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The Effect of Amoxicillin on The Fetuses of Albino Mice

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ABSTRACT

Introduction: The present study was carried out to evaluate the effect of the beta-lactam antibiotic amoxicillin on the fetuses of albino mice from the morphological and skeletal points of view.

Material and methods: Twenty four adult pregnant mice were used in the present study. They were allocated into 3 groups (8 mice each). The first group served as a control and were injected intraperitoneally (i.p.) with the solvent of the drug and the second and third groups were treated with 205 and 820 mg/kg body weight of amoxicillin for 8 days (gestation days 7-14), respectively.

Results: The morphological examination of the fetuses of treated groups showed growth retardation of mice fetuses as represented by the conspicuous decrease in the average body weight and body length in the two treated groups. No external malformations were recorded among fetuses maternally treated with the low dose of the drug. On the other hand, the fetuses maternally treated with the high dose showed mild external morphological malformations. In addition, the skeleton of the two treated groups exhibited incomplete ossification in most skeletal elements.

Conclusion: The beta-lactam antibiotic amoxicillin had exerted mild morphological malformations and skeletal abnormalities in mice fetuses maternally treated during organogenesis period of gestation.

Keywords: Amoxicillin-Morphology-Skeleton-Fetuses of albino mice.

INTRODUCTION

The beta-lactam antibiotic drugs are useful agents used for the treatment of many cases of urinary tract infections [1], acute otitis media[2] and pneumonia [3].

They were classified into four categories according to their chemical structure[4] . The first category was the penicillin derivatives like amoxicillin, cephalosporis like cefprozil, monobactams like aztreonam and carbapenes like meropenem. β-lactam antibiotics are bacteriocidal, and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. The peptidoglycan layer is important for cell wall structural integrity being the outermost and primary component of the wall[4] . In spite of the beneficial role of β-lactam antibiotics[5] . reported that amoxicillin crosses the human placenta readily during the first trimester. Also, growth retardation and malformations of the skeletal elements were observed in mice fetuses maternally treated with 8mg/kg of Doxycycline [6]. Delaying in development of skull bone and cartilage calcification due to the administration of carbamazepine were also reported by **Gerenutti *et al.*** [7]

Higher incidence of bone developmental variations was seen in rats fetuses whose mothers were treated with D-penicillamine in GD15-19 [8] . **Xia, *et al.*** [9] observed bone malformation in rat fetuses maternally treated with adriamycin in gestation days (GDs) 8-9. **Kotsios *et al.*** [10] recorded skeletal anomalies in rat fetuses maternally treated with adriamycin in GDs 6-9.

There is even less information concerning the most common generation of beta-lactam antibiotics, amoxicillin. Few reports have been presented on the effects of amoxicillin on fetuses covering the gestation days of organogenesis (GDs 7-14). In the present study we aim to investigate the potential teratogenic effects of amoxicillin on morphology and skeletal structures of mice fetuses maternally treated during gestation days 7-14.

MATERIAL AND METHODS

The beta-lactam antibiotic drug used in the present investigation is amoxicillin which is available in the form of vials, each containing 1000mg of the active ingredient . It was obtained from 1 A Pharma. The low dose and the high dose of this drug for mice were calculated according to **Paget and Barnes** [11] .The chosen doses were nearly comparable to the human effective therapeutic dose (ETD). Two doses of amox were used in the present study; 205 and 820 mg / kg body weight 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 21-23g obtained from the

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breeding unit of Theodor Bilharz Research Institute(TBRI), Imbaba,Giza .

Female and male mice were housed separately in plastic cages, free access of food and tap water were allowed. 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 [12] Females which give positive vaginal smears are considered pregnant and the day of detection was taken to indicate gestation day (GD) 1. Twenty four pregnant female mice were divided into three groups (8 mice each). The first group is considered as the control group (C) and the other two groups (A&B) are the drug treated groups. The treatment of these groups was achieved in the following manner:

Group (C):Each pregnant female was injected intraperitoneally with 0.1ml saline solution (the solvent of the drug) daily for 8 days during pregnancy from day 7 till day 14 of gestation (organogenesis period).

Group (A): Each pregnant female was injected intraperitoneally with 205mg/kg body weight of the amox for 8 days during pregnancy from day 7 till day 14 of gestation.

Group(B): Each pregnant female was injected intraperitoneally with 820mg/kg body weight of the amox for 8 days during pregnancy from day 7 till day 14 of gestation.

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 . The mean number, mean body weight and mean body length of fetuses were recorded and statistically analyzed using Independent 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** [13]. 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 preparations of control and maternally treated fetuses.

RESULTS

Morphological studies:

On the day 19 of gestation, the pregnant mice of the control (C) and the treated groups (A &B) were sacrificed and the percentage of alive fetuses, mean body weight and mean body length of fetuses are recorded (Table 1 and Figures 1&2).

The data show that treatment with 205 and 820 mg/kg body weight of amox 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 amox during GDs 7-14), while the highest rate of decrease in mean fetal body weight was noticed in members of group B (treated with the high dose of amox during GDs 7-14) as illustrated in Table 1 and figures1&3.

The results displayed in Table 1 and Figures 2 &3 showed a significant decrease in the mean body length of fetuses maternally treated with the low (205 mg/kg) and high (820 mg/kg) doses of amox in compared with those obtained in the control group. The highest rate of decrease in mean fetal body length was observed in fetuses maternally treated with the high dose (group B) of the drug during GDs 7-14. However, the minimal decrease in mean fetal body length was recorded among fetuses of group A (maternally treated with the low dose of amox during GDs 7-14).

Incidence of gross malformations:

In the present investigation, the gross examination of maternally treated fetuses with low or high doses of the beta lactam antibiotic amoxicillin showed that the reported significant decrease in mean body weight and body length was accompanied with a noticeable reduction in litter size. No external malformations were recorded among fetuses of the control group and fetuses of group A (maternally treated with the low dose of amox during GDs 7-14). While mild degree of external malformations were recorded among fetuses of group B (maternally treated with the high dose of amox during GDs 7-14). These malformations appeared in the form of: stunting in size, malrotated limbs and tail defects (Figs. 4&5).

Skeletal studies:

The cleared cartilage and bone preparations of the control and maternally treated fetuses are shown in figures 8-13. The control fetuses showed normal ossification, the dermal bone of the skull is well ossified and consists of paired nasals, roofing the nasal cavities, followed posteriorly by the paired frontals and parietals. The triangular interparietal bone located between the posterior ends of the parietals, as seen in figure 8.The upper jaw bones is premaxilla, maxilla, jugal forming most of the zygomatic arch and temporal, completing the zygomatic arch and covering the sides of the skull, including the tympanic bulla. The lower jaw or mandible is composed of single pair of bones, the dentaries, joined anteriorly as designated in figure 8.The chondral bones or replacing bones of the skull include the basioccipital, two exooccipitals, basisphenoid, presphenoid and orbitosphenoids which are clearly ossified. The supraoccipital, squamosals and ethmoid and tympanic regions are not ossified. The vertebral column is composed of 7 cervical, 13 thoracic, 6 lumber, 4 sacral and caudal vertebrae. The sternum is composed of six well ossified sternebrae. The mouse fetus have 13 pairs of ribs. Each rib consists of a bony vertebral portion and a cartilaginous sternal one. The sternal parts of the ribs, except the last three pairs, articulate with the sternum. The pectoral girdle consists of well ossified two dorsal broad triangular chondral bones, the scapulae: two small cartilaginous supra-scapulae attached to the dorsal ends of the scapulae and two small slender dermal bones; the clavicles (fig.8).The fore limbs are composed of humerus, radius and ulna, carpals, metacarpals and phalanges. The humerus is well ossified long bone with cartilaginous head and trochlea. The radius and ulna are slender and slightly curved bones with ossified middle portion. The carpals are not ossified and are shown as cartilaginous elements. The metacarpals are five slender elements in which the first one is very much reduced and cartilaginous while the remaining metacarpals are clearly ossified. The phalanges are not well ossified .The pelvic girdle is composed of three well ossified bones; an anterior ilium, a posterior ischium and a ventral pubis on each side as designated in figure 11.The hind limb is composed of femur, tibia and fibula, tarsals, metatarsals and phalanges. The femur is well ossified long bone with cartilaginous head and condyles. The tibia is a straight bone with well ossified middle portion fused distally with the tibia. The tarsals are composed of elements arranged in rows. The metatarsals consist of five elements in which the first one is rudimentary and cartilaginous while the remaining metatarsals are ossified. The phalanges are not well ossified.

Fetuses maternally treated with amox (205 or 820 mg/kg b.wt.) showed overall incomplete ossification of the dermal bones, reduction in the size of the ossified bones and shortening of mandible bone. The tip of the hypoplastic mandible appeared posterior to the tip of maxilla (Figs.9&10). The skeletal elements of the fore limbs of maternally treated fetuses were considerably shorter and less ossified, hypoplastic sacral and caudal vertebrae as well as reduction in the size and ossification of caudal vertebrae as compared to control fetuses (Figs.12&13). 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	100	0	0	0	1.52±0.0078	3.004±0.00286
A	100	0	0	0	1.305±0.0068 0.054	2.611±0.0077 0.059
B	100	0	4	1.84%	1.076±0.0117 0.000	2.0804±0.01104 0.000

C : fetuses of control group

A: fetuses maternally treated with 205mg/kg body weight of amox for 8 days (GDs 7-14)

B: fetuses maternally treated with 820mg/kg body weight of amox for 8 days (GDs 7-14)

*P<0.05=significant and **p<0.01=highly significant.

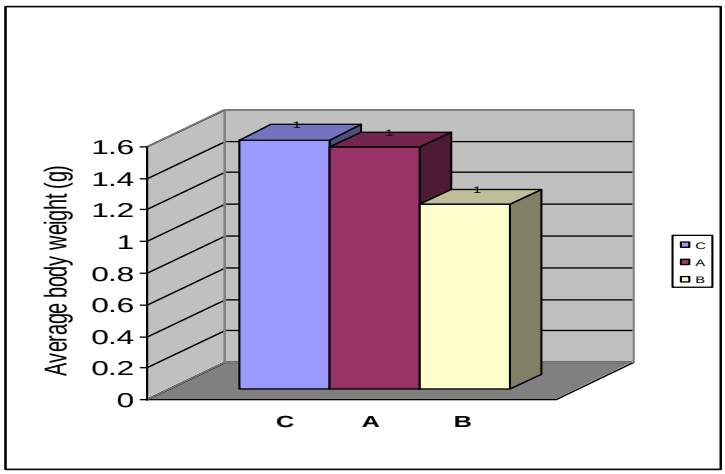

Fig.1: Illustrating the average body weight (g) of mice fetuses maternally treated with 205mg/kg body weight (group A) and 820mg/kg body weight (group B) of the antibiotic amox for 8 days during organogenesis period from day 7 till day 14 of gestation and their corresponding control(C).

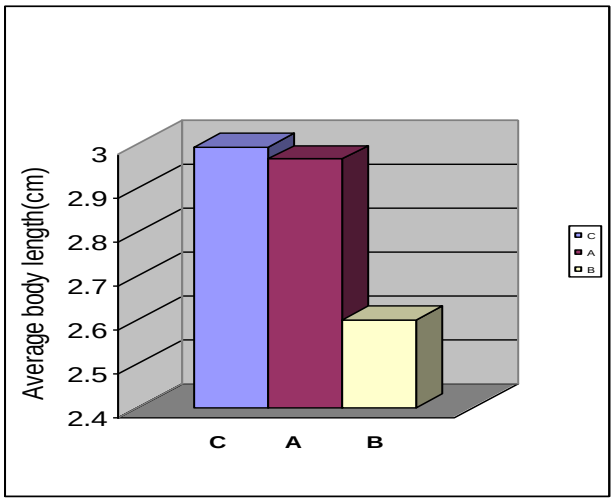

Fig.2: Illustrating the average body length (cm) of mice fetuses maternally treated with 205mg/kg body weight (group A) and 820mg/kg body weight (group B) of the antibiotic amox for 8 days during organogenesis period from day 7 till day 14 of gestation and their corresponding control(C).

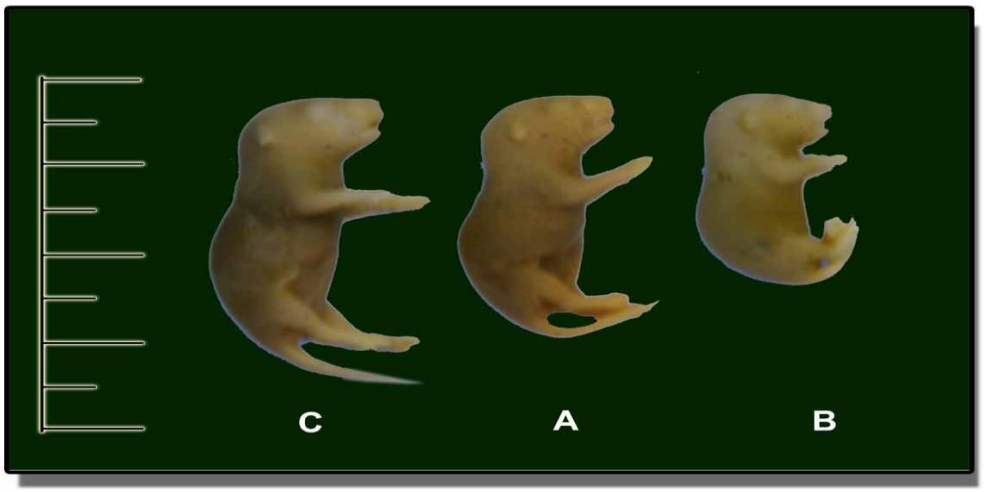

Fig.3: Photograph of 19-day old mice fetuses of the control (C) and maternally treated (A&B) fetuses showing growth retardation and stunting in size in the two treated groups. (A) fetuses maternally treated with

205 mg/kg b.wt. of amox for 8 days during GDs 7-14**.** (B) fetuses maternally treated with 820 mg/kg b.wt. of amox for 8 days during GDs 7-14**.**

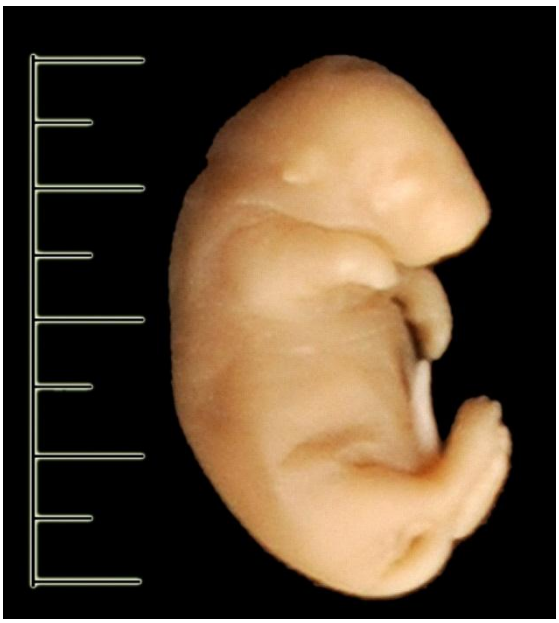

Fig.(5)

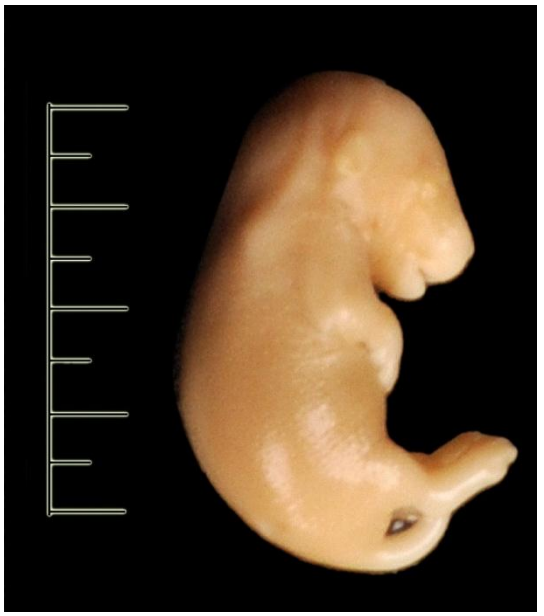

Fig.(4)

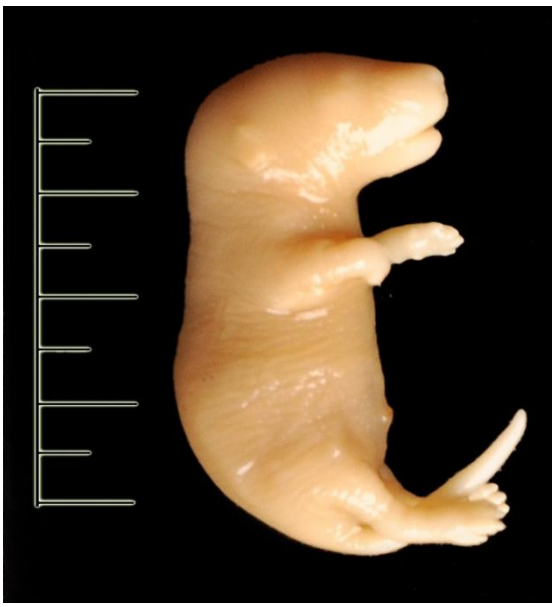

Fig.(6)

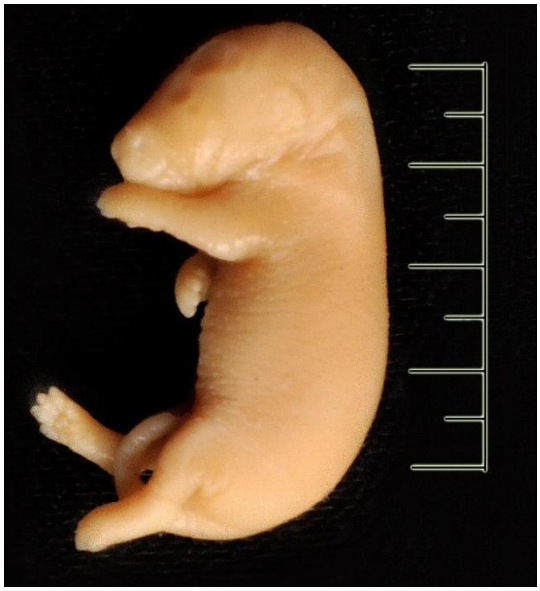

Fig.(7)

Figs.4-7: Photographs of 19-day old fetuses maternally treated with 820 mg/kg b.wt. of amox for 8 days during GDs 7-14, showing types of malformations in the form of malrotated fore and hind limbs (4-6) and tail defects (7).

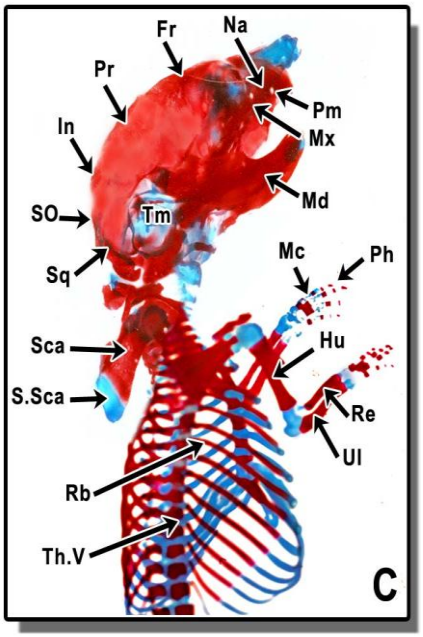

Fig.(8)

Fig.(9)

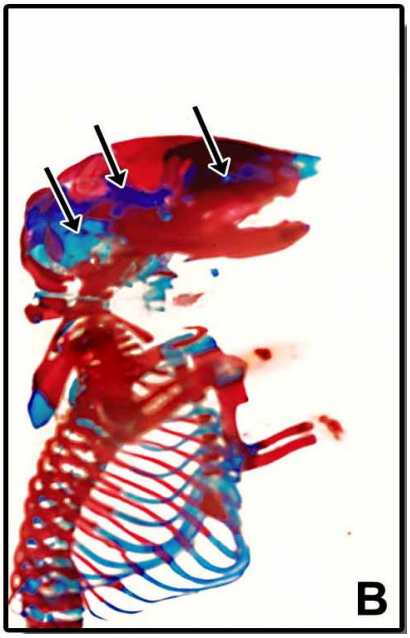

Fig.(10)

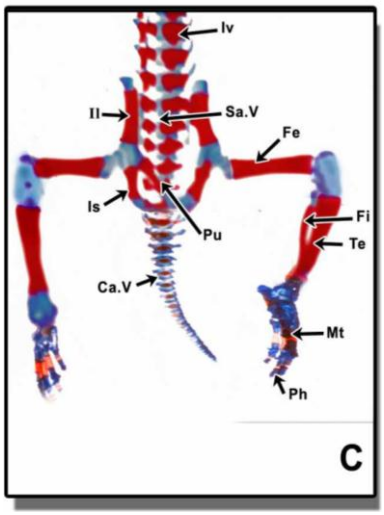

Fig.(11)

Fig.(12)

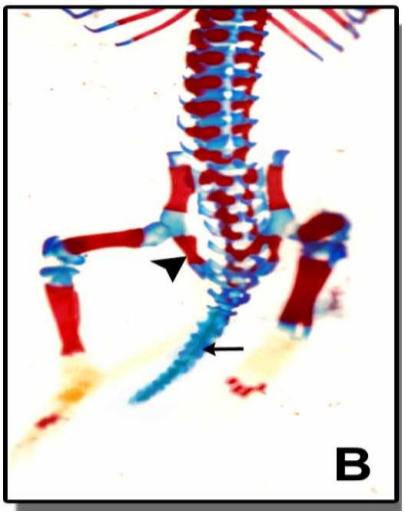

Fig.(13)

Fig. 8: 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(Th.V), sternum(ST), scapula(Sca),supra scapula (S.Sca) humerus(Hu), radius(Re), ulna(Ul), metacarpals(Mc) and phalanges(Ph). **(X 34)**

Fig. 9: Photograph of the anterior region of the skeleton of 19-day old fetus maternally treated with 205 mg/kg b.wt. of amox for 8 days during GDs 7-14 (group A) manifesting incomplete ossification of the dermal bones of the skull (arrows) . **(X 34)**

Fig. 10: Photograph of the anterior region of the skeleton of 19-day old fetus maternally treated with 820mg/kg b.wt. of amox for 8 days during GDs 7-14(B) manifesting incomplete ossification of the dermal bones of the skull (arrows) . **(X 34)**

Fig. 11: Photograph of the posterior region of the skeleton of 19-day old fetus of control group (C) showing lumbar vertebrae(Lv), sacral vertebrae(Sa.V), caudal vertebrae(Ca.V), ischium(Is), ilium(Il), pubis(Pu), femur(Fe), tibia(Te), fibula(Fi), metatarsal(Mt), and phalanges(Ph). **(X 34).**

Fig. 12: Photograph of the posterior region of the skeleton of 19-day old fetus maternally treated with 205 mg/kg b.wt. of amox for 8 days during GDs 7-14(A), showing incomplete ossification of the sacral and caudal vertebrae (arrow). **(X 34)** **Fig. 13:**Photograph of the posterior region of the skeleton of 19-day old fetus maternally treated with 820 mg/kg b.wt. of amox for 8 days during GDs 7-14(B), displaying less ossification of the sacral and caudal vertebrae (arrow). **(X 34)**

DISCUSSION

The present study was conducted to investigate the effects of the beta-lactam antibiotic amoxicillin on mice fetuses maternally treated during organogenesis. The morphological and skeletal features were assessed.

Morphological studies

The present results showed that pregnant mice treated with 205 and 820 mg/kg body weight of amox for 8 days (during organogenesis) caused growth retardation in maternally treated fetuses of the two experimental groups. The effect on the body length and body weight was 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 was dose dependent. Such effects of amoxicillin on fetal morphological features during gestation is consistent with the findings of **Lin *et al.*** [14] who found that the use of amoxicillin was associated with an increased risk of oral cleft. A positive association between treatment with antibiotics during pregnancy and growth retardation was reported by **Siddiqui and Naqvi** [15] who recorded that oral administration of 60 mg/kg/day ciprofloxacin from 6th to 12th days of gestation caused a highly significant decrease in mean body weight of pups of maternally treated animals. The growth retardation of maternally treated fetuses observed in the present investigation is probably due to an impairment of blood flow to the placenta which reduces the uterine blood flow by the effect of the drug thus leading to reduction in the supply of nutrients and oxygen to the fetal circulation.

The present results also illustrated that maternal amoxicillin treatment on gestation days 7-14 resulted in a various degrees of external malformations characterized by stunting in size , in addition to limb and tail defects. The incidence of these malformations was found in the high dose treated group. It is worth of mentioning that no growth malformations were recorded among fetuses maternally treated with the low dose of amoxicillin during GDs 7-14(organogenesis stage) . In the same line **Molgaard-Nielsen *et al.*** [16] reported congenital malformation outcomes after exposure to fluconazole during the first trimester of pregnancy.

Skeletal studies:

The results of the present investigation clearly illustrated that intra uterine growth retardation (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 amoxicillin during organogenesis (GDs 7-14). These hypoplasia and retarded ossification of skeletal elements observed in this study might by interpret to result from maternal toxicity due to amoxicillin dosing. The role of maternal toxicity in abnormal fetal development has been previously emphasized [17] **.** Such results may be correlated with the inhibition of osteoclastic activity. Also growth retardation of the skeletal elements were also observed in mice fetuses maternally treated with norfloxacin [18] as well as clarithromycin [19] .

The mechanisms of the action of antibiotics on osteogenesis is reported by **Duewelhenke *et al.*** [20] who illustrated that antibiotics impaired mitochondria of primary human osteoblasts (PHO) caused mitochondrial activity results in a reduction of pyruvate to lactate by ADH formed via glycolysis, which is catalyzed by cytosolic lactate hydrogenase. At the same time, glycolysis is stimulated with the aim of maintaining the ATP production constant over time, resulting in an enhancement of lactate production and secretion in the cell culture supernatant . PHO compensated for the complete inhibition of aerobic ATP production via glycolysis . primary human osteoblasts reacted by reducing cell proliferation and saving energy .

In conclusion, we have shown that the beta-lactam antibiotic amoxicillin had adverse or devastating effects that appeared in the form of growth retardation along with various external 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 amoxicillin in human are warranted to elucidate the mechanism of teratogenicity of amoxicillin. Before more information in human becomes

available, the use of amoxicillin (especially large therapeutic doses) in pregnant women should be treated with caution.

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