Maternal and Neonatal Toxicities induced by three Antirheumatic Drugs in Albino Rats

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Abstract: Because of their analgesic and anti-inflammatory properties especially for patients with rheumatoid arthritis, nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most often ingested drugs during pregnancy. The aim of the present work was to evaluate and compare some of the maternal and neonatal toxicity induced by three presently marketing antirheumatic drugs namely meloxicam, celecoxib and leflunomide. The study revealed an ascendent retardation in the body weight gain of experimental dams during gestation compared to control in a drug dependent manner for meloxicam, celecoxib and leflunomide, respectively. Moreover, maternal atrophy of femur cartilage thickness associated with lacking of the integrity was observed in the treated dams. Significant retardation in weight, size and length of the maternally treated newborns was also detected compared to control. A number of congential malformations associated with a significant decrease in ossified lengths of certain axial and appendicular bones and evident missing of ossification centers were observed in the maternal treated litters. The mentioned maternal and neonatal toxicity showed direct dependency on the applied drug. The results indicate that the tested drugs should be avoided during pregnancy and if necessary, this should be done with caution.
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1. Introduction

The use of nonsteroidal anti-inflammatory drugs NSAIDs is increasing because they remain first–line during therapy for a wide range of rheumatic conditions and they are the drugs of choice for the treatment of inflammatory arthritis. It is well known that NSAIDs provide analgesia and suppress inflammation by inhibiting the enzyme cyclooxygenase, resulting in decreased prostaglandin synthesis (Urban, 2000). Many rheumatic diseases affect women of childbearing age, and the medications used to treat these diseases may affect both maternal and fetal organs (Janssen and Genta, 2000). The deleterious side effects that accompany the chronic use of NSAIDs on human health are of concern, particularly their impact on reproduction (Fort, 1999). NSAIDs readily cross the placenta and contribute alongside maternal and placental toxicity in blocking prostaglandin synthesis in a variety of foetal tissues (Burdan *et al.*, 2009 b). The risk of NSAIDs during pregnancy is best searched for aspirin including a number of complications such as diaphragmatic hernia, midline (gastro-sichisis/ umbilical hernia) and cardiac septal defects (Kauffman, 1989, Cook *et al.*, 2003 and Kozer *et al.*, 2002 &2003, Burdan *et al.*, 2006 a &b, Ofori *et al.*, 2006). Examples of other NSAIDS that have been searched for are indomethacin, sulindac, naproxen, ibuprofen, ketoprofen, and diclofenac (Ostensen, 2001) mostly because of their ability to suppress premature labour.

Animal studies may provide some guidance in the usage of drugs during pregnancy though they cannot be easily extrapolated to the human situation. A few reports have demonstrated that NSAIDs exerted embryotoxicity and teratogenicity among experimental animals including isopropylantipyrine (Burdan, 2000), Diclofenac (Chan *et al.*, 2001), acetylsalicylic acid (Espiridiao *et al.*, 2002), ibuprofen and tolmetin (Burdan, 2004) and piroxicam (Burdan, 2005a). The latter study showed that piroxicam caused maternal toxicity, intrauterine growth retardation, and increase of external and skeletal variations in rats, while the decrease of fetal length was the only signs of developmental toxicity observed in pups. More recently, a number of histopathological side effects have been reported for both celecoxib and leflunomide on liver and kidney of both neonatal and pregnant albino rats (El-Sayyad *et al.*, 2010).

As immunosuppressant drug, leflunomide has been found to be teratogenic in a wide range of experimental animals. In rabbits, fused and incomplete ossification of the sternebra are seen when treatment began during fetal organogenesis (Sanofi-Aventis, 2003). In mice, leflunomide causes multiple malformations over the entire body of the fetus when administered to pregnant mice during gestation day 6-15 (Fukushima *et al.*, 2007). The latter study showed that the characteristic

malformations induced by leflunomide are exencephaly, cleft palate, tail deformity anomalies of the axial skeleton, and cardiovascular malformations. A more recent studies (Fukuschima *et al*., 2009 & 2010) demonstrated that leflunomide induced limb malformations in mice fetuses via inhibition of dihydroorotate dehydrogenase, a key enzyme in the *de novo* synthesis of pyrimidine found in lymphocytes and other cells with subsequent inhibition of RNA and DNA synthesis. The same study demonstrated that the coadmistration of uridine inhibited most of the teratogenicity caused by leflunomide.

In human, it has been reported that NSAIDs can cross the placenta and therefore have been detected in foetal tissues (Siu *et al*., 2000). A few reports confirmed the animal findings and indicated that the use of prescribed NSAIDs was found to be highly associated with miscarriage beside certain teratological effects and an increased risk for defects of oral facial clefts (Ericson and Kallen, 2001). Santis *et al.* (2005) reported higher incidence of congenital malformations in neonatal outcome among women subjected to leflunomide-treatment during pregnancy. Leflunomide was also found to cause dose-related teratogenicity and fetotoxicity including malformations of the skeleton and central nervous system and prenatal exposure resulted in decreased birth weight and increased mortality in the offspring (Brent 2001 and Casanova *et al.,* 2005). It is well known that cyclooxygenase inhibitors caused intrauterine growth retardation and increased the number of skeletal developmental variations (Gross *et al.,* 1998, Reese *et al.,* 2000 and Burdan *et al.*, 2003). Such observations were partially confirmed in manufacturer studies with celecoxib (PDR, 2003). However, those results were never fully published in available journals.

The present experimental study was therefore aimed to evaluate and compare some of the possible morphological and skeletal maternal and neonatal teratogenic effects of certain marketing NSAID, namely, meloxicam and selective COX-2 inhibitor i.e. celecoxib as well as leflunomide as immunosuppressant drugs.

2. Material and Methods

2.1. Animals and Housing

Forty eight sexually mature healthy Wister albino rats (*Rattus norvegius*), 12-15 weeks of age and weighing 125 ± 5 g were purchased from the breeding center of experimental animals at Helwan University, Helwan, Egypt and used for experimentation. The rats were acclimated for at least 2 weeks, housed and maintained in an animal care facility. The experiments were approved by the Animal Research Ethics Committee at Menoufiya University and were conducted in accordance with the guidelines for animal experiments at Menoufiya University. Free access of standard diet composed of 50 % grinding barley, 20% grinding yellow Maize, 20% milk and 10% vegetables was supplied and tap water was allowed *ad-libitum*. Rats were housed in individual cages and maintained in a room at 23 ºC. They were kept under good ventilation with a 12 hour light/dark photocycle. Feed and water consumption were monitored daily after the acclimatization period. Females were made pregnant by keeping them with healthy fertile male rats overnight (at a ratio of 1 male: 3 females). On the next morning, vaginal plugs were examined. Vaginal smears were carried out to give a precise determination of the onset of gestation i.e. the designation of gestation day 0. The pregnant rats were arranged into four groups nine female each including one control group and three experimental groups.

2.2. Applied drug-treatment

Three anti-rheumatic drugs were used in this study, namely, celecoxib and meloxicam as non-steroidal cyclooxygenase inhibitor drugs and leflunomide *(Avara)* as a disease-modifying antirheumatic drug. The three drugs were bought from a local pharmacy and manufactured by Amoun pharmaceutical Company, El-Obour city, Cairo, Egypt. The drug tablets were ground separately with Tween-80 (Sigma Chemical Co., St. Louis, Mo, USA). Using distilled water, the suspension was freshly prepared and orally administered by the use of gastric tube to experimental pregnant rats every other day beginning on gestational day 6 and ending on the day 20 few hours before spontaneous delivery which occurred on day 21, while the control group had no treatment. The drug administration period was therefore prolonged to the end of pregnancy, according to the FDA guidelines (Christian, 2001). Care was taken just before the anticipated day of parturition to prevent dams from eating any malformed offspring. The applied therapeutic dose for celecoxib (Pulbutr and Sookvanichsilp, 2002) and leflunomide (Menor and Dunham, 1999) was 0.2 mg/kg body weight whereas it was 0.4 mg / kg body weight for meloxicam (Salhab *et al.,* 2001).

2.3. Maternal investigation

Dam body weight was monitored every other day before drug administration from day 6 until the end of pregnancy. All animals were observed at least once daily before treatment and two times a day during treatment. The total numbers of delivered newborns were recorded for both control and experimental groups. At parturition, the pregnant rats of both control and experimental groups were sacrificed and ten femur bones were incised and

fixed by immersion in 10% neutral formalin for 24 hours and decalcified in 5% nitric acid for 24 hours, after then washed carefully in water and returned to 10% neutral formalin. Consequently, the femur bones were dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in molten paraplast 58-62ºC. 5 μ m histological sections were cut, stained with Harris hematoxylin, counter stained with eosin and investigated under bright field Leitz microscope. The obtained sections were subjected to morphometric analysis. Some sections were photographed with "Letiz-Laborlux-S" binocular photomicroscope.

2.4. Delivered newborns investigation

When fetuses were spontaneously delivered on gestational day 21, they were routinely examined. External variations and different morphological abnormalities were examined. The size (cm^3), weight (g.) and crown-rump length (cm) of delivered newborns of both control and experimental groups were therefore determined and recorded. The size of the newborns was determined using measuring cylinder containing 10 cm^3 fixative solution (10 % neutral formalin). The final size after putting the newborn was subtracted from the constant volume of the fixative i.e. 10 cm^3 to find out the exact size of the specimen which represents the displacement volume.

After analyzing the morphological teratogenic effects, both experimental and control delivered newborns were further investigated for the possible adverse effects on bone elements. This has been done by eviscerating and fixing the whole specimens in 10% neutral buffered formalin followed by treatment with 2% potassium hydroxide for 5 days till ossified areas were clearly visible through the soft tissue. The whole specimens were stained following transparency technique for demonstration of bones by Alizarin red "S" method (Mahony, 1973).

The axial and appendicular bones were examined for the occurrence or absence of ossification centers. Lack of alizarin staining was interpreted as bone missing. The incidence of missing bones and length reduction of axial and appendicular bones were determined. Length measurements of mandibular bone (axial region) as well as of ischium, ilium and fore- (humerus, radius and ulna) and hind (femur, tibia and fibula) limbs were carried out in 10 specimens per each group using an ocular micrometer to assess the degree of length reduction displayed by experimental groups comparing with the control.

2.5. Statistical Analysis

The data are presented as the means ± standard error of the mean (SEM) of different groups. Statistical analysis was carried out using normal deviates (z-scores) and Student's t test to compare the experimental groups with the control using Minitab 12 computer program (Minitab Inc. State College, PA). A difference was regarded as statistically significant at a value $P<0.05$.

The following four integrated teratogenic parameters were therefore investigated for both control and experimental groups:

1- Body weight gain of dams during gestation as well as the total number of delivered newborns.

2- Histological alterations of maternal femur.

3- Body weight (g.), size (cm^3) and crown-rump length (cm) *of delivered newborns.*

4- Morphological *and skeletal abnormalities of the delivered newborns including morphometric assessments of both axial and appendicular* skeletal bones.

3. Results

3.1. Maternal toxicity examination

Within treated groups, food consumption exhibited retardation compared with control in an ascending order for celecoxib, meloxicam and leflunomide respectively.

3.1.1. Body weight gain of dams during pregnancy

Fig.1 demonstrates the significant decrease in the accumulated body weight gain of experimental groups during gestation compared to control. Statistical analysis showed that the reduction magnitude in weight gain was 19.67, 28.96 and 31.97% of that of the control for celecoxib, meloxicam and leflunomide, respectively.

3.1. 2. Histological examination of femur

The femur of the control dams exhibited normal epiphyseal cartilage with regular arrangement of cartilage column. The epiphyseal cartilage differentiates into resting layer, cartilage column, hypertrophied zone and calcified zone and bone trabeculae (Fig. 2 A). However, femur of dams treated with celecoxib (Fig. 2 A1) and meloxicam (Fig.2 A2) showed a marked disruption of cartilage cells including reduction of cartilage column, lacking their integrity, deranged epiphyseal line and lacked most of their building cartilage cells. The cartilage stromata became widened with widely separated cartilage cells. The bone trabeculae attained a considerable reduction. Leflunomide treated dams exhibited disruption of cartilage column cells including reduction of the epiphyseal cartilage associated with irregularity of cartilage column cells and reduction of trabeculae bone (Fig. 2 A3).

3.1. 3. Morphometric assessments of femur

Fig. 3 illustrates the depths of epiphyseal cartilage (µm) of femur bone of control and experimental dams. The antirheumatic drug-treatment induced a significant atrophy of epiphyseal cartilage in an ascending manner i.e. celecoxib, meloxicam,

and leflunomide groups.

3.2. Developmental toxicity

3.2.1. Morphological abnormalities of delivered newborns

Table 1 shows three of the investigated parameters of developmental toxicity. Fetal weight was significantly reduced and followed the same trend of the experimental dams. It therefore has an ascending order for celecoxib, meloxicam then leflunomide respectively. The table also shows the evident reduction of body size (cm^3) and crown-rump length (cm) *of* delivered newborn rats maternally treated with the tested anti-rheumatic drugs.

Examining the gross morphology of maternally treated newborns shows the presence of some pattern of congenital malformations including kyphotic body, uni-& bilateral malformation of both fore -& hind limb, kinky tail and presence of superficial spotty regions of skin hemorrhage in head, neck and trunk regions (superficial haematomas). These abnormalities are observed at higher rates in delivered newborns maternally treated with either celecoxib or leflunomide. Table 2 summarizes all the external malformations and Figs. 4 A1 & A2 demonstrates two malformed examples compared to control (Fig. 4 A).

3.2.2. Effects of the tested drugs on ossification of delivered newborn skeleton

Contrarily to the control group (Fig. 4 B) delivered newborns maternally-treated with the tested antirheumatic drugs show a significant decrease in the length of the primary ossification centers in both axial and appendicular bones. Fig. 4 B1 & B2 demonstrate examples of skeletal preparations. As can be noted from the figures, the decrease in the length of the primary ossification centers were restricted mainly in nasal , parietal, interparietal ,zygomatic arch of squamosal , hyoid arch , squamosal, tympanicum , exoccipital , supraoccipital , sternum , ischium , pubis and distal phalanges. As shown in table 3, delivered newborn rats of the three experimental groups exhibit significant reduction in the ossified length of mandibular, scapula, ilium, humerus, radius, ulna, femur, tibia and fibula. It is also evident from the table that the highest reduction of ossified length is detected post- leflunomide and celecoxib-treatment.

Assaying the incidence of missing ossified bones in newborns of different experimental groups revealed that the most affected bones are distal phalanges of both fore-& hind limbs, tympanic region, hyoid arch, exoccipital, and pubs. Developmental skeletal variations are seen in the treated litters (Table 4).

4. Discussion

The majority of rheumatologic disorders have been increased frequently in women (Temprano *et al*., 2005). Rheumatic diseases in women of childbearing years may necessitate drug treatment during pregnancy to control maternal disease activity and to ensure a successful pregnancy outcome. The illness may be severe enough that it requires therapy even during pregnancy and lactation. Health care providers need to be aware of the potential adverse effects of these antirheumatic drugs during conception and pregnancy and should carefully weigh the risks and the benefits of these medications for both mother and child. It is worth mentioning that observations concerned prenatal toxicity are out of the scope of this study since the present data are limited only to the maternal and neonatal toxicity. The latter is attracting special interest particularly in view of the present FDA initiative to encourage clinical trials in children (FDA, 1998).

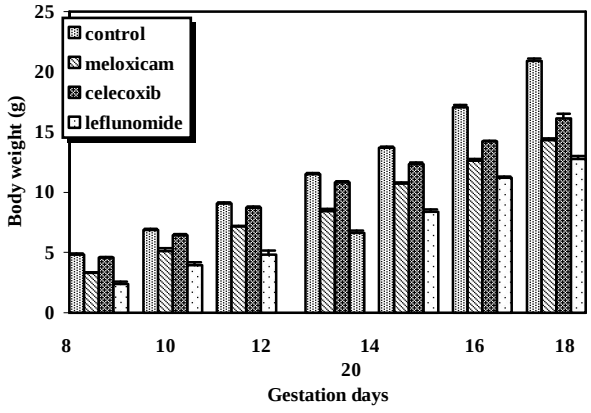

Fig. (1): Maternal increase in body weight gain of control and experimental dams.

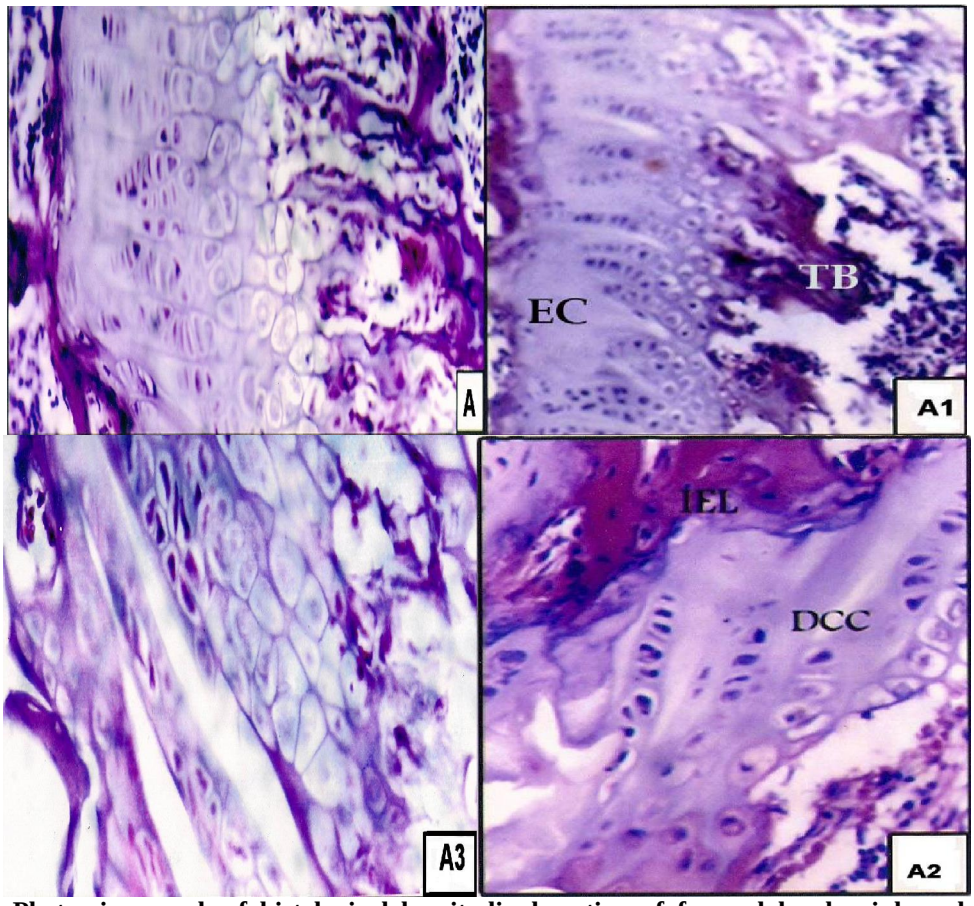

Fig. (2): A: Photomicrograph of histological longitudinal section of femoral head epiphyseal cartilage of control dam showing normal arrangement of epiphyseal cartilage column. (H&E. X100). A1 & A2: Photomicrographs of histological longitudinal sections of femoral head epiphyseal cartilage (EC) of celecoxib and meloxicam-treated dams showing marked atrophy of cartilage thickness, irregular epiphyseal line (IEL) and degenerated cartilage cells (DCC). The arrangement of cartilage column is disturbed and the trabeculae bone (TB) is reduced. (H&E. X200).
A3: Photomicrograph of histological longitudinal section of femoral head epiphyseal cartilage of leflunomide-treated dam showing reduction of epiphyseal cartilage thickness associated with irregularity of cartilage column cells and reduction of trabeculae bone. (H&E. X100).

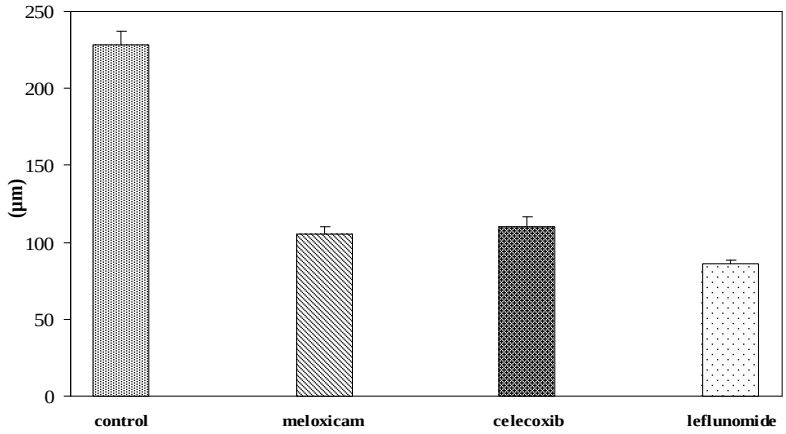

Fig. (3): The depths of epiphyseal cartilage (µ m) of 10 femur bones of both control and experimental dams.

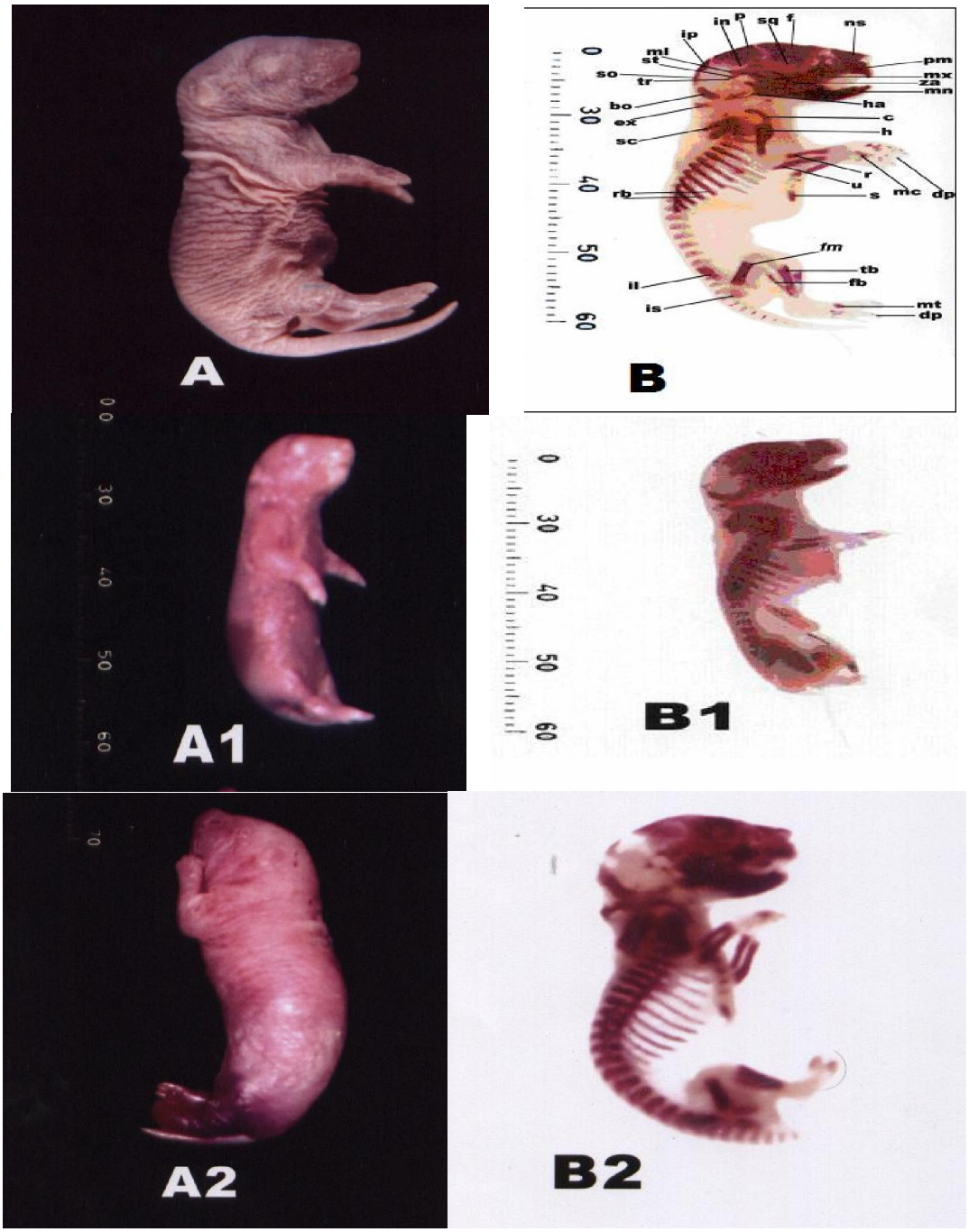

Fig. (4): A: Lateral view photograph showing the gross morphology of control delivered newborn rat. A1: Lateral view photograph showing the gross morphology of delivered newborn maternally treated with leflunomide. A2: Lateral view photograph showing the gross morphology of delivered newborn maternally treated with celecoxib. B: Lateral view photograph of alizarin red preparation of control delivered newborn rat showing normal skeletal elements. nasal (ns),frontal (f), parietal (p), interparietal (ip), premaxilla (pm), maxilla (mx), zygomatric arch (za), hyoid arch(ha), mandibular (mn), squamosal(sq), incus (in), ml: malleus(ml), stapes (st), tympanic ring (tr), basioccipital (bo), exoccipital (ex), supraoccipital (so), scapula (sc), clavicle (c), sternum (s), ribs (rb), ischium (is), ilium (il), humerus (h), radius (r), ulna (u), distal phalanx (dp), metacarpal (mc), femur (fm), tibia (tb), fibula (fb), metatarsal (mt). B1 & B2: Lateral view photographs of alizarin red preparation of delivered newborn maternally-treated with meloxicam and leflunomide showing reduction in the lengths of the ossification centers.

Table (1): Morphological assessments of control and experimental delivered newborn rats.

Parameter	*Control*	*Meloxicam*	*Celecoxib*	*Leflunomide*
Body weight (g)	0.04±5.67	0.19±4.82	0.09±5.29	0.17±3.69
Body size (cm^3)	o.12±6.8	0.19±4.8	0.15±4.65	0.23±3.69
Crown- rump length (cm)	0.47±41	0.54±37.4	0.75±40.6	1.07±38.4

Values are means ± Standard error of the mean.

Table (2): Morphological abnormalities of control and experimental delivered newborn rats.

	Control	Meloxicam	Celecoxib	Leflunomide
Total number	20	20	20	20
Superficial haematomas	0	4	8	3
Reduced neck region	0	5	3	0
Kinky tail	0	10	10	1
Mild-kyphotic body	0	2	0	0
Abnormal fore limb				
-Unilateral	0	10	13	7
-Bilateral	0	1	1	1
Abnormal hind limb				
-Unilateral	0	8	5	3
-Bilateral	0	2	3	0
Oedematous skin	0	1	0	0
Abnormal skin appearance.	0	2	0	0

Table (3): Morphometric assessments of control and experimental delivered newborn rats.

Skull	*Control*	*Meloxicam*	*Celecoxib*	*Leflunomide*
Ossified length (mm) of mandibular bones	0.02±8.09	0.46±4.64	0.21±4.16	0.14±2.57
Ossified length (mm) of scapula bones	0.04±4.30	0.07±1.79	0.07±1.31	0.12±1.18
Ossified length (mm) of ilium bones	0.07±2.84	0.23±1.43	0.08±0.72	0.05±0.82
Ossified length (mm) of humerus	0.03±5.45	0.11±2.08	0.07±1.79	0.08±1.45
Ossified length (mm) of radius	0.06±5.07	0.07±1.79	0.10±0.74	0.09±1.14
Ossified length (mm) of ulna	0.04±4.85	0.10±2.14	0.05±0.84	0.09±1.59
Ossified length (mm) of femur	0.01±4.08	0.11 ±1.61	0.06±1.13	0.07±1.09
Ossified length (mm) of tibia	0.05±4.48	1.07±1.79	0.12±0.50	0.16±1.32
Ossified length (mm) of fibula	0.03±3.82	1.10±1.67	0.06±1.30	0.11±1.05

Values are means ± Standard error of the mean.

Table (4): Incidence of missing ossified bones of both control and experimental delivered newborn rats.

Missed bones	Control	Meloxicam	Celecoxib	Leflunomide
Total number	20	20	*15*	20
Nasal	*0*	*2*	*2*	*3*
Interparietal	*0*	*4*	*1*	*0*
Pre maxilla	0	2	1	0
Hyoid arch	0	5	3	2
Zygomatic arch	*0*	*2*	*3*	*0*
Squamosal	0	4	4	0
Ossicles(incus, malleus & stapes(	0	4	4	2
Tympanic ring	0	4	4	11
Basioccipital	0	0	2	7
Exoccipital	0	4	3	1
Supraoccipital	0	4	4	0
Incomplete ossification of sternum	0	4	4	0
Ischium	*0*	*4*	*2*	*0*
Pubis	*0*	*4*	*4*	*4*
Tibia & Fibula	*0*	*2*	*2*	*0*
Distal phalanx	*0*	*10*	*12*	*12*

The present study showed that the investigated drugs caused morphological as well as skeletal changes in both dams and their newborn albino rats. Thus, a reduction in the weight gain of pregnant dams compared with control was largely noticed with leflunomide, meloxicam and celecoxib respectively and therefore, the decrease in the rate of body weight gain varied with the drug type. It is well known that maternal toxicity reflects adversely on the offspring (Chahoud *et al.*, 1999 and Burdan *et al.*, 2009 b) and consequently, similar findings were also noticed among the newborn rats, where the body parameters and bone ossification were adversely affected. These adverse effects would be exemplified by the magnitude of weight gain reduction, which is a symbol of the overall drastic change of these drugs. Thus, it could be stated that the drastic effect on health as reduction in weight gain of the studied animals exhibited significant dependency upon the applied drug. Hewiston *et al.*, (2000) showed that among its adverse side effects, leflunomide caused weight loss. Other animal data indicated that exposure to leflunomide at normal therapeutic levels during pregnancy has teratogenic and foetotoxic effects including reduced foetal weight (Hazes *et al.*, 2004). The observed developmental effects as expressed by delayed ossification are related to the fetal growth retardation (Burdan, 2005a).

The morphological abnormalities were evident in delivered newborn rats maternally treated with the tested drugs. According to the present data, the incidences of abnormalities are found to appear markedly higher in newborn maternally treated with either leflunomide or celecoxib more than meloxicam. The observed morphological abnormalities were kyphotic body, malformed fore-& hind limb, reduced neck region, superficial haematomas and kinky tail. These abnormalities were associated with reduction in body weight, size and crown-rump length. According to Manson and Kang (1994) the body weight, the crown length of the fetuses were considered sensitive indicators of animal's response to xenobiotics. However, superficial haematomas can be interpreted as a side effect of inhibition of cyclooxygenase enzyme (Burdan, 2000). In accordance with the present data, decreased foetal length was reported post piroxicam treatment by Burdan *et al.* (2005a) and similar pattern of congenital abnormalities were observed post diclofenac-treatment (Chan *et al*., 2001).

Number of animal and human studies showed that cyclooxygenase inhibitors may disturb bone physiology and decrease synthesis of cartilage matrix (Seidenberg and An, 2004 and Burdan 2005b). Studies stem from variety of animal models indicating that NSAIDs have adverse effects on the skeletal system. According to Allen *et al*., 1980; Tornkvist and Lindholm, 1980; Keller *et al*., 1987 Ho *et al*., 1995, the NSAIDs induced cytotoxicity of cartilage cells, the element components of epiphyseal cartilage of the femur. The defects of epiphyseal cartilage may be attributed to the inhibition of glycosaminoglican and collagen synthesis (McKenzie *et al*., 1976; Palmoski and Brandt, 1979; Herman *et al*., 1984). Meanwhile, the disruption of cartilage column and degeneration of cartilage cells manifesting the apoptic cell death as a result of drug toxicity. These may be attributed to the slow physiological death leading to apoptosis (Kuhn *et al*., 2004).

The direct effects of NSAIDs on cartilage have frequently been reported to be adverse (Smith *et al.*, 1995) but generally ignored owing to the fact that they are not visible during clinical evaluation and are shadowed by the effects on inflammation. Despite that, there is a significant amount of evidence that cartilage is sensitive to certain NSAIDs which inhibit the synthesis of cartilage proteoglycans (Dingle, 1999). Decrease in alizarin staining, as a qualitative sign of mineralization reduction, was observed in bones whose development occurred slowly and / or late in fetal life e.g. cranial, phalanges, metacarpal and metatarsal (Zoetis *et al.*, 2003). Lack of the alizarin staining in well formed cartilage structures, indicating a delay of ossification as a result of intrauterine growth retardation. Poorly ossified metacarpal, metatarsal and caudal vertebrae were also observed in the present work after drug treatment with highest average in leflunomide-treatment. The results obtained from this study indicated that ossification centers of different parts of axial and appendicular regions were retarded especially in tympanic region, sternbrae, clavicle, ischium, distal phalanges of fore-& hind limbs as well as caudal vertebrae which represented by higher incidence of missing ossified bones or delayed formation. Length measurements of ossified bones in mandibular, humerus, radius, ulna, femur, tibia, fibula, scapula and ilium appeared markedly reduced in maternally treated newborns. These findings are in agreement with the report of Brent (2001) in that leflunomide given to pregnant rats and rabbits in doses equivalent to human doses induced skeletal malformations in the offspring. Other studies have shown similar pattern of retarded ossifications including coccygeal, sarcococcygeal vertebrae missing, reduced ossification of skull bones, wavy ribs and decreased ossification of appendicular bones in newborn maternally treated with the highest dose of cox-2 inhibitors NSAIDs including tolmetin, ibuprofen and piroxicam (Burdan *et al*., 2005 & 2009a). Similar results have been reported by Fukuschima *et al.*

(2009 & 2010) on mice fetuses. *In vitro* experiments revealed that NSAIDs including celecoxib suppress proliferation, delay the endochondral ossification and induce cell death of cultured osteoblasts of fetal rats (Chang, *et al.*, 2006). However, according to available data (Fritz, 1975, Khera, 1981, Beck, 1990 and Solecki *et al.*, 2003) growth restriction and most of the skeletal variations, including treatment-related decrease of bone ossification, could be normalized during postnatal life and thereafter. However, more recent studies indicated that delayed fetal mineralization may be the cause of complications in adulthood (Burr, 2002).

Taken together, the spectrum and frequency of bone malformation presented here are similar to those observed in fetuses exposed prenatally to paracetomol and caffeine (Burdan and Wyskiel, 1999; Burdan, 2001). The maternal toxicity is manifested by decreased body weight gain and both hepato-and nephrotoxicity lead to fetotoxicity (El-Sayyad *et al.*, 2010). Most of the observed developmental defects, e.g., morphological and skeletal malformations are possibly related to the foetal growth retardation (Burdan, 2003).

Based on the present data, it could be concluded that celecoxib, meloxicam and leflunomide induced maternal and neonatal toxicity and consequently, it is highly recommended to delay the treatment with these drugs until the termination of pregnancy.

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