The Egyptian Journal of Hospital Medicine (July 2011) Vol., 44: 272 – 283

The effect of the non-steroidal anti-inflammatory drug diclofenac sodium on the fetuses of albino mice

Mohamed A. Shahin, Ramadan A. Ramadan, Samia M. Sakr and Sahar A. Sabry
Department of Biological and Geological Sciences,
Faculty of Education, Ain Shams University

Abstract

Introduction: The present study was carried out to evaluate the effect of the non-steroidal anti-inflammatory drug diclofenac sodium (DS) on the fetuses of albino mice from the morphological and skeletal points of view.

Material and methods: Sixty adult pregnant female mice were used in the present study. They were allocated into 6 groups (10 mice each). The first two groups served as control and were injected intraperitoneally (i.p.) with the solvent of the drug, and the 3rd and 5th groups were treated with 1.5 and 3mg/kg body weight of diclofenac sodium for 6 days (gestation days 1-6), respectively ; the 4th and 6th groups were treated with 1.5and 3mg/kg body weight of the drug for 8 days (gestation days 7-14), respectively.

Results: The morphological examination of the fetuses of treated groups showed conspicuous decrease in the average body weight and body length in all treated groups. The fetuses maternally treated with the drug showed noticeable external morphological malformations and their skeletons exhibited mild retardation in skeletal elements.

In conclusion: The non-steroidal anti-inflammatory drug diclofenac sodium had exerted marked morphological malformations and mild skeletal alterations in mice fetuses maternally treated during different periods of gestation.

Keywords: Diclofenac sodium- Morphology – Skeleton- Fetuses of albino mice.

Introduction

The non-steroidal anti-inflammatory drugs (NSAIDs) have three major therapeutic actions: reduce inflammation (anti-inflammatory), pain (analgesic), and fever (antipyretic). However, they differ in their anti-inflammatory, analgesic and antipyretic activities (Laurance *et al.*, 1997). Cyclooxygenase (COX) exists as two isoforms, COX-1 and COX-2, and inhibition of COX-2 is the mechanism responsible for the therapeutic anti-inflammatory NSAID characteristics (Vane *et al.*, 1998).

Siu *et al.* (2000) reported that diclofenac crosses the human placenta readily during the first trimester. Some case studies have linked aspirin (acetylsalicylic acid [ASA]) and indomethacin use with a higher risk of congenital abnormalities and low birth weight, whereas others have not found an association (Nielsen *et al.*, 2001). The experimental studies showed that these drugs could increase post-implantation loss, decrease fetal number, induce skeletal, and heart defects as well as fetal growth retardation (Cappon *et al.*, 2003; Cook *et al.*, 2003; Ostensen and Skomsvoll, 2004). The bone and/or cartilage examination showed that in utero exposure to COX inhibitors (NSAIDs) may disturb skeletal formation (Ostensen and Skomsvoll, 2004). Higher incidence of bone developmental variations was seen in fetuses whose mothers were treated with high toxic doses of non-selective COX inhibitors (Burdan, 2004). It is well known that NSAIDs may cause miscarriage, intrauterine growth retardation (IUGR) and ductus arteriosus constriction in human (Ostensen *et al.*, 2006). Burdan *et al.*(2009) reported that piroxicam, ibuprofen and

tolmetin caused IUGR, increased skeletal developmental variations, and decreased bone ossification in fetuses of pregnant rats exposed to high doses .

There is even less information concerning the most common generation of NSAIDs, diclofenac sodium (DS). Few reports have been presented on the effects of DS on fetuses covering the stages of preimplantation and implantation; gestation days (GDs) 1-6 as well as the sensitive days of organogenesis and differentiation ; GDs 7-14. In the present study we aim to investigate the potential teratogenic effects of diclofenac sodium (DS) on morphology and skeletal structures of mice fetuses maternally treated on two different periods of gestation (GDs 1-6 and GDs 7-14).

Material and Methods

The non-steroidal anti-inflammatory drug (NSAID) used in the present investigation is diclofenac sodium (DS). DS (declophen) is available in the form of vials, each containing 75mg of the active ingredient. The therapeutic dose (1.5 mg/kg b.wt.) of this drug for mice was calculated according to Paget and Barnes (1964). The chosen dose was nearly comparable to the human effective therapeutic dose (ETD). Two doses of DS were used in the present study; the therapeutic dose (1.5 mg/kg b.wt.) and double the therapeutic dose (3mg/kg b.wt.) and were considered as the low and high doses, respectively. The doses were estimated according to weight of the mouse and injected intraperitoneally (i.p.).

The present investigation was carried out on mature albino mice of pure CD-1 strain with an average body weight of 25g obtained from the breeding unit of Theodor Bilharz Research Institute (TBRI), Imbaba, Giza.

Female and male mice were housed separately in plastic cages, and were allowed free access to food and tap water *ad libitum*. Pregnancy was achieved by housing one adult virgin female with one well marked fertile male overnight, from 5 pm until 9 am of the next day. Successful mating was indicated either by the presence of a vaginal plug or by the presence of spermatozoa in the vaginal smears (Snell, 1956). Females which give positive vaginal smears are considered pregnant and the day of detection was taken to indicate gestation day (GD) 1. Sixty pregnant female mice were divided into six groups (10 mice each). The first two groups (C_1& C_2) are the control groups and the last four groups (A, B, D & E) are the experimental groups and treatment of these groups was achieved in the following manner:

Groups C_1 & C_2 : Each pregnant female was injected intraperitoneally with 0.1ml distilled water (the solvent of the drug) daily for 6 days during pregnancy from day 1 till day 6 of gestation (GDs 1-6) and for 8 days during pregnancy from day 7 till day 14 of gestation (GDs 7-14), respectively.

Groups A & D: Each pregnant female was injected intraperitoneally with 1.5 and 3mg/kg body weight of DS, respectively for 6 days during pregnancy from day 1 till day 6 of gestation (GDs 1-6).

Groups B & E: Each pregnant female was injected intraperitoneally with 1.5 and 3mg/kg body weight of DS, respectively for 8 days during pregnancy from day 7 till day 14 of gestation (GDs 7-14).

On day 19 of pregnancy , before the onset of labor , females of both control and experimental groups were sacrificed, dissected and their uteri were removed, placed in normal saline solution and the fetuses were taken out for morphological and skeletal studies. Living fetuses were distinguished from dead ones by their spontaneous movement. The mean number, mean body weight and mean body length (crown-rump length) of fetuses were recorded and statistically analyzed using Student *t*-test. The fetuses were carefully examined externally for any morphological malformations using a binocular microscope. For skeletal studies, fetuses of control and experimental groups were fixed in 95% ethanol for 7 days then placed in acetone for 7 days and were double stained for cartilage and bone using alcian blue and alizarin red-S according to the method described by McLeod (1980). The stained preparations of the skeletons were carefully examined under the dissecting binocular microscope. Photographs were performed for control and maternally treated fetuses as well as for skeletal systems of control and maternally treated fetuses.

Results

Morphological studies:

On the 19th day of gestation, the pregnant mice of the control (C_1 & C_2) and the treated (A, B, D & E) groups were sacrificed and the percentage of alive fetuses, mean body weight and mean body length of fetuses are recorded (Table 1). The data show that treatment with 1.5&3mg/kg body weight of DS caused obvious growth retardation of mice fetuses. Growth retardation was indicated by the significant reduction of both fetal body weight and body length. The minimal decrease in mean fetal body weight was recorded among fetuses of group A (maternally treated with the low dose of DS during GDs 1-6), while the highest rate of decrease in mean fetal body weight was noticed in members of group E (maternally treated with the high dose of DS during GDs 7-14) as illustrated in Table 1.

The results displayed in Table 1 and Figures 1&2 showed a significant decrease in the mean body length of the fetuses of the four experimental groups, maternally treated with the low (1.5mg/kg b.wt.) and high (3mg/kg b.wt.) doses of DS as compared with those obtained in the control groups. The highest rate of decrease in mean fetal body length was observed in fetuses maternally treated with the low (group B) and high (group E) doses of the drug during GDs 7-14 (Table 1 and Figs. 1&2). However, the minimal decrease in mean fetal body length was recorded among fetuses of groups A&D (maternally treated with the low and high doses of DS during GDs 1-6, respectively). No fetal mortality was observed in control groups, but cases of dead fetuses were observed in treated groups. The lowest percentages (11.6 & 14 %) of dead fetuses were recorded among those of groups A&B (maternally treated with the low dose during GDs 1-6 and 7-14, respectively). On the other hand the highest percentages (17.24 & 30.76 %) of dead fetuses were recorded in groups D & E (maternally treated with the high dose of DS during GDs 7-14, respectively).

Incidence of gross malformations:

No external malformations were recorded among fetuses of the control groups and fetuses of group A (maternally treated with the low dose of DS during GDs 1-6). While various degrees of external malformations were recorded among fetuses of group B (maternally treated with the low dose of the drug during GDs 7-14) and groups D&E (maternally treated with the high dose of DS during GDs 1-6 and 7-14 , respectively) .These malformations appeared in the form of ; stunting in size, grossly malformed body, subcutaneous hemorrhage on body surface, edema in different parts of the body, eye abnormalities, and limb and tail defects (Figs. 3-5). The incidence and severity of gross malformations were pronounced in the high dose treated groups. The minimal percentage of fetuses with gross malformations (4%) was recorded among those maternally treated with the low dose of DS during GDs 7-14 (group B) and included stunting in size and subcutaneous hemorrhage on body surface (Fig. 3: I&II). In group D (fetuses maternally treated with the high dose of the drug during GDs 1-6) the external malformations (reached a percentage of 8.6%) were represented in stunting size , grossly malformed body, bulged or open eyes, microcephaly, malrotated limbs, absence of tail and subcutaneous hemorrhage on body surface (Fig. 4: I&II). The highest percentage (35.9%) of external malformations was noticed in members of group E (maternally treated with the high dose of DS during GDs 7-14). The predominant malformations in this group were stunting in size, grossly malformed body, subcutaneous hemorrhage, absence of eyes, malformed left and hind limbs and adactyly (Fig. 5: I, II& III)

Skeletal studies

The cleared cartilage and bone preparations of the control mice fetuses are shown in Figures 6 &11.

Maternal treatment with DS (1.5 or 3 mg/kg b.wt.) did not result in major skeletal defects in the fetuses. However, IUGR observed grossly had its skeletal basis in terms of an overall reduction in the size of the ossified bones (Figs. 7-10 & 12-15) as compared to control fetuses (Figs. 6 & 11). The DS- maternally treated fetuses (groups B, D & E) are growth restricted to varying extents and have poorly ossified skeletons. Craniofacial bones of fetuses

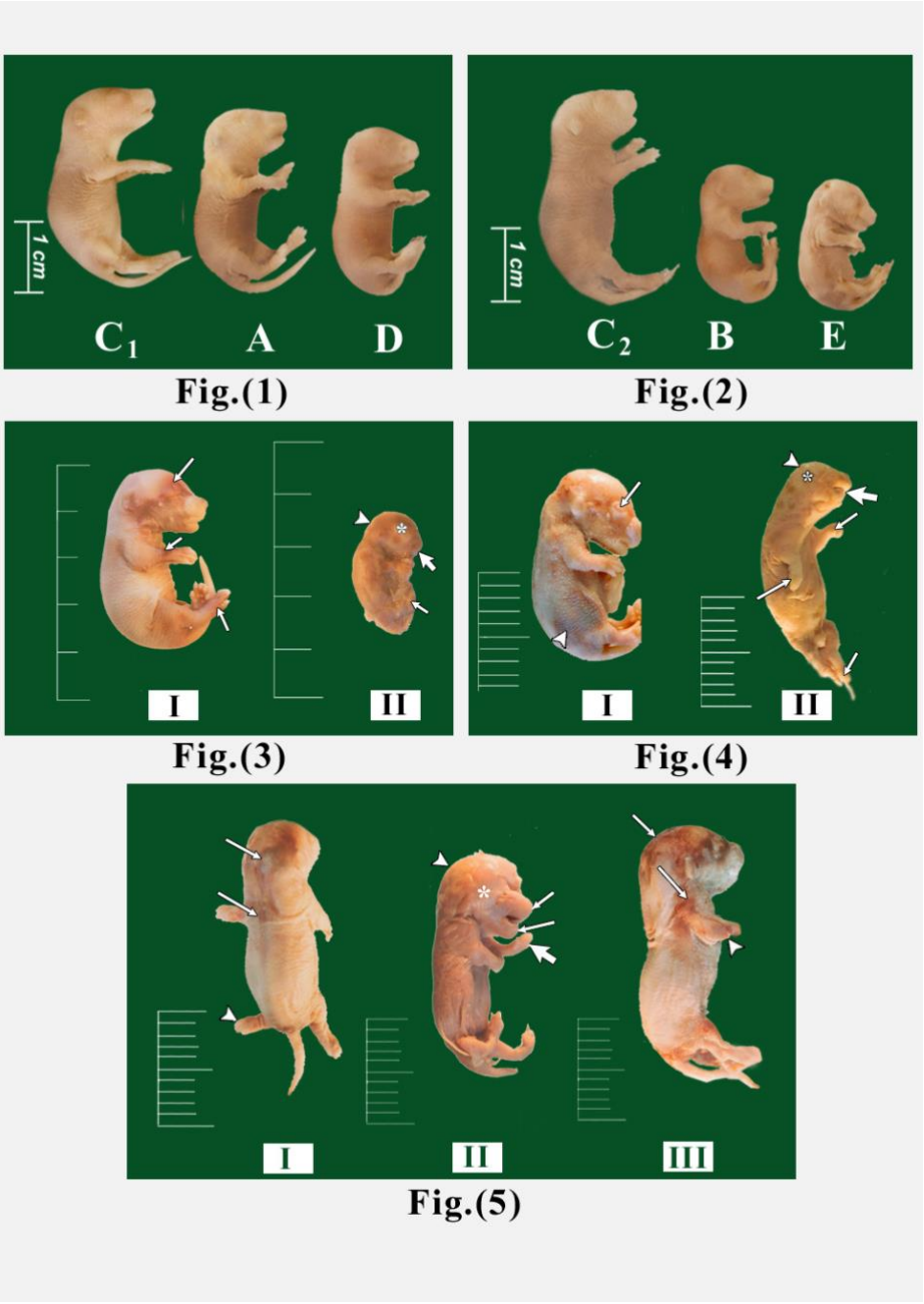
maternally treated with the high dose of the drug during GDs 7-14 (group E) exhibited hypoplasia and shortening of mandible bone. The tip of the hypoplastic mandible appeared posterior to the tip of maxilla (Fig. 10). The skeletal elements of the fore limbs of maternally treated fetuses were considerably shorter and less ossified compared to those of control fetuses (Figs. 7-10). This was pronounced in fetuses maternally treated with the high dose of the drug, during GDs 7-14 (Fig. 10). On the other hand, fetuses maternally treated with this high dose of DS during GDs 1-6 and 7-14 (groups D & E, respectively) showed hypoplastic sacral and caudal vertebrae as well as reduction in the size and ossification of caudal vertebrae as compared to controls (Figs. 14&15). Apart from the mentioned defects the rest of the skeletal elements did not show prominent malformations

.

Table 1: *Illustrating the percentages of alive, dead and malformed fetuses, the mean body weight (g) and the mean body length (cm) of mice fetuses of control and experimental groups.*

Animal groups	Developing fetuses					
	Alive %	Dead %	Malformed fetuses		Body weight (Mean±SE)	Body length (Mean±SE)
			NO.	%		
C_1	100%	-	-	-	1.56 ±0.24	2.96 ±0.40
A	88.32%	11.68%	-	-	1.30 ±0.22**	2.79±0.36*
D	82.76%	17.24%	5	8.6%	1.28±0.39**	2.23 ±0.18**
C_2	100%	-	-	-	1.56 ±0.24	2.96 ±0.40
B	86%	14%	2	4%	1.03±0.30**	2.14 ±0.62**
E	69.24%	30.76%	14	35.9%	1.01 ±0.38**	2.06±0.61**

C_1, and C_2: Fetuses of control groups.
A: Fetuses maternally treated with 1.5mg/k body weight of DS for 6 days (GDs 1-6).
D: Fetuses maternally treated with 3mg/k body weight of DS for 6 days (GDs 1-6).
B: Fetuses maternally treated with 1.5mg/k body weight of DS for8 days (GDs 7-14).
E: Fetuses maternally treated with 3mg/k body weight of DS for 8 days (GDs 7-14).
*P< 0.05 = significant and** P< 0.01 =highly significant.



Figs. 1-2: Photographs of 19-day old mice fetuses of the control and DS treated groups showing growth retardation in the four treated groups. ($C_{1\&}$ $C_{2)}$ fetuses of control groups, (A) fetus maternally treated with 1.5 mg/kg b.wt. of DS for 6 days during GDs 1-6, (B) fetus maternally treated with 1.5 mg/kg b.wt. of DS for 8 days during GDs 7-14, (D) fetus maternally treated with 3mg/kg b.wt. of DS for 6 days during GDs 1- 6, (E) fetus maternally treated with 3mg/kg b.wt. of DS for 8 days during GDs 7-14.

Fig. 3: Photographs of 19-day old fetuses maternally treated with 1.5 mg/kg body weight of DS for 8 days during GDs 7-14, showing different types of malformations.

I) Illustrating subcutaneous hemorrhage in the head region and different parts of the body surface (arrows).

II) Showing severe growth retardation, microcephaly (arrow head), open eyes (*), micrognathia (large arrow) and meromelia of hind limbs (arrows) with absence of tail.

Fig. 4: Photographs of 19-day old fetuses maternally treated with 3 mg/kg body weight of DS for 6 days during GDs 1-6, showing different types of malformations.

I) Illustrating stunting in size, open eyes (arrow), hemorrhage on left side of body surface (arrow head).

II) Showing grossly malformed body, microcephaly (arrow head), micrognathia (large arrow), absence of eyes (*), adactyly of both fore and hind limbs with malrotated limbs (arrows).

Fig. 5: Photographs of 19-day old fetuses maternally treated with 3 mg/kg body weight of DS for 8 days during GDs 7-14, showing different types of malformations.

I) Illustrating stunting in size, subcutaneous hemorrhage on head and neck (arrows), malrotated left hind limb (arrow head).

II) Showing grossly malformed body, large head (arrow head) with extending in the large lower and upper jaws (arrows), absence of eyes (*), adactyly of fore limbs (large arrow) and short tail.

III) Illustrating grossly malformed body, subcutaneous hemorrhage on head and fore limb(arrows), and syndactyly(arrow head).

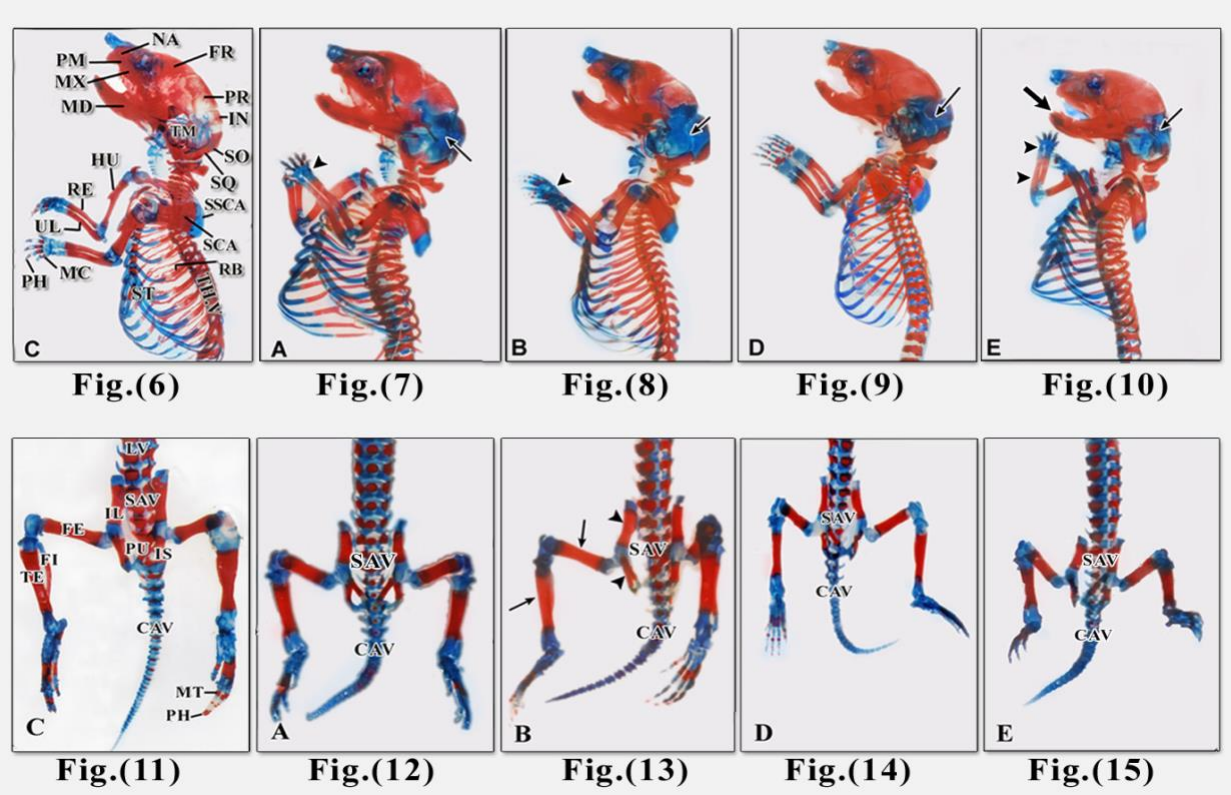


Fig.6: Photograph of the anterior region of the skeleton of 19-day old fetus of a control group (C) showing nasal (NA), frontal (FR), parietal (PR), interparietal (IN); squamosal (SQ), supraoccipital (SO), temporal bones (TM), premaxilla (PM), maxilla (MX), mandible (MD), ribs (RB), thoracic vertebrae (THV), sternum (ST), scapula (Sca), humerus (HU), radius (RE), ulna (UL), metacarpals (MC) and phalanges (PH). **(X 34)**

Fig.7: Photograph of the anterior region of the skeleton of 19-day old fetus maternally treated with 1.5mg/kg body weight of DS for 6 days during GDs 1-6 (A) manifesting incomplete ossification of the dermal bones of the skull (arrow). The figure also shows less degree of ossification of metacarpals (MC) and phalanges (PH) (arrow head). **(X 34)**

Fig.8: Photograph of the anterior region of the skeleton of 19-day old fetus maternally treated with 1.5mg/kg body weight of DS for 8 days during GDs 7-14 (B), displaying incomplete ossification of the dermal bones of the skull (arrow) with less affected ossification of the skeletal elements of the fore limb (arrowhead). **(X 34)**

Fig.9: Photograph of the anterior region of the skeleton of 19-day old fetus maternally treated with 3mg/kg body weight of DS for 6 days during GDs 1-6 (D), manifesting incomplete ossification of the bones of the skull (arrow). **(X 34)**

Fig.10: Photograph of the anterior region of the skeleton of 19-day old fetus maternally treated with 3mg/kg body weight of DS for 8 days during GDs 7-14 (E), manifesting incomplete ossification of the bones of the skull (arrow), and elements of the fore limb **(**arrow heads**).** Notice that the tip of the hypoplastic mandible (large arrow) is markedly posterior to the tip of the maxilla. **(X 34)**

Fig. 11: Photograph of the posterior region of the skeleton of 19-day old fetus of a control group (C) showing lumbar vertebrae (LV), sacral vertebrae (SAV), caudal vertebrae (CAV) ischium (IS), ilium (IL), pubis (PU), femur (FE), tibia (TE), fibula (FI), metatarsals (MT) and phalanges (PH) **(X34)**

Fig. 12: Photograph of the posterior region of the skeleton of 19-day old fetus maternally treated with 1.5mg/kg body weight of DS for 6 days during GDs 1-6 (A), showing incomplete ossification of the sacral (SAV) and caudal (CAV) vertebrae**. (X 34)**

Fig. 13: Photograph of the posterior region of the skeleton of 19-day old fetus maternally treated with the same drug dose for 8days during GDs 7-14 (B), showing less affected skeletal elements of the pelvic girdle (arrow heads) and hind limb (arrows)as well as incomplete ossification of the sacral (SAV) and caudal (CAV) vertebrae**.** **(X 34)**

Fig. 14: Photograph of the posterior region of the skeleton of 19-day old fetus maternally treated with 3mg/kg body weight of DS for 6 days during GDs 1-6 (D), displaying less ossification of the sacral (SAV) and caudal (CAV) vertebrae**. (X 34)**

Fig. 15: Photograph of the posterior region of the skeleton of 19-day old fetus maternally treated with 3mg/kg b.wt. of DS for 8days during GDs 7-14 (E), displaying incomplete ossification of the sacral (SAV) and caudal (CAV) vertebrae**. (X 34)**

Discussion

The present study was conducted to investigate the effects of the non-steroidal anti-inflammatory drug diclofenac sodium (DS) on mice fetuses maternally treated during two different periods of pregnancy. The morphological and skeletal features were assessed.

Morphological studies

The present results showed that pregnant mice treated with 1.5 and 3mg/kg body weight of DS for 6 days (during preimplantation and implantation stages) and for 8 days (during organogenesis period) caused growth retardation in maternally treated fetuses of the four experimental groups. The effects on the body length and body weight were statistically significant. The difference in weight and length compared between the low and high doses treated groups was also significant, implying thereby that the effect on the body weight and body weight was dose dependent. Such retarding effect of DS on fetal growth during gestation is consistent with the findings of Bogdanenko *et al*. (1999) who reported that the general development of mice fetuses was delayed post

injection with a 10-fold therapeutic dose of DS on days 16-18 of pregnancy. A positive association between use of NSAIDs during pregnancy and growth retardation along with stunting in size was reported by Nielsen *et al*. (2001). COX inhibitors (ibuprofen and tolmetin) were toxic to pregnant rats in the highest doses evaluated, which caused a significantly greater incidence of IUGR and developmental variations (Burdan, 2004). In the same line, Sahu (2009) reported adverse effects on pregnancy outcome in pregnant mice treated at different phases of gestation with ibuprofen. The growth retardation of maternally treated fetuses observed in the present investigation, is probably 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 transfer of nutrients and oxygen to the fetal circulation.

The present results illustrated also that maternal DS treatment on gestation days (GDs) 1-6 and 7-14 resulted in an increase in the percentage of dead fetuses and various degrees of external malformations characterized by stunting in size, grossly malformed body, subcutaneous hemorrhage on body surface, eye abnormalities, and limb and tail defects. The incidence of these malformations was pronounced in the high dose treated groups. It is worth of mentioning that no gross malformations were recorded among fetuses maternally treated with the low dose of DS during GDs 1-6 (preimplantation and implantation stages). With few exceptions, the present findings are similar to those obtained by other investigators using salicylates in multiple dose paradigms in rats (Nakatsuka and Fuji, 1979; Mankes *et al*., 1982; Hamed *et al*.,1994).

On the other hand and contrary to our results (no gross malformations were recorded among fetuses maternally treated with the low dose of DS during GDs 1-6 ; preimplantation and implantation stages) it was stated by several authors that pharmacologically diverse agents such as chlorambucil, retinoic acid, methylnitrosurea, ethanol and streptozotocin administered at preimplantation stages induce embryonic loss , gross malformations, and IUGR (Giavini *et al.*, 1984 ; Padmanabhan & Hameed, 1988 ; Nagao *et al*., 1991 ; Rutledge *et al*., 1994 ; Padmanabhan and Shafiullah, 2004). Such contradiction might be due to the difference of the used doses as well as to the variance in the mechanism of action of these drugs. It is worth to mention that the high dose of DS, in the present study, caused external malformations in fetuses maternally treated during pre-implantation and implantation stages (GDs 1-6). Along the same line, Lou *et al*. (1996) reported that embryotoxicity and fetal malformations were induced by treatment with aspirin before implantation in a dose dependent manner.

Regarding subcutaneous hemorrhage observed on different parts of body surface of DS maternally treated fetuses , several reports postulated that the potential adverse effects of NSAIDs (indomethacin , naproxen , ketoprofen) on the fetus include increased cutaneous and intracranial bleeding , and reduced amniotic fluid volume and the dose , duration , and period of gestation are important determinants of these effects (Nelson and Ostensen , 1997; Janssen and Genta, 2000).

In normal mice the eyelids grow across the eye and fuse together during days 15 and 16 of gestation, and the mice are born with their eyes closed (Harris and Juriloff, 1986). In the present study growth and fusion of the eyelids did not occur in some fetuses maternally treated with DS, and the 19 days old fetuses appeared with their eyes opened (bulged eyes). Various eye malformations including microphthalmia and opened eyes were detected by Sutcliffe *et al*. (1998) and Afshar *et al*. (2010) in newborn and mice fetuses maternally treated with the antiepileptic drug carbamazepine, respectively.

Skeletal studies:

The results of the present investigation clearly illustrated that IUGR observed grossly had its skeletal basis in terms of an overall reduction in the size of ossified bones of maternally treated fetuses as compared with control ones. The most marked skeletal variations included facial (mandibular) hypoplasia (diminution in size of the lower jaw), considerable shortening and less ossification of the skeletal elements of the limbs. These malformations were pronounced in fetuses maternally treated with the high dose of DS during organogenesis (GDs 7-14). These

hypoplasia and retarded ossification of skeletal elements observed in this study might be interpreted to result from maternal toxicity due to DS dosing. The role of maternal toxicity in abnormal fetal development has been previously emphasized (Rogers *et al.*, 2005). Such results may be correlated with the inhibition of osteoclastic activity (osteoclastic bone reabosrption). Inhibition of the process of bone formation and calcium replacement or precipitation in the long bones and other skeletal defects were reported in the offspring of pregnant mice treated with indomethacin (Kusanag *et al.*, 1977), or xylocaine (EL-Shabaka, 1992). The latter author added that such treatment induced also cleft palate malformation as well as marked retardation in the dermal bones of maternally treated fetuses. Also growth retardation and malformations of the skeletal elements were also observed in mice fetuses maternally treated with ethanol and nicotine (Mohamed, 1996), as well as the antiepileptic drug vigabatrin (Abdulrazzaq *et al*., 1997).

Several mechanisms were reported by various authors to explain the action of NSAIDs on osteogenesis. Kotake *et al*. (1997) reported that receptor activator of NF-kB ligand (RANKL) is the factor inducing murine osteoclastogenesis, through PGE2. In 2009, Karakawa *et al*. demonstrated that DS has a direct effect on mouse osteoclast differentiation and activation, and that, in part, inhibition of phosphorylated NF-kB translocation is indispensable for diclofenac efficacy. Interestingly, the diclofenac dose inhibiting osteoclast activation is lower than the dose that inhibits osteoclast differentiation; thus diclofenac sodium inhibits NF-kB transcription in mouse osteoclasts (Kotake, 2010).

Prostaglandins, the products of cyclooxygenase enzyme activity, have been recognized as key molecules in reproductive biology (Jabbour *et al*., 2006). They have also been implicated in modulating angiogenesis and vascular function, allowing the delivery of oxygen and nutrients to tissues, and are involved in endothelial cell sprouting (Namkoong *et al*., 2005). It is conceivable that NSAIDs mediated teratogenicity may be a result of vascular disruptions, given the relationship between COX inhibitors, prostaglandins and their vascular and endothelial effects (Ofori *et al.*, 2006).

In conclusion, we have shown that the non-steroidal anti-inflammatory drug diclofenac sodium had adverse or devastating effects that appeared in the form of growth retardation along with various external and some skeletal malformations of maternally treated mice fetuses. Although results from animal teratogenicity studies may not reflect the circumstances in humans, findings in our study suggest that further investigations and monitoring of possible embryo toxic effects of DS in human are warranted to elucidate the mechanism of teratogenicity of DS. Before more information in human becomes available, the use of DS (especially large therapeutic doses) in pregnant women should be treated with caution.

References

Abdulrazzaq YM , Bastaki SM and Padmanabhan R (1997): Teratogenic effects of vigabatrin in TO mouse fetuses. Teratology, 55: 165-176.

Afshar M , Moallem SA , Baharara J, Takjo T and Golalipour M (2010): Preventive effect of vitamin B6 on developmental toxicity of carbamazepine in mice. Iran. J. Basic Med. Sci., 14: 99-106.

Bogdanenko EV , Sviridov IUV, Sadovnikov VB and Zhdanov RI (1999): The effect of orthofen (diclofenac) on the embryogenesis of laboratory mice**.** Eksp. Klin. Farmakol.,62(2): 55-57.

Burdan F, Szumilo J, Klepacz R, Dudka J, Korobowicz A, Tokarska E, Cendrowska-Pinkosz M, Madej B and Klepacz L (2004): Gastrointestinal and hepatic toxicity of selective and nonselective cyclooxygenase-2 inhibitors in pregnant and nonpregnant rats. Pharmacol. Res., 50: 533–543.

Burdan F, Szumilo J and Klepacz R (2009): Maternal toxicity of nonsteroidal anti-inflammatory drugs as an important factor affecting prenatal development. Reprod. Toxicol., 28: 239–244.

Cappon GD, Gupta U, Cook JC, Tassinari MS and Hurtt ME (2003): Comparison of the developmental toxicity of aspirin in rabbits when administered throughout organogenesis or during sensitive windows of development. Birth Defects Res. Part B: Dev. Reprod. Toxicol., 68:38–46.

Cook JC , Jacobson CF , Gao F , Tassinari MS , Hurtt ME and De-Sesso JM (2003): Analysis of the non-steroidal anti-inflammatory drug literature for potential developmental toxicity in rats and rabbits. Birth Defects Res. Part B: Dev. Reprod. Toxicol., 68: 5–26.

El-Shabaka HA (1992): Congenital malformations in fetuses of mice treated with xylocaine. 1- Dose assessment, morphological and skeletal studies. J. Egypt. Ger. Soc. Zool., 9 (B): 27-42.

Giavini E, Bonanomi L and Ornaghi F (1984): Developmental toxicity during the preimplantation period: embryotoxicity and clastogenic effects of chlorambucil in the rat. Teratog. Carcinog. Mutagen., 4 : 341-348.

Hamed MR, Al-Assy YS and Ezzeldin E (1994): Influence of protein malnutrition on teratogenicity of acetylsalicylic acid in rats. Hum. Exp. Toxicol., 13: 83–88.

Harris MJ and Juriloff DM (1986): Eyelid development and fusion induced by cortisone treatment in mutant, lidgap-Miller , foetal mice. A scanning electron microscope study. J. Embryol. Exp. Morphol., 91: 1-18.

Jabbour HN, Sales KJ, Smith OP, Battersby S and Boddy SC (2006): Prostaglandin receptors are mediators of vascular function in endometrial pathologies. Mol. Cell Endocrinol., 252: 191-200.

Jansen NM and Genta MS (2000): The effects of immunosuppressive and anti-inflammatory medications on fertility, pregnancy and lactation. Arch. Intern. Med., 160: 610-619.

Karakawa A , Fukawa Y , Okazaki M , Takahashi K, Sano T, Amano H, Yamamoto M and Yamada S (2009): Diclofenac sodium inhibits NFkappa B transcription in osteoclasts . J. Dent. Res., 88: 1042-1047.

Kotake S , Schumacher HR , Yarboro CH , Arayssi TK . Pando JA , Kanik KS , Gourley MF , Klippel JH and Wilder RL (1997) : *In vitro* gene expression of type 1 and type 2 cytokines in synovial tissues from patients in early stages of rheumatoid , reactive, and undifferentiated arthritis. Proc. Assoc. Am. Physicians, 109: 286-301.

Kotake S, Yago T, Kawamoto M and Nanke Y (2010): Effects of NSAIDs on differentiation and function of human and murine osteoclasts _ Crucial ' human osteoclastology '. Pharmaceuticals, 3: 1394-1410.

Kusanag T, Ihara T and Mizutani M (1977): Teratogenic effects of non-steroidal anti-inflammatory agents in mice. Congen. Anom., 17:177–185.

Laurence DR , Bennett PN and Brown HS (1997): Comparative cytotoxicity of non-steroidal anti-inflammatory drugs in primary cultures of rat hepatocytes. Toxicol. *In vitro*, 8:55-66.

Lou YJ , Ding GS and Tu ZH (1996): Toxicity to transferred rat embryos after aspirin treatment during preimplantation stage *in vivo*. Acta Pharmacol. Sin., 17:52–54.

Mankes RF, Rosenblum I, Benitz KF, LeFevre R and Abraham R (1982): Teratogenic and reproductive effects of ethanol in Long-Evans rats. J. Toxicol. Environ. Health, 10: 267–276.

McLeod MJ (1980): Differential staining of cartilage and bone in whole mouse fetuses by alcian blue and alizarin red-S. Teratology, 22: 299-301.

Mohamed MIE (1996): The effect of alcohol and nicotine on fertility, pregnancy and development in albino mice. Ph.D. Thesis, Department of Zoology, Faculty of Science, Ain Shams University, Egypt.

Nagao JH , Morita Y, Ishizuka Y, Wada A and Mizutani M (1991): Induction of fetal malformations after treatment of mouse embryos with methylnitrosourea at the preimplantation stages. Teratog. Carcinog. Mutagen., 11: 1-10.

Nakatsuka T and Fujii T (1979): Comparative teratogenicity study of diflunisal (MK-647) and ASA in the rat. Oyo Yakuri, 17: 551–557.

Namkoong S, Lee SJ, Kim CK, Kim YM, Chung HT, Lee H, Han JA, Ha KS, Kwon YG and Kim YM (2005): Prostaglandin E2 stimulates angiogenesis by activating the nitric oxide / cGMP pathway in human umbilical vein endothelial cells. Exp. Mol. Med., 37: 588-600.

Nelson JL and Ostensen M (1997): Pregnancy and rheumatoid arthritis. Rheum. Dis. Clin. North Am., 23: 195-212.

Nielsen GL , Sorensen HT , Larsen H and Pedersen L (2001): 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., 322:266–270.

Ofori B, Oraichi D , Blais L , Rey E and Be'rard A (2006): Risk of congenital anomalies in pregnant users of non-steroidal anti-inflammatory drugs: A nested case-control study. Birth Defects Res. (Part B), 77: 268-279.

Ostensen M and Skomsvoll JF (2004): Anti-inflammatory pharmacotherapy during pregnancy. Expert. Opin. Pharmacother., 5: 571–580.

Ostensen M, Khamashta M, Lockshin M, Parke A, Brucato A, Carp H, Doria, A , Rai R, Meroni P, Cetin I, Derksen R, Branch W, Motta M, Gordon C, Ruiz-Irastorza G, Spinillo A, Friedman D, Cimaz R, Czeizel A, Piette JC, Cervera R, Levy RA, Clementi M , De Carolis S, Petri M, Shoenfeld Y, Faden D, Valesini G and Tincani A (2006): Anti-inflammatory and

immunosuppressive drugs and reproduction. Arthritis Res. Ther., 8 (3): 209–228.

Padmanabhan R. and Hameed MS (1988) : Effects of acute doses of ethanol administered at preimplantation stages on fetal development in the mouse. Drug Alcohol Depend., 22: 91- 100.

Padmanabhan R and Shfiullah M (2004) : Effect of maternal diabetes and ethanol interactions on embryo development in the mouse. Mol. Cell. Biochem., 261 : 43-56.

Paget GE and Barnes JM (1964): Toxicity tests. In Evaluation of Drug Activities: Pharmacometrics. Laurence, D.R. and Bacharach, A.L. (eds), Academic Press, London and New York, 135-166.

Rogers JM, Chernoff N , Keen CL and Daston GP (2005): Evaluation and interpretation of maternal toxicity in Segment II studies. Issues, some answers, and data needs. Toxicol. Appl. Pharmacol., 207 (2 suppl.): 367- 374.

Rutledge JC, Shourbaji AG, Hughes LA, Polifka JE, Cruz YP, Bishop JB and Generoso WM (1994) : Limb and lower-body duplications induced by retinoic acid in mice .Proc. Natl. Acad. Sci. USA, 91: 5436-5440.

Sahu CR (2009): Developmental toxicity of ibuprofen treated mice. Intern. J. Pharmaceutical Science, 1: 92-102.

Snell GD (1956): Biology of the laboratory mouse. 5th ed., the Blakiston Company, Philadelphia.

Siu SSN, Yeung JHK and Lau TK (2000): A study on placental transfer of diclofenac in first trimester of human pregnancy. Hum. Reprod., 15: 2423-2425.

Sutcliffe AG , Jones RB and Woodruff G (1998): Eye malformations associated with treatment with carbamazepine during pregnancy. Ophthalmic Genet., 19: 59-62.

Vane JR, Bakhle YS and Botting RM (1998): Cyclooxygenases 1 and 2. Annu. Rev. Pharmacol. Toxicol., 38: 97–120.

تاثير العقار مضاد الالتهاب غير الستيرويدى "دايكلوفيناك الصوديوم" على أجنة الفئران المهقاء

محمد عبد الحميد شاهين - رمضان عبد الصادق رمضان- سامية محمد صقر- سحر أحمد صبري
قسم العلوم البيولوجية والجيولوجية- كلية التربية- جامعة عين شمس

يعتبر عقار دايكلوفيناك الصوديوم المعروف تجاريا باسم "ديكلوفين" أحد أدوية مضادات الالتهاب غير الستيرويدية التي تستخدم في الساحة الطبية في مصر على نطاق واسع. وبالرغم من الاستخدامات المفيدة لهذا العقار إلا أن هناك بعض التقارير الطبية التى تشير إلى دوره في إحداث بعض الآثار الجانبية عند استعماله. ومن ثم فان الدراسة الحالية تهدف لتوضيح وتقييم الآثار الجانبية المحتملة لعقار دايكلوفيناك الصوديوم على أجنة الفئران المعاملة أمهاتها بهذا العقار .

أجريت الدراسة على 60 من إناث الفئران المهقاء الحوامل، قسمت إلى ست مجموعات واعتبرت المجموعتان الأولى والثانية مجموعتين ضابطتين وحقنت إناثهما الحوامل عن طريق التجويف البريتونى بالمذيب وهو الماء المقطر وذلك لمدة ستة أيام متتالية (من اليوم الأول إلى اليوم السادس من الحمل) وثمانية أيام متتالية (من اليوم السابع إلى اليوم الرابع عشر من الحمل), على الترتيب . وبالنسبة لحيوانات المجموعتين الثالثة والرابعة فقد حقنت بجرعة 1.5 ملجم / كجم من وزن الجسم لمدة ستة أيام متتالية (من اليوم الأول إلى اليوم السادس من الحمل) و ثمانية أيام متتالية (من اليوم السابع إلى اليوم الرابع عشر من الحمل) ، على الترتيب. وأما حيوانات المجموعتين الخامسة والسادسة فقد حقنت بجرعة 3 ملجم / كجم من وزن الجسم لمدة ستة أيام وثمانية أيام، على الترتيب، مثل الذي حدث في المجموعتين الثالثة والرابعة. وقد تم تشريح الأمهات الحوامل وفحص أجنتها فى اليوم التاسع عشر من الحمل .

وقد أوضحت النتائج المورفولوجية أن المعاملة قد تسببت فى حدوث تأخر فى نمو الأجنة تمثل فى نقص أوزانها وأطوالها مقارنة بالمجموعتين الضابطتين، فضلا عن حدوث تشوهات خارجية فى بعض الأجنة شملت تقزم فى الحجم، نزيف تحت الجلد، وتشوهات فى العينين والأطراف. وفيما يتعلق بعناصر الجهاز الهيكلى فقد تسببت المعاملة بعقار دايكلوفيناك الصوديوم فى تأخر نمو بعض العظام ونقص التعظم فى البعض الآخر، وأن هذه التأثيرات ازدادت بزيادة الجرعة .