs, and un-ossification of metacarpals and metatarsals. Such results may be correlated with the inhibition of osteoclastic bone reabsorption activity (inhibition of the process of bone formation and calcium replacement or precipitation in the long bones).

This current findings are agreed with the reported data that the less ossifications of the skeletal elements of the limbs of mice were pronounced in fetuses maternally treated with the high dose 3mg/kg body weight of diclofenac sodium during organogenesis (GDs 7-14) [15]. Diclofenac potassium also induced skeletal abnormalities in the feti when administered orally in pregnant rats at therapeutic and double therapeutic doses from the 1st to the 4th day of pregnancy at the period of organogenesis[12].

Teratogenic effects were also recorded on the CD-1 mouse embryo exposed to concurrent doses of ethanol and aspirin[20].Salicylate induced fetal death and malformations in two mouse strains[21]. Anomalies of ribs and vertebrae showed the highest incidence after injection on the 9th day. Cekanova observed interactions between salicylic acid and pyridy;-3-methanol anti-inflammatory and teratogenic effects. Salicylate-induced skeletal malformations were obtained after treatment in early organogenesis[22].

Several mechanisms were reported by various authors to explain the action of NSAIDs on osteogenesis. Diclofenac sodium has a direct effect on mouse osteoclast differentiation and activation and in part inhibition of phosphorylated NF-kB translocation[23]. Interestingly, the dose of diclofenac inhibiting osteoclast activation is lower than the dose that inhibits osteoclast differentiation; thus diclofenac sodium inhibits NF-kB transcription in mouse osteoclasts[24].

Chang and his team investigated the influences of NSAIDs on cell cycle kinetics, cytotoxicity, and cell death pattern in osteoblast cultures from rat fetal calvarias. They documented that NSAIDs arrested cell cycle at the G (0)/G (1) phase and induced cytotoxicity and cell death of osteoblasts, contributing in this manner to the suppressive effect on bone formation[25].

Many reactive Oxygen species produced by biological system are mediators for several enzymatic and non-enzymatic reactions that have important role in the body. Reactive oxygen species (ROS)is formed during normal physiological processes that occur when the cell is not under stress[26].Cells have complex antioxidant systems and chemical sequesters that help to prevent oxidative damage caused by high free radical concentrations. These defense systems are classified as endogenous e.g. Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSH), Malondialdehyde (MDA), and exogenous antioxidants as vitamin C, vitamin E, beta-carotenes and flavonoids [27-29] .

The current study concerned with the effect of diclofenac potassium on endogenous defense system. It was concluded that diclofenac potassium showed clear alteration in all levels of GSH, CAT, SOD and MDA in placenta tissue, maternal and fetal liver in each of the two treated groups.

GSH level showed significant decrease in placenta, significant increase in maternal liver, and slight increase in fetal liver in both of two treated groups G2and G3.CAT level was significantly increased in placenta but showed significant decrease in fetal and maternal liverin both of two treated groups G2and G3. SOD level showed significant reduction in placenta, maternal liver, and fetal liver in both of two treated groups G2and G3. Finally, MDA level was significantly elevated in placenta and also slightly elevated in fetal and maternal liverin both of two treated groups G2and G3.

These findings partially agreed with findings that administration with diclofenac sodium at dose 13.5 mg/kg for 14 days to adult of rats induced marked increase in lipid peroxidation product, MDA content and a significant decrease in GSH value in liver and kidney at the second and fourth weeks of the experiment[30].

The status of lipid peroxidation as well as the reduction of GSH altered levels of certain endogenous radical scavengers is taken as direct evidence for oxidative stress affecting functional as well as structural integrity of cell and organelles membrane[31].

It has been reported by Ishiyama et al that diclofenac induced hepatotoxicity in rats was associated with the production of reactive oxygen species (ROS) [32]. Diclofenac can result in liver damage through various mechanisms such as generation of ROS [33].

In conclusion, the diclofenac potassium considered as a teratogen due to its embryotoxic effects on the fetus of rats. The teratogenicity of diclofenac potassium may be mediated by its ability to elevate the reactive oxygen species levels and imbalance the antioxidant system. Depends on the previous comments diclofenac potassium should be avoided during the gestation period or taken in very narrow range. Finally, further clinical studies should be done in order to evaluate the impact effect of diclofenac potassium in the human and make a clear decision about usage of diclofenac potassium during the pregnancy.

REFERENCES

1. Wise L.D., Numeric estimates of teratogenic severity from embryo-fetal developmental toxicity studies. Birth Defects Res. B Dev. Reprod. Toxicol. 107(1), 2016, 60-70.
2. Navarro-Yepes J., Zavala-Flores L., Anandhan A., Wang F., Skotak M., Chandra N., Antioxidant gene therapy against neuronal cell death. Pharmacology & Therapeutics, 142, 2014, 206–230.
3. Coopersmith H., Kerbel N.C., Drugs and congenital anomalies. Canadian Mediccal Association Journal, 1962, 87-193.
4. Montenegro M.A., Palomino H., Induction of cleft palate in mice by inhibitors of prostaglandin synthesis. Journal of Craniofacial Genetics and Developmental Biology, 10, 1990, 83–94.
5. McClain R.M & Becker B.A., Teratogenicity, fetal toxicity and placental transfer of lead nitrate in rats. Toxicology and applied pharmacology, 931, 1975, 72-82.
6. Shannon R., Minakshi N., Nihal A., Dose translation from animal to human studies revisited.Federation of American Societies for Experimental Biology Journal, 22, 2008, 659-661.
7. Young A.D., Phipps D.E., Astroff A.B., Large-scale double-staining of rat fetal skeletons using alizarin red S and alcian blue. Teratology, 61, 2000, 273–276.
8. Beutler E., Duron O., Kelly M.B., Journal of Laboratory and Clinical Medicine, 61, 1963, 882.
9. Aebi H., Catalase in vitro. Methods Enzymology, 105, 1984, 121- 126.
10. Beauchamp C., Fridovich I., Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical biochemistry, 44(1), 1971, 276-87.
11. Ohkawa H., Ohhhishi W., Yagi K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95, 1979, 35.
12. El-Sayed M.G.A., Evaluation of Teratogenic Potentials of Diclofenac in Pregnant Female Rats. Benha Veterinary Medical Journal, 26(1), MARCH 2014, 54-66.
13. Okamoto M., Kihara T., Tanimura T., and Developmental Toxicity of Aspirin Prenatally Given to Rats: Assessment in Slc: Wistar- KY Rats. Congenital Anomalies, 28(4), 1988, 265-278.
14. Carp H.J., Fein A., Nebel L., Effect of diclofenac on implantation and embryonic development in the rat. European Journal of Obstetrics & Gynecology and Reproductive Biology, 28(3), 1988, 273-277.
15. Mohamed A.Shahin., The effect of the non-steroidal anti-inflammatory drug diclofenac sodium on the fetuses of albino mice. The Egyptian Journal of Hospital Medicine, 44, July 2011, 272-283.
16. Bogdanenko E.V., Sviridov I.U.V., Sadovnikov V.B., Zhdanov R.I., The effect of orthofen (diclofenac) on the embryogenesis of laboratory mice. Eksp. Klin. Farmakol, 62(2), 1999, 55-57.
17. Nielsen G.L., Sorensen H.T., Larsen H., Pedersen L., Risk of adverse birth outcome and miscarriage in pregnant users of non-steroidal anti-inflammatory drugs. Population based observational study and case- control study, B.M.J, 322, 2001, 266-270.
18. Burdan F., Developmental toxicity evaluation of ibuprofen and tolmetin administered in triple daily doses to Wistar CRL (WI) WUBR rats. Birth Defects Research Part B. Developmental and Reproductive Toxicology, 71 (5), 2004, 321-330.
19. Sahu C.R., Developmental toxicity of ibuprofen treated mice. Intern. J. Pharmaceutical Science, 1, 2009, 92-102.
20. Guy J.F., and Sucheston M.E., Teratogenic effects on the CD-1 mouse embryo exposed to concurrent doses of ethanol and aspirin. Teratology, 34 (3), 1986, 249-261.
21. Larsson K.S., Eriksson M., Salicylate Induced Fetal Death and Malformations inTwo Mouse Strains. Acta Paediatrica, 55(6), 1966, 569-576.
22. Cekanova E., Larsson K.S., Mörck E., Aberg G., Interactions between Salicylic Acid and Pyridy;-3-methanol: Anti-inflammatory and Teratogenic Effects. Acta Pharmacologica et Toxicologica, 35(2), 1974, 107–118.
23. Karakawa A., Fukawa Y., Okazaki M., Takahashi K., Sano T., Amano H., Yamamoto M., Yamada S., Diclofenac sodium inhibits NFkappa B transcription in osteoclasts. J. Dent. Res, 88, 2009, 1042-1047.

24. Kotake S., Yago T., Kawamoto M., Nanke Y., Effects of NSAIDs on differentiation and function of human and murine osteoclasts _ Crucial. Human osteoclastology Pharmaceuticals, 3, 2010, 13941410.

25. Chang JK., Wang GJ., Tsai ST., Ho ML., Nonsteroidal anti-inflammatory drug effects on osteoblastic cell cycle, cytotoxicity, and cell death.Connect. Tissue Res. 46 (4-5), 2005, 200-10.

26. Duchen M.R., Role of mitochondria in health and disease. Diabetes; 53 Suppl 1, 2004, 96-102.

27. Parker D.V., Nutrition antioxidants and disease prevention: mechanism of action. In: Cansda TK (editor). Antioxidant in human health and disease, 1999.

28. 28. Duthie G.G., Duthie S.J., Kyle J.A.M., Plant polyphenols in cancer and health disease: implications as nutritional antioxidant. Nutr Res Rev, 13, 2000, 79-106.

29. Thomas J.A., Estres oxidativo y defense oxidante. In: Nutricion en salud y enfermedad. 9th ed. McGrawHill. Mexico, 2002.

30. El-Maddawy, Z.K.,El-Ashmawy, I.M., Hepato-Renal and Hematological Effects of Diclofenac Sodium in Rats. Global Journal of Pharmacology, 7(2), 2013, 123-132.

31. Khan M.R., Ahmed D., Protective effects of Digera muricata (L.) Mart. on testis against oxidative stress of carbon tetrachloride in rat. Food and Chemical Toxicology, 2(47), 1393-1399.

32. shiyama H.,T., Ogino K., Shimomura Y., Kanke T., Hobara T. Hepatotoxicity of diethyldithiocarbamate in rats. Pharmacol. Toxicol., 67, 1990, 426-430.

33. Cantoni L., Valaperta R., Ponsoda X., Castell J.V., Barelli D., Rizzardini M., Mangolini A., Hauri L., Villa P.Induction of hepatic heme oxygenase-1 by diclofenac in rodents: role of oxidative stress and cytochrome P-450 activity.J. Hepatol., 38(6), 2003, 776-83.

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