

## The Congenital Malformations in White Pregnant Mice Fetus Induced by Metformin Drug During 6-18 Days of Gestation

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### Abstract

The metformin drug is anti-hyperglycemia and known to cross the placenta which leads to the fetus during pregnancy. The aim of this study is to define the drug effects in the fetus growth. The doses used, therapeutic dose (0.18 & 0.53) mg\25g body weight and over dose (1.8 & 2.85) mg\ 25g body weight, administrated orally at the beginning organogenesis stage at (6 -18) day of pregnancy in the morning. A total (50) animal were divided into five groups. The first group control not treated, 2nd group treated with (0.18) mg, 3rd group with (0.53) mg , 4th group with ( 1.8 ) mg and 5th group with ( 2.85 ) mg . In this study noted some congenital malformations in the fetus compared with normal control group , which included in the 2nd and 3rd group ( irregular body level , exophthalmia , micromelia , elevated some hand fingers , simple club foot , torsion tail and wrinkle skin ) while the defects was larger in the 4th and 5th group ( trunk abnormalities like C Shape , big head ( scaphocephaly ) , cranial flexure , prosencephaly, short neck , micromelia , syndactyly some of hand fingers and others hemimelia , torsion tail and some aquiline , sharply club foot and spina bifida ) . It is mentioning that the malformations were less in the 2nd group treated with (0.18) mg of the drug. The treatment with metformin drug during pregnancy induced the congenital malformations.

**Keyword:** Metformin Drug, Congenital Malformation, Defect.

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## 1. Introduction

The metformin drug a biguanide compound used to treat diabetes [ 1 ] Anti-hyperglycemia [2] soluble in water , chemical name (N.N. Dimethyl Limido Dicarbonimidic ) [3] , The therapeutic dose recommended 500 mg , maximum 2.5 g [ 1 ] , tablet form taken orally , absorbs in the intestine and excrete by urine and bile [ 4 ] . It has ability to a cross the placenta during pregnancy and reach to the fetuses [5]. Some studies indicated the metformin was safe if used during pregnancy [6] , the pregnant women used the drugs frequently despite of warning especially in the first three months which causes the malformation in the embryo [ 7 ] , the malformations caused by some factors , metabolic factors ( e.g Diabetes ) , physical factors ( e.g Heat ) [ 8 ] , In addition to drugs , so the embryologists considered for study the malformations and reasons , most of them are focused on neural tube defects .The organogenesis or embryonic stage of pregnant is the most sensitivity period for malformations which extend from ( 3-8 ) weeks in human [ 9 ]and from ( 7-11 ) days in mice [ 10 ] .

## 2. Materials and Methods

### The animals:

The animals total (50) pregnant mice were used with age (12-16) weeks, weight (25±2) g, they were obtained from the animals reproduction which brought from Mosul university , Veterinary medicine . They were arised in plastic cages in special room with standard laboratory conditions, temperature (24) °C and arrangement photo cycle, special food eating, contents (Wheat 34%, Barley 20%, Corn 25% , Animal protein 10% and salt 1% ) mixed with water and eating after dry [ 11 ] .

### The drug:

The form used of metformin drug was tablet, made from (Merck san , Lyon france ) ,were concentrated ( 500 & 1000 ) mg , In this study, the doses used were therapeutic dose & over dose , the therapeutic dose is concentrated ( 0.18 & 0.53 ) mg of mice weight which represents ( 500 & 1500 ) mg in human , over dose is concentrated ( 1.8 & 2.85 ) mg of mice weight whose concentrate is ( 5000 & 8000 ) mg in human . The doses are determined in mice by depending on the recommended dose in human (500-2500) mg, according to the following equation [ 12]: -

$$Y1 \cdot X2 = Y2 \cdot X1$$

**Y1= Unknown dose in mice**

**X1= Adult mice weight ( 25 ) g**

**Y2= Known dose in human (500 ) mg**

**X2= Adult human weight ( 70 \*1000)**

$$Y1 = \frac{X1 \cdot Y2}{X2} = \frac{25g \cdot 500 \text{ mg}}{70000 \text{ g}} = 0.18 \text{ mg}$$

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$$Y1 = 0.18 \text{ mg}$$

(Unknown dose in mice)

**The resulting dose is dissolved in distilled water to prepare the dose in ml to each animal, according to the following equation: -**

$$V1 * Y2 = V2 * Y1$$

**V1= Dispelled water size**

**V2= Unknown value**

**Y1= Dose in mice**

**Y2= Dose in human**

$$V2 = \frac{V1 * Y1}{Y2} = \frac{250 \text{ ml} * 0.18 \text{ mg}}{500 \text{ mg}} = 0.09 \text{ ml} \quad (12)$$

**Note: Therapeutic dose dissolved in 250 ml while over dose in 500 ml of D.W to best solubility . Table (1).**

### **The mating:**

In the night two female isolated with one male in each cage, then pregnancy diagnosed in the morning by finding vaginal plug . The fertilization day is zero of pregnancy and the day after it is the first day of pregnancy [ 13 ].

### **Experimental design**

The animals were divided in to five groups :

The first group : Control group not treated

The second group : Treated with 0.18 mg from metformin

The third group : Treated with 0.53 mg from metformin

The fourth group : Treated with 1.8 mg from metformin

The five group : Treated with 2.85 mg from metformin

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**Table (1): shows the groups, required doses and concentrations**

Groups	Does in human	Dose in mice		Animals numbers
	Mg	Mg	MI	
Control	-----	-----	-----	10
Therapeutic Dose	500	0.18	0.09	10
	1500	0.53	0.09	10
Over Dose	5000	1.8	0.18	10
	8000	2.85	0.18	10

The treatment was orally by cavage tube from (6-18) day of pregnancy in the morning with start of the organogenesis stage, were the (6.75) day is considered beginning the formation of neural tube in fetus [14].

### The dissecting:

The animals were anesthetized with chloroform in ( 18 ) days of gestation before birth , then fetuses removed from uterus horns and saved in formalin 10% to study the malformations by naked eye and dissecting microscope ( 10 X ) and then photographed by the camera ( Samsung ) ( 9.6 Meqa pixel ) .

### 3. Results and Discussion

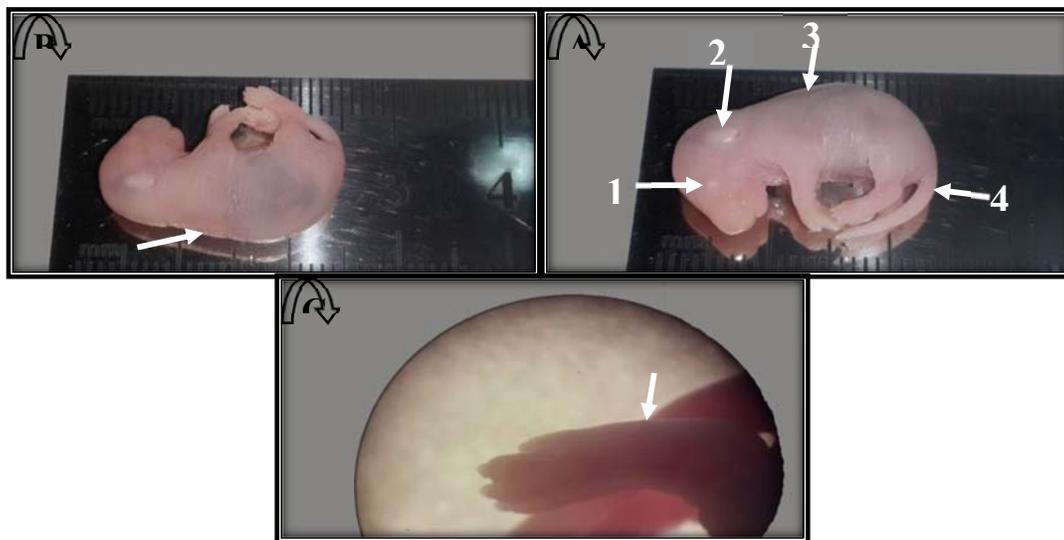
The results showed some of the congenital malformations caused by the treatment with drug compared with control that normal of (body length, eyes, ear pinna, trunk and tail), figure (1). while The statistical analysis showed no significant changes in fetuses body length in all doses compared with control as shown in the table (2). The malformations were represented in the therapeutic dose in concentrate ( 0.18 ) mg ( big head , cranial flexure , exophthalmia , micromelia , elevated some of hand fingers , simple club foot , prosencethaly , torsion tail and wrinkle skin ), figure (2) . In addition to those defects noted ( Irregular Body Line and swelling toe of club foot ) in high concentrate ( 0.53 ) mg , figure ( 3 ) . These defects were larger in over dose in both concentrates represented in ( 1.8 ) mg concentrate ( abnormalities of trunk like C shape ,big head( scaphocethaly ) , short neck , prosencethaly ,cranial flexure , exophthalmia, micromelia, torsion tail and sharply club foot ) , figure ( 4 ) . and in (2.85 ) mg concentrate shows more sharpness ( large number of fetuses with trunk like C shape , big of head ( scaphocethaly ) more than others , exophthalmia, sharply club foot , exophthalmia, micromelia and some hand fingers syndactyly and others hemimelia , spina bifida ( swelling in the back ) , torsion tail and some aquiline ) , figure ( 5 ) .

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**Table (2): shows effect of metformin in fetus body length average ( cm ) ± standard deviation**

Groups	Metformin drug Concentration	Fetus body length averag ( cm ) ± standard deviation
Control	—	0.13±3.57 a
Therapeutic Dose	0.18	0.07 ±3.80 a
	0.53	0.05 ±3.52 a
Over Dose	1.8	0.15 ±3.57 a
	2.85	0.00 ±3.60 a

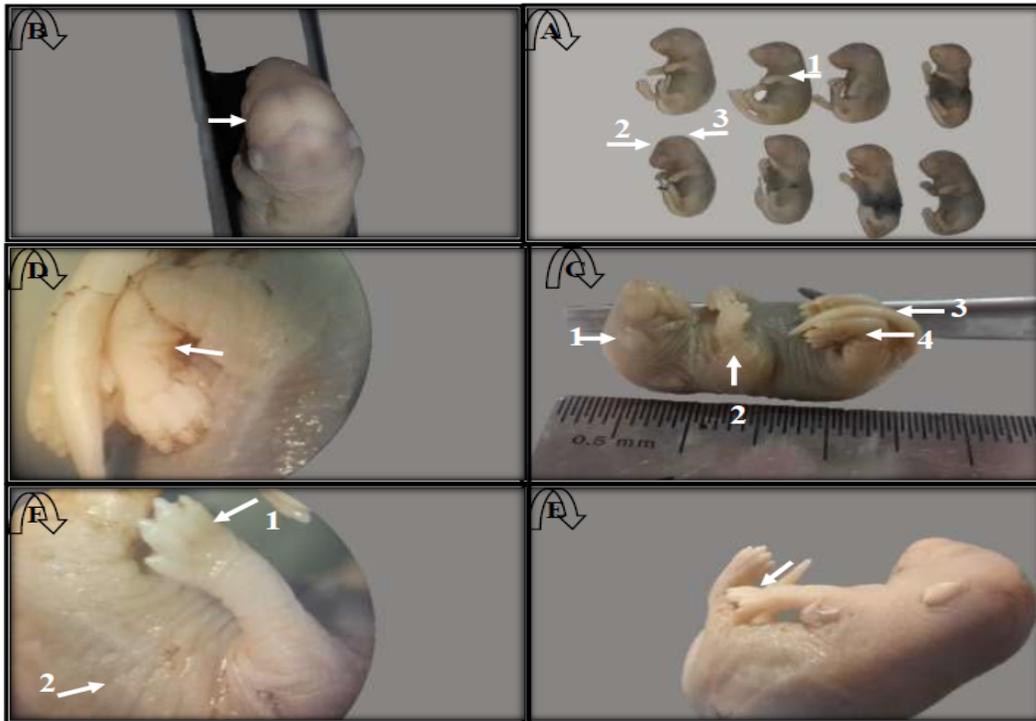


**Figure (1): Control mice fetus show :**

- A. Anterior view : Eyes ( 1 ) Ear Pinna ( 2 ) ,Trunk ( 3 ) , Tail ( 4 ) .**  
**B. Posterior view : Trunk ( Arrow )**  
**C. Foot ( Arrow ) 20x.**

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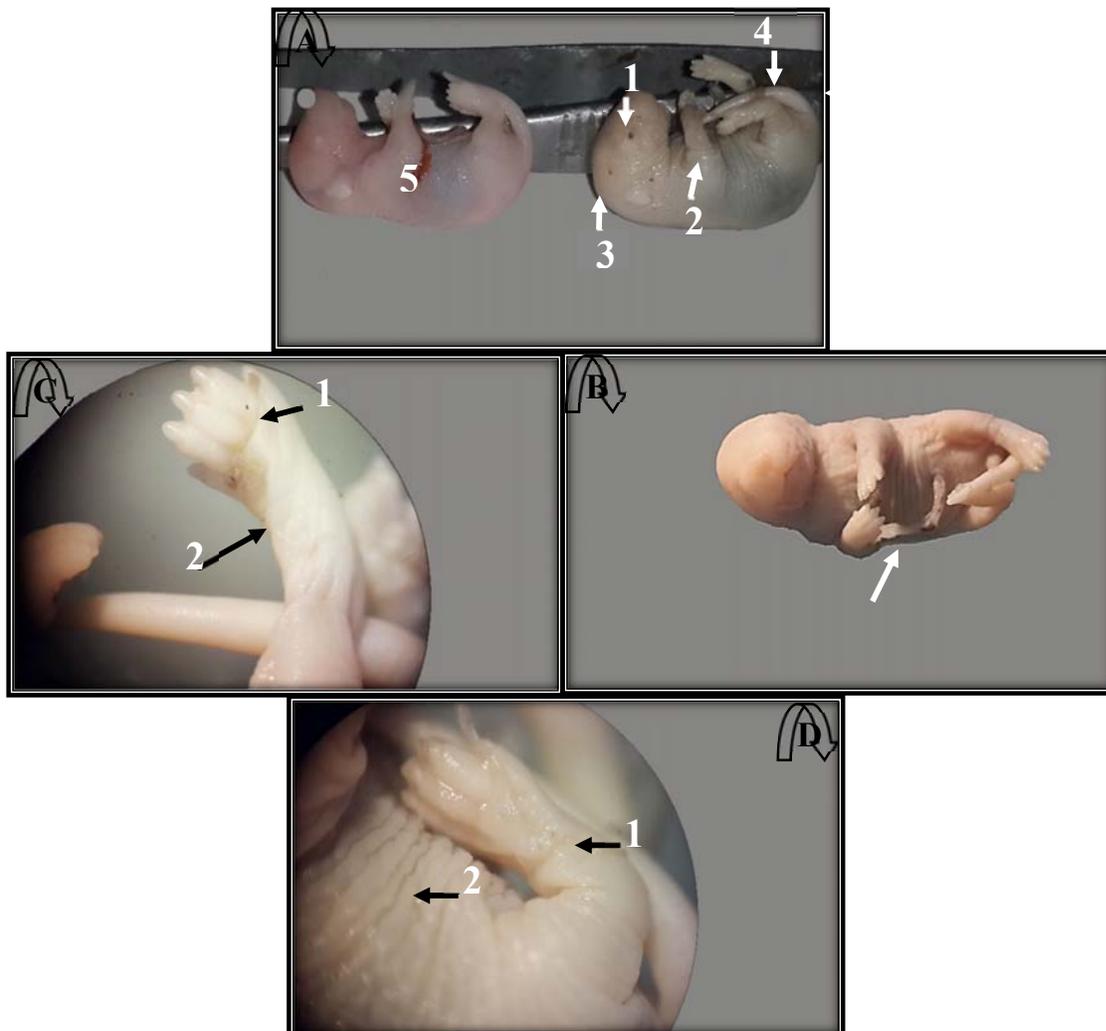


**Fig (2):- Abnormal mice fetuses treated with ( 0.18 ) mg of metformin show :-**

- A. Micromelia ( 1 ) , Big Head ( 2 ) , Cranial Flexure ( 3 ) .**
- B. Prosencephaly ( Arrow ) .**
- C. Exophthalmia ( 1 ) , Micromelia ( 2 ) , Torsion Tail ( 3 ) , Club Foot ( 4 ) .**
- D. Club Foot ( Arrow ) 20x.**
- E. Elevate hand fingers ( Arrow ) .**
- F. Elevate hand fingers ( 1 ) , Wrinkle Skin ( 2 ) . 20x.**

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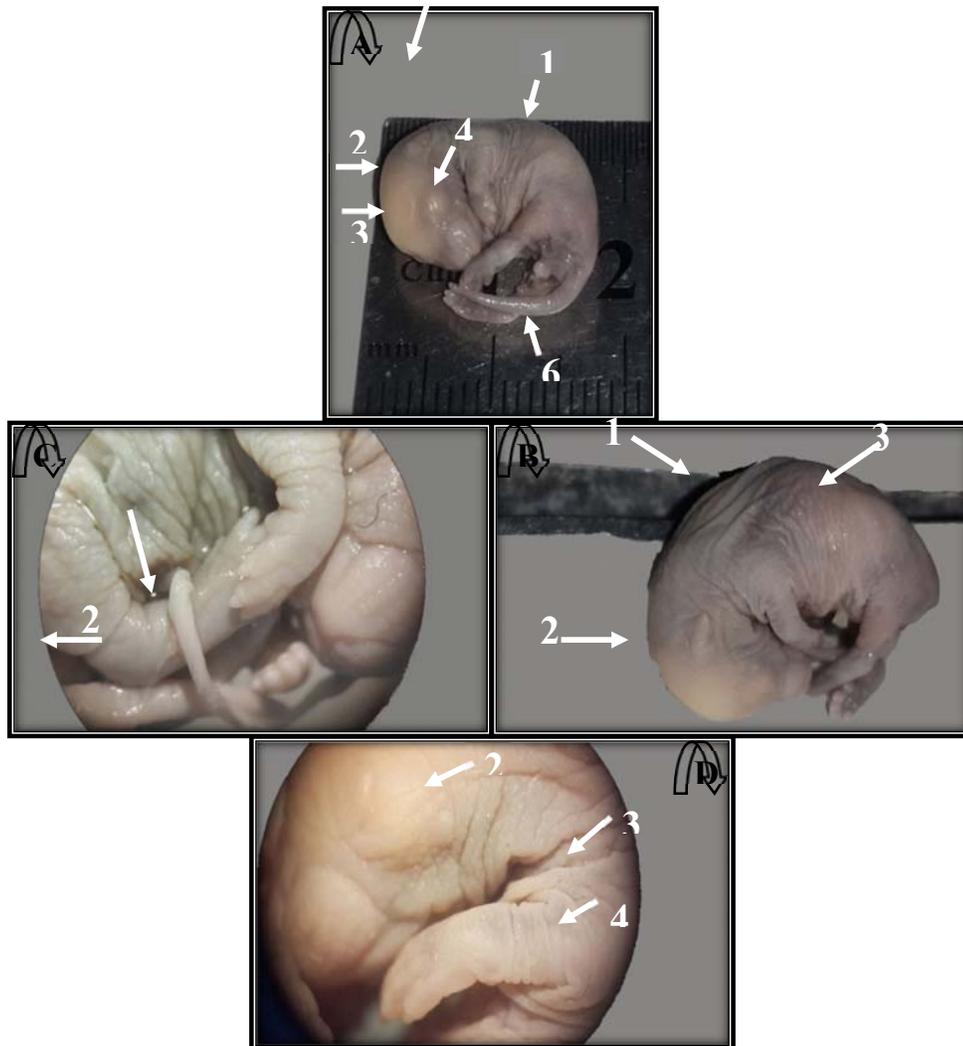


**Figure (3): Abnormal mice fetuses treated with (0.53) mg of metformin show :-**

- A. Exophthalmia (1), Micromelia (2), Big Head (3), Torsion Tail (4), Control Fetus (5)**  
**B. Irregular Body Line (Arrows).**  
**C. Elevate foot toe (1), Abnormal Foot (2) 20x..**  
**D. Swelling toe (1), Wrinkle Skin (2). 20x.**

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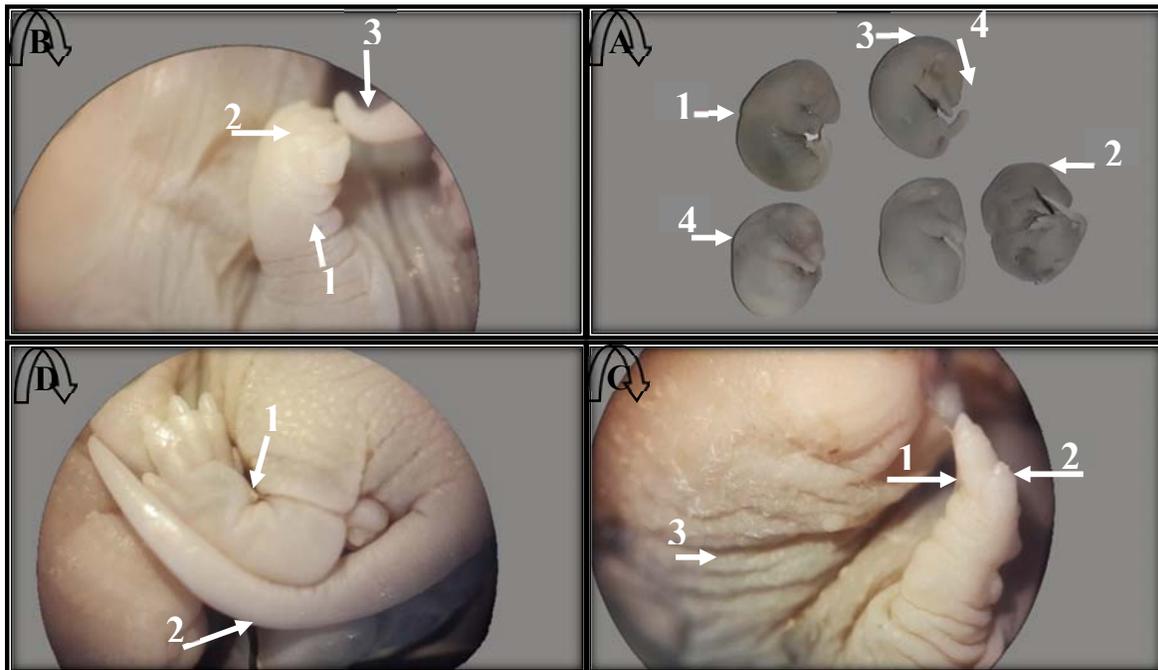


**Figure (4): Abnormal mice fetuses treated with ( 1.8 ) mg of metformin show:-**

- A. Anterior Veiw :** Trunk Like C Shape ( 1 ), Big Head ( Scaphocephly) ( 2 ), Prosencephaly ( 3 ), Exophthalmia ( 4 ), Micromelia ( 5 ), Torsion Tail ( 6 ).
- B. Side Veiw :** Trunk Like C Shape ( 1 ), Cranial Flexure ( 2 ), Wrinkle Skin ( 3 ).
- C. Club Foot ( 1 ), Torsion Tail ( 2 ). 20x.**
- D. Big Head ( 1 ), Exophthalmia ( 2 ), Short Neck ( 3 ), Micromelia ( 4 ). 20x.**

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**Figure (5): Abnormal mice fetuses treated with ( 2.85 ) mg of metformin show :-**

- A- Trunk Like C Shape ( 1 ), Big Head ( Scaphocephly ) ( 2 ), Cranial Flexure ( 3 ), Spina Bifida ( Swelling in the back ) ( 4 ).**  
**B- Micromelia ( 1 ), Syndactyly Fingers ( 2 ), Aquiline Tail ( 3 ) 20x.**  
**C- Elevate Hand Finger ( 1 ), Hemimelia Hand Finger ( 2 ), Wrinkle Skin ( 3 ). 20x**  
**D- Club Foot ( 1 ), Torsion Tail ( 2 ) . 20x.**

The results do not agree with what they mentioned kumar and khan ( 2012) [ 15 ] that reported was the use of metformin in pregnant women with ovary cyst syndrome was not caused any malformation , also it does not agree with bertoldo *et al.* ( 2014 ) [ 16 ] was showed adoption of metformin does not effect on the embryos formation during pregnancy .while in this study it was noted the malformations in all doses , these results agree with jabr ( 2009 ) [17 ] show the same defects in mice fetus which caused by treated the pregnant with therapeutic and over dose of acetaminophen .

The formation of primitive streak in mice start in ( 6.5-7.5 ) days of pregnancy [14 ] , so the exposure of pregnant to effects in this period causes defect in the formation , the trunk anomalies result from neural tube closure defect [ 18 ] and a neural tube fusion failure in early time of neurolation causes defect in embryonic development [ 19 ]. Anural tube defects have two types, open and closed type , the open type is in brain and spinal cord defect state ( skull & vertebrae ) , closed type is in spina defect which covered by the skin [ 20 ] , in this study it was noted both types occurred in the fetuses by brain and spinal cord defect and skin

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covered of spina defect ( swelling in the back ) which refers to spina bifida state and this agrees with Al-tuhami ( 1999 ) [ 21 ] reported the swelling in the back of spinal cord place that refers to spina bifida type cystica .

Head malformation result from early closure of one suture or more, skull form depends on suture type which closed, the sagittal suture early closure causes the frontal suture expansion (scaphocephaly) [9]. The formation of head fold starts in (7. 5) day in mice (14) and the exposure to metformin began in (6) which caused early closure and defect in head formation

Al-Hamodi and Yousif ( 2005 ) [ 18 ] pointed to limb malformation included ( syndactyly , club foot , etc ) which resulted from bones growth defect.

Copp and Green (2010) [ 22 ] pointed to tail defect, included (torsion tail and aquiline) which resulted from closure delay of posterior neural aperture.

The disturbance of skin differentiation caused wrinkle skin (Van *et al.* ;1970 ) [ 23 ].

Our malformations in this results attributed to high homocysteine in pregnant animals which caused by metformin drug , the drug causes an increase in homocysteine ( Seto *et al.* ; 2010 ) [ 24 ] , some studies have suggested that high homocysteine levels in pregnant mothers causes the malformations in the nervous system and limbs of embryos ( Smith *et al.* ; 2008 ) [ 25 ] .

The treatment with metformin drug during pregnancy induced some congenital malformations in the fetus in both therapeutic and over dose.

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