THE EFFECT OF ORDINARY BENZENEON THE OSSIFICATION CENTERS IN THE LONG BONES OF MICE EMBRYOS

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ABSTRACT

Present study was to detect the ordinary benzene on ossification in the long bones of the mouse embryos *Musmusculus L.*conducted the current study, 56 mice adult (16 male& 40 female) the females were divided into four groups, first for the control of the three treatment groups wasexposed to ordinary benzene concentration (4.5ppm, 9ppm, 45ppm) hours / day for 45 days and then married with intact males and took 20-day embryos,the results refer that the treatment groups showed significant decrease p< o.o5 revealedin the centers of ossification compared with control group.

INTRODUCTION

Benzene which is a volatile, colorless and highly flammable liquid was first discovered in 1825 by Michael Faraday, who isolated it from a liquid condensed from compressed oil gas (1).

Benzene is found in the air from emissions from burning coal and oil, gasoline service stations, and motor vehicle exhaust. Acute (short – term) inhalation exposure of humans to benzene may cause drowsiness , dizziness , headaches, as well as eye , skin , and respiratory tract irritation , and , at high levels , unconsciousness. Chronic (long – term) inhalation exposure has caused various disorders in the blood, including reduced numbers of red blood cells and a plastic anemia. In chronic exposures, benzene metabolites are considered the chronic exposures, benzene metabolites are considered, the toxic agents, not the parent compound (2). The relative contribution of different benzene metabolic pathways may be dose related, with more toxic agents produced by high affinity low capacity pathways (3). Chronic benzene exposure can cause bone marrow stem cell depression, apparently through a

cytotoxic effect on all lineages of haematopoietic progenitor cells, although there is some evidence for a mechanism involving injury to marrow stromal cells. Bone marrow macrophages have been shown to metabolize phenol to reactive compound that bind irreversibly to protein and DNA (4).

Benzene crosses the placenta and is present in cord blood in concentrations equal to or greater than maternal blood (5). Animal experiment exposing pregnant mice and rats to inhaled benzene in general demonstrated increased fetal skeletal variants and reduced fetal weight, but failed to demonstrate consistent convincing evidence of teratogenicity. Rats exposed to 313ppm for 24 hours/day on days 9 to 14 of gestation demonstrated reduced fetal weight and increased skeletal variants (6).

During skeletal development, the majority of the bones in the body are established by the endochondral bone formation process which is initiated by mesenchymal cell condensation and subsequent mesenchymal cell differentiation in to chondrocytes and surrounding perichondrial cells. Primary ossification occurs with osteoblast-mediated bone formation, which initially occurs on the calcified cartilage template. Chondrocyte maturation and the endochondral bone development process is factors, including bone morphogenetic proteins (BMPs), fibroblast growth factors(FGFs), parathyroid hormone-related protein(PTHrP) (7).

(8) reported that the hormone androgen in the chicken combines with estrogen to cause ossification as that of the hormones the thyroid and neighbor thyroid and calcitonin connection with the composition of the bone, because the thyroid hormone is important in the process of differentiation and maturation of cartilage by stimulating the production of material interstitial and the process of ossification it plays a role in stimulating inflation cartilage cells.

MATERIALS AND METHODS

Experimental Animals:

Used (56) adult*Musmusculus* mice(16 male, 40 female), 12weeks old, and of 23-26 grams weight were group housed in well ventilated cagesand provided with adequate food and water.The light was adjusted in 12 h light/ dark cycle.

Thefemale mice were divided into four groups (10 mice each) exposed groups to

concentrations (45ppm,9ppm and 4.5ppm) of ordinary benzene for 1 hour /day for 45days set LC50, 13700ppm , the treatment groups mated with male control.

Determine the age of the fetus through the development of males with females in the evening and isolated morning the next day, then females examined by investigating the presence of sperm in the vagina supply only by injecting a physiological solution 0.9 % sodium chloride in the females vagina and then take the contents through a special absorbent and examined the contents placing them on a glass slide and optical microscopy as the day has shown sperm per day zero (9).

Pregnant females sacrificed on day 20 of pregnancy after drugged with chloroform at room temperature using anesthesia fund made was quickly out of the uterus and placed in a petri dish contains 10% formalin , open the uterus and punctured embryonic membranes were directed embryos from the uterus.

Determination of the primary ossification center.(for 20 day mouse embryos)

Primary ossification center length for long bones were determined by using method (10).

1- Opens the skin and removed the viscera carefully avoiding the damage in bony or cartilaginous skeleton or mesenchyme tissue.
2- Fixed the embryos at 100% ethanol for 4 days without stirring and then three days with stirring.
3- imprisoned in the solution of Alizarin red and Alcian blue stain for five days with stirring washed by tap water and imprisoned in solution of glycerol 20% with sodium hydroxide 1% in ratio 1: 1 for 16h in room temperature to removed tissue completely and stores in 15 glycerol for examined and photographed.
   Using Ocular Micrometer, where the measured lengths ossification centers of the long bones in the mid-body bone.

RESULTS

Effect of benzene on the ossification centers in the long bones

Table (1) and figure (1) show ossification centers in the long bones after exposed to ordinary benzene in mice embryos in both the treated and control groups, Group A records

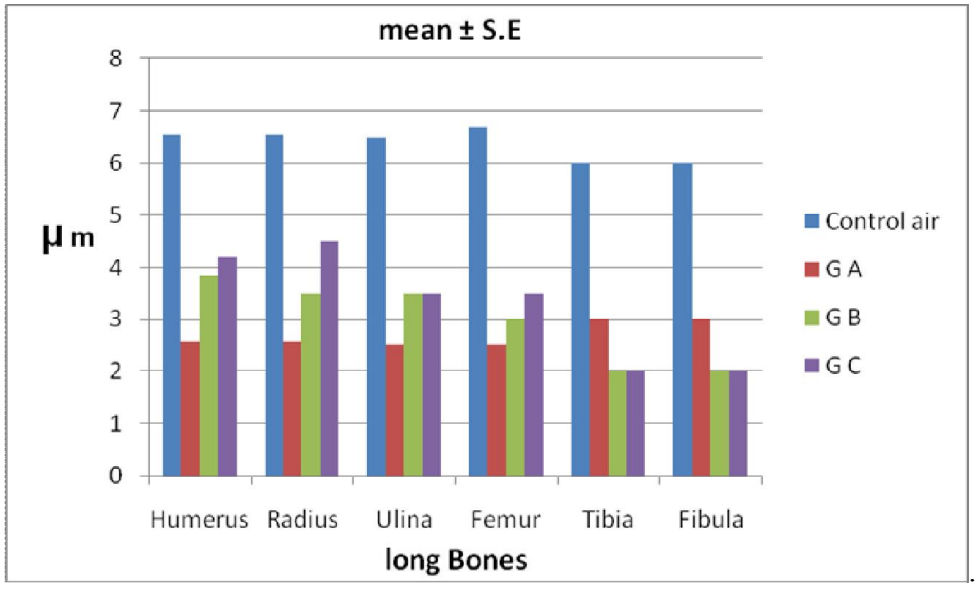
significant decrease (p< o.o5) with the highest mean valve among all group. Al so Group A shows a significant decrease (p< o.o5) when compared with control group.

The results in table(1) and figure(1) show more a significant decrease (p< o.o5) in the ossification centers of (humerus, radius) bones of Group A compared with Groups B and C. in addition, a significant decrease (p< o.o5) revealed in the ossification centers ulina bone of Group A compared with Groups B and C, while no significant deference was observed between Groups B and C as in figure (3, 4, 5).

The results show a significant decrease (p< o.o5)in the ossification centers femur bone Group A compared with Groups B and C.in addition, a significant decrease (p< o.o5) revealed in the ossification centers (tibia, fibula) bones of Group A compared with Groups B and C as in figure (7, 8, 9).

The results showed in the 45pmm Group A some distortion in the ossification centers of long bones in mice embryos marked by the lack of symmetry in the ossification centers of the long bones as in figure (9).In addition, the lack of symmetry in the background of existing centers of ossification from the right and the left as shown in figure (10, 11).

Fig.1. Values of ossification centers in the long bones of the exposed to ordinary benzene in mice embryos.



Significantly different (p< 0.05)
GA: 45ppmGB: 9ppmGC: 4.5ppm

Table .1.Values of ossification centers in the long bones of the exposed to ordinary benzene in mice embryos.

Long bones / Groups	Humerus	Radius	Ulina	Femur	Tibia	Fibula
Control air	A 6.55 ± 0.45 a	A 6.55 ± 0.41 A	A 6.5 ± 0.35 a	B 6.7 ± 0.35 a	A 6 ± 0.27 A	A 6 ± 0.14 a
G A	A 2.55 ± 0.31 b	A 2.57 ± 0.51 B	A 2.5 ± 0.14 b	A 2.5 ± 0.35 b	A 3 ± 0.23 B	A 3 ± 0.13 b
G B	A 3.85 ± 0.88 c	A 3.5 ± 0.32 c	B 3.5 ± 0.19 c	C 3 ± 0.65 b	C 2 ± 0.37 C	C 2 ± 0.21 b
G C	A 4.2 ± 0.91 d	A 4.5 ± 0.31 d	A 3.5 ± 0.34 d	B 3.5 ± 0.31 b	B 2 ± 0.31 C	B 2 ± 0.23 c

*Significant differences at (P <0.05). Duncan's multiple range tests.
*Small letters refer to significant differences vertically.
*large letters refer to significant differences between rows.
*S. E. stander error

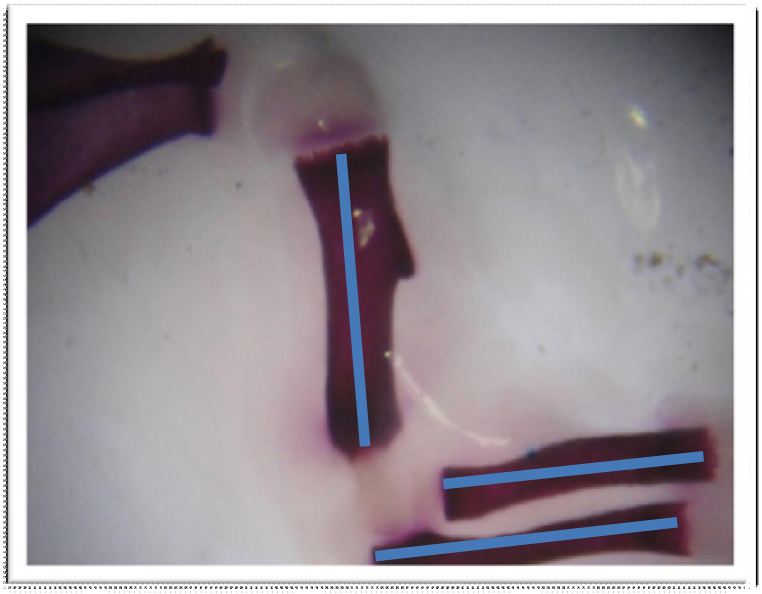


Fig .2.Normal formation of ossification centers in (Humerus, Radius&Ulina) bones of mice embryos. (Alizarin red stain 140X

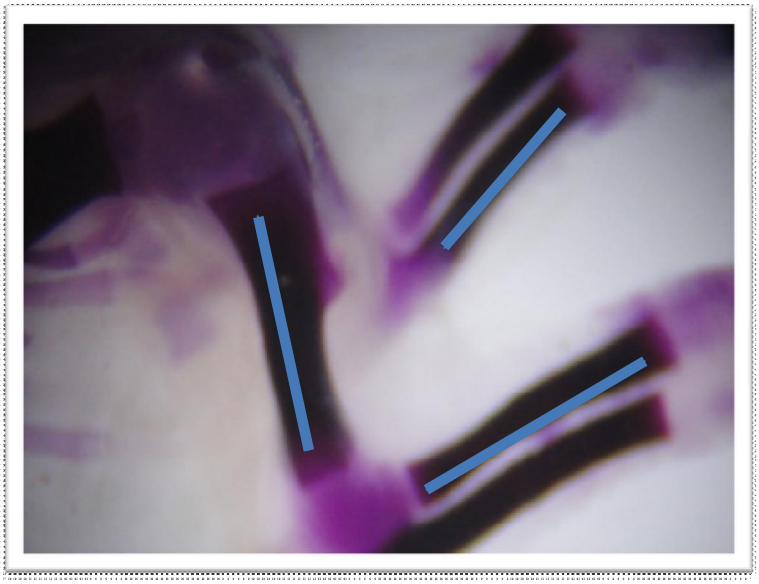


Fig .3.Delayed formation of ossification centers (Humerus, Radius&Ulina) bones exposed of benzene 4.5ppm in mice embryos (Alizarin red stain 140X)

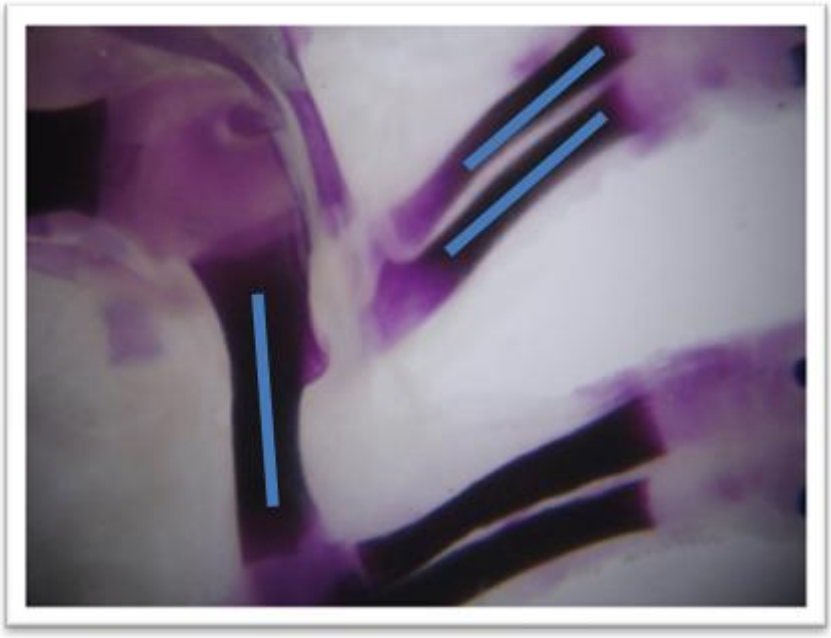


Fig .4.Delayed formation of ossification centers in (Humerus, Radius&Ulina) bones exposed of benzene 9ppm in mice embryos.(Alizarin red stain 140X)

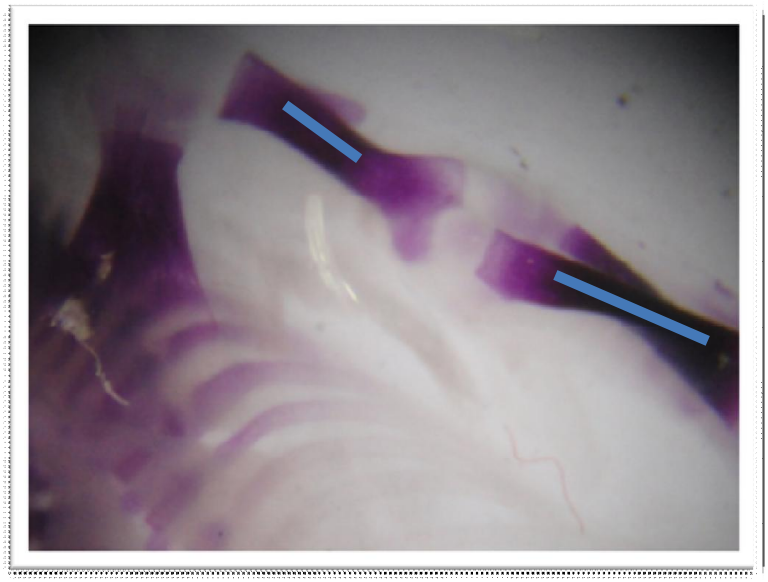


Fig .5.Delayed formation of ossification centers in (Humerus, Radius, &Ulina) bones exposed benzene of 45ppm in mice embryos.(Alizarin red stain 140X)

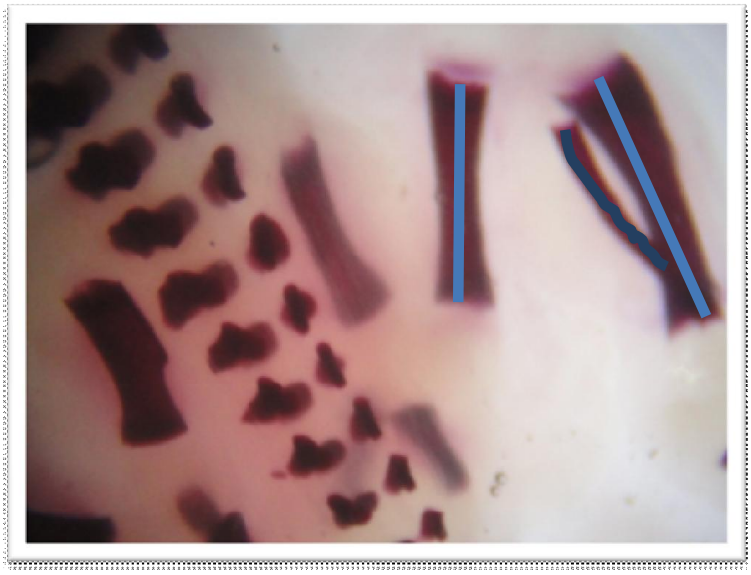


Fig .6:Normalformation of ossification centers in (Femur, Tibia&Fibula)bones in mice embryos.(Alizarin red stain 140X)

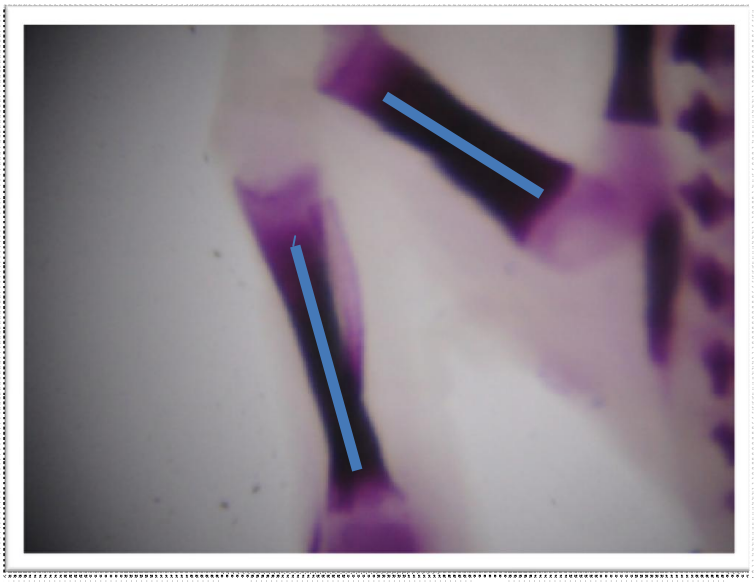


Fig .7:Delayed formation of ossification centers in (Femur, Tibia&Fibula)bones exposed benzene of 4.5ppm in mice embryos.(Alizarin red stain 140X)

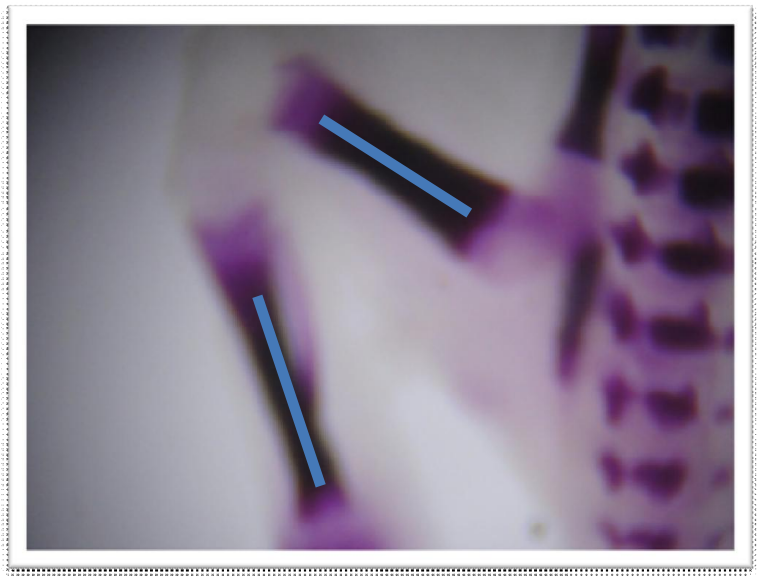


Fig .8:Delayed formation of ossification centers in (Femur, Tibia&Fibula) bones exposed benzene of 9ppm in mice embryos. (Alizarin red stain 140X)

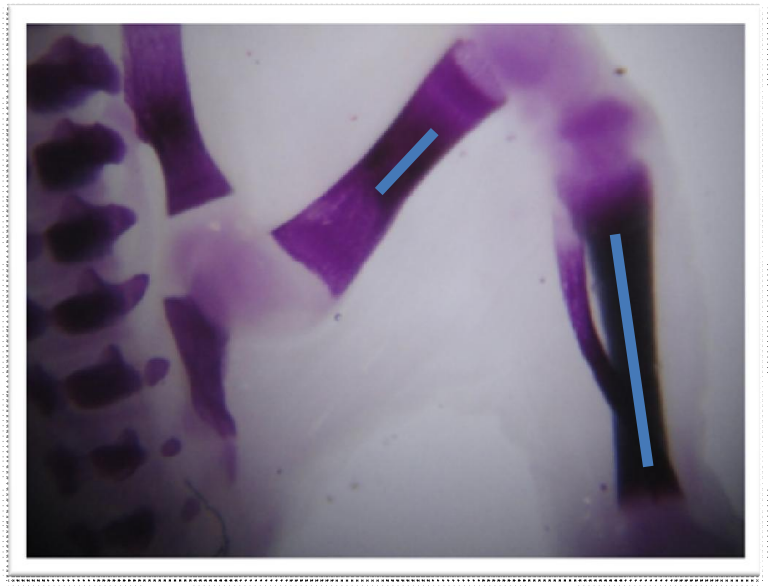


Fig .9:Delayed formation of ossification centers in (Femur, Tibia&Fibula)bones exposed of benzene 45ppm in mice embryos.(Alizarin red stain 140X)

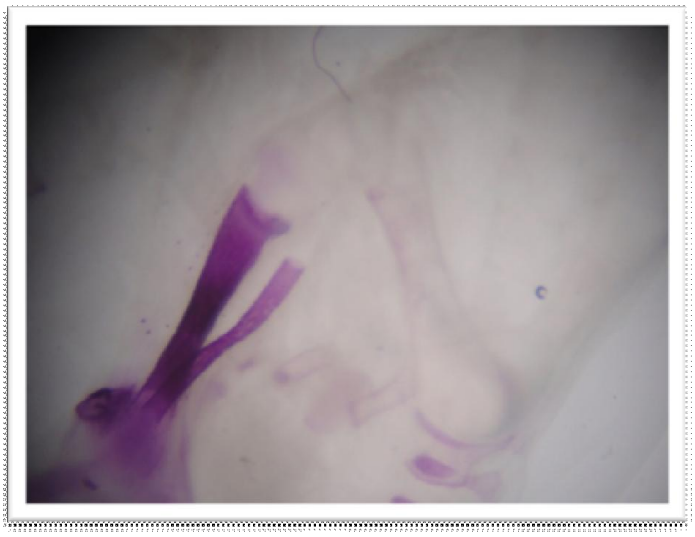


Fig .9: Delayed formation of ossification centers in (Femur, Tibia &Fibula)bones exposed of benzene 45ppm in mice embryos.(Alizarin red stain 140X)

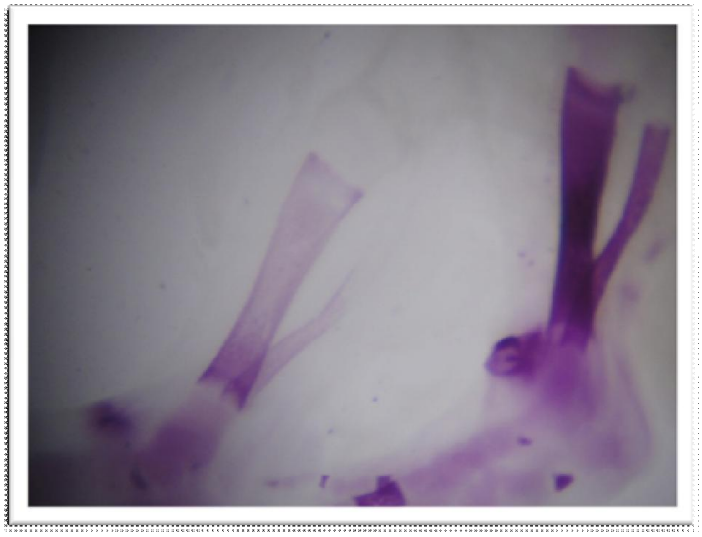


Figure.11: show lack of symmetry in the ossification centers of (Tibia, Fibula) bones between the right and the left, exposed benzene 45ppm in mice embryos (Alizarin red stain 140x).

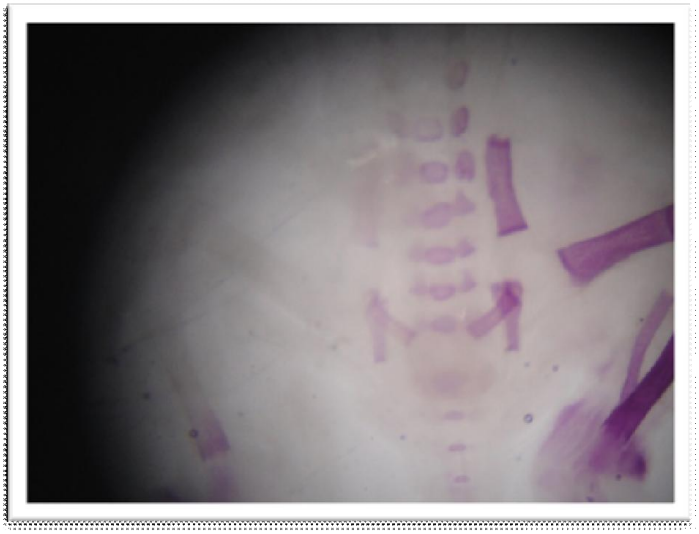


Figure.12: show lack of symmetry in the ossification centers of (Femur, Tibia, & Fibula) bones between the right and the left, exposed benzene 45ppm in mice embryos (Alizarin red stain 140x)

DISCUSSION

Effect of benzene on the ossification centers in the long bones of mice embryos:

In the present study showed that the effect of ordinary benzene on the ossification centers in the long bones (Humerus, Radius, Ulina, Femur, Tibia, and Fibula)were decrease on the ossification centers, these effect may be caused by embryotoxicity of benzene.

(11) suggested that several factors may be responsis for the embryotoxicity of benzene :- first, benzene can pass the placenta barrier and affect the embryonal cells directly; second, phenol (a major metabolite of benzene) was shown to inhibit DNA synthesis in bone marrow in vivo can also pass the placental barrier; third, benzene can damage the maternal circulation and cause a bone marrow depression, resulting in adverse nutritional conditions for the fetus.The bone marrow is a complex matrix harboring stem cells of blood cells, and stromal cells, which

provide growth factors necessary for the proliferation and differentiation of stem and progenitor cells (12).

(7) reported that the embryotoxicity caused a decrease of growth factors and transcription factors (BMPs, FGFs, IHH, PTHrP and 2(RunX2) responsible for the ossification centers of embryos mice.

(13) reported that the skeleton of the mice of the type 57B1 that mice-free protein Conexin leads to delayed ossification membrane and cartilage and therefore the delay in the centers of ossification indicating that this protein has an abundance of the barriers between cells osteoblasts bones as the decreasing leads to lack of proteins responsible bone deposition Therefore, this protein is important in the process of ossification and bone function of osteoblasts and bone characterized this protein-free easily break and lack of diploe space.

تأثيرالبنزين العادي على مراكز التعظم في العظام الطويلة لأجنة الفئران

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الخلاصة

درس تأثير استنشاق البنزين العادي على مراكز التعظم في العظام الطويلة لأجنة فئران *Musmusculus L.*اجريت الدراسة الحالية على ٥٦ فأر بالغ (١٦ ذكر ،٤٠ اناث) قسمت الاناث الى اربعة مجاميع الاولى للسيطرة وثلاث مجاميع عرضت الى البنزين العادي بتركيز (4.5ppm , 9ppm , 45ppm)ساعة/يوم لمدة 45 يوم بعدها زوجت مع ذكور سليمه وأخذت الأجنة بعمر 20 يوم فكانت النتائج تشير إلى أن المادة أحدثت انخفاض معنوي $p<0.05$ في مراكز التعظم الاولية وازداد الانخفاض بزيادة التركيز مقارنة مع السيطرةوقد تناسب التأثير طرديا مع زيادة التركيز .

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