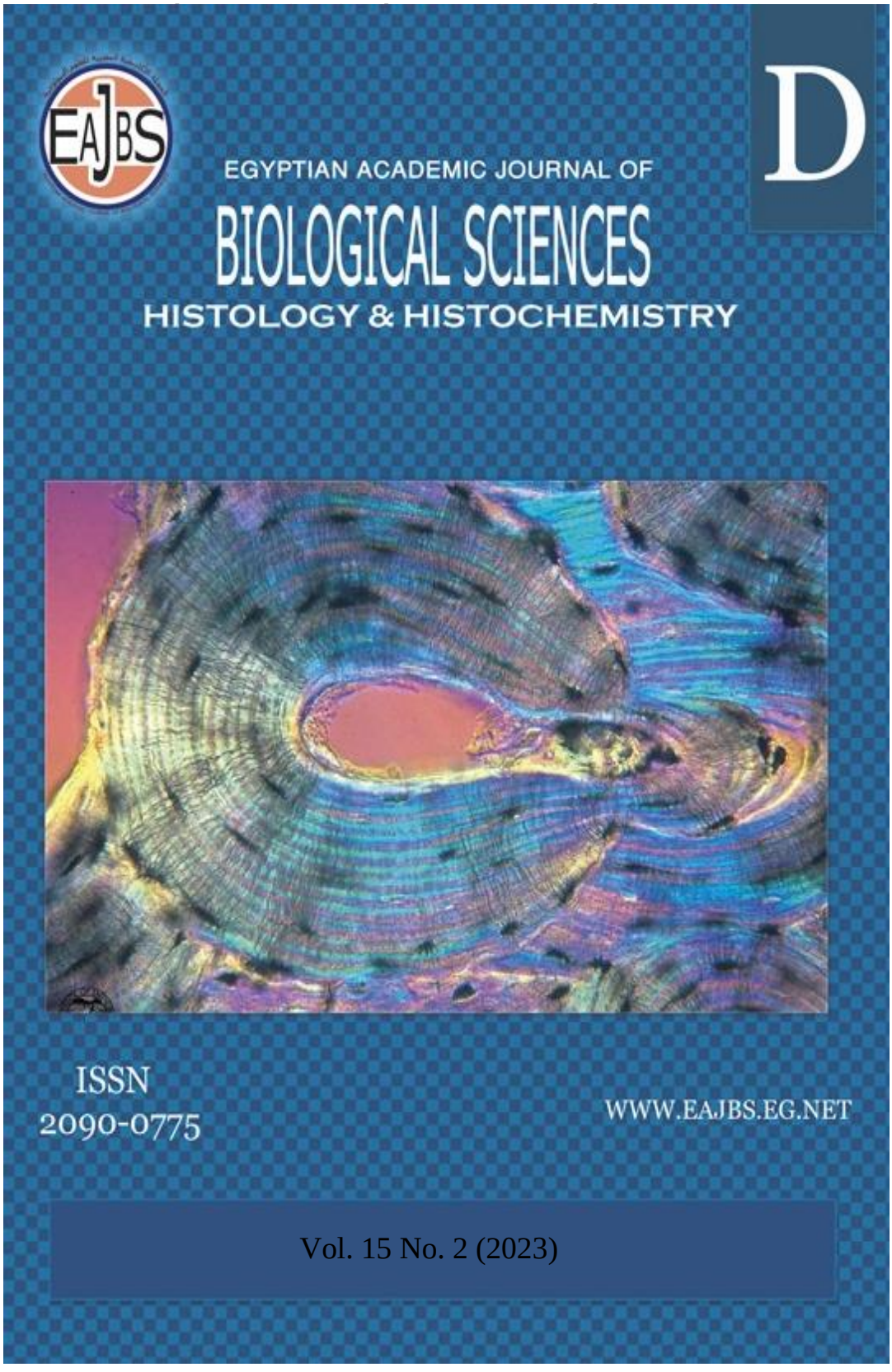


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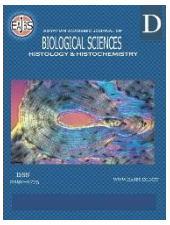

# Toxicological Studies on The Effect of Methotrexate on Prenatal Rat Foetuses

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**ABSTRACT**

**Background:** Methotrexate (MTX), is a folate antagonist that inhibits cell development and is used to treat a wide range of disorders. The goal of the current study was to investigate the toxic effect of MTX on the bone, heart and kidney of albino rat foetuses. **Methods:** Thirty-five rats were divided into 5 groups, MTX was given orally (0.03 mg/kg) for 10 days to G1 & G2 (males and females) and before being allowed to mate with the control group (females and males respectively). G3 (males and females) was given orally (0.03 mg/kg) for 10 days before being allowed to mate with each other. G4 pregnant females were given (0.03mg/kg) of MTX orally every day from the 1st to the 20th day of pregnancy. **Results:** The changes suggested that MTX treatment caused developmental abnormalities including partial ossification of some skull bones, and vertebrae of limbs, and a notable decrease in the length of most long bones in both limbs. The kidney of MTX-treated groups showed changes such as capsular dilatation, renal corpuscle distortion, shrunken glomeruli, edoema, and necrosis in renal tubules of the renal medulla. It also induced histopathological changes in cardiac muscle tissue, including widely distributed vacuolated spaces, congested blood vessels, leukocytic infiltration, cardiomyocyte disarrangement with pyknotic nuclei, necrosis and a lack of regular arrangement.

## INTRODUCTION

Methotrexate (MTX) is a dihydrofolate medication that is commonly used to treat autoimmune disorders, inflammatory diseases, cancer, rheumatoid arthritis, and gestational trophoblast diseases (Heidari *et al*., 2018 and Nancy, 2022). Additionally, it is employed to induce abortion, both for elective procedures and in the treatment of ectopic pregnancy (Sun *et al*., 2014). MTX is an antimetabolite that prevents folic acid metabolism. It binds to dihydrofolate reductase and prevents the transformation of dihydrofolate to tetrahydrofolate1-3, which produces thymidine and purine, both of which are necessary for DNA synthesis (Hyoun *et al*., 2012 and Howard *et al*., 2016). It is useful in the treatment of cancer due to its ability to suppress cellular proliferation (Chan & Cronstein, 2010).

Growth plate dysfunction brought by MTX drug results in bone growth arrest, increased bone marrow fat content, and decreased metaphysis trabecular bone volume (Xian *et al.*, 2008; Fan *et al.*, 2009 and Georgiou *et al.*, 2012). Additionally, it is believed that MTX has direct toxicity on osteoblasts, preosteoblasts, osteoprogenitors, bone marrow stromal progenitor cells, and osteocytes interferes with bone remodelling and osteogenesis (Fan *et al.*, 2009; King *et al.*, 2012 and Shandala *et al.*, 2012).

Several studies have been conducted on the effects of high doses of MTX on pregnancy. It has an immediate effect on dividing cells, such as trophoblast cells and blood stem cells, and it also causes cells to stop dividing (Gol *et al*., 2009). MTX caused increasing apoptotic cells, delaying in S-phase, decreasing the number of mitotic cells and reduction in the number of cells of the foetuses of developing zebrafish (Lee *et al*., 2012). Also, it was found in the umbilical blood and placenta of a woman who received MTX causing foetal chromosomal aberrations (Al-Saleh *et al*., 2007). Studies in rats, mice, and rabbits revealed embryotoxicity and teratogenicity (Al-khateeb *et al*., 2014). Foetal MTX syndrome or MTX embryopathy is a congenital malformation disease caused by the mother's exposure to MTX during treatment for the maternal disease, misdiagnosed ectopic pregnancy, or failed medical abortion. It is characterized by growth deficiency, microcephaly, craniosynostosis, facial dysmorphic features, spina bifida cystica, or a bony defect in the spinal column, abnormal skull development, abnormal positioning of the hands at the wrist, missing bones in the arm and feet and limb defects (Lewden *et al*., 2004 and Hyoun *et al*., 2012).

When planning to become pregnant and during pregnancy, both partners should avoid MTX (Nancy, 2022). So, the current study investigated the toxic effect of a low dose of MXT on foetal body weight and length, skeletal system, kidney and heart of albino rats who's paternally treated with MTX.

## MATERIALS AND METHODS

Methotrexate MTX ($C_{20}H_{22}N_8O_5$) with molar mass (454.447 g/mol-1) was obtained from the Egyptian company 'ORION Pharm', Cairo. Based on previous studies, one dose of MTX (0.03 mg/kg) was chosen (Saka & Aouacheri, 2017). This treatment is parallel to the therapeutic dose applied to humans (2.5mg/100kg b.w of MTX once daily for 10 days. MTX LD50 in rats is 317 mg/kg (Saka & Aouacheri, 2017).

**Mating Procedure:**

Adult virgin females between the ages of 8 and 12 weeks were selected. Each of them was housed in a cage with one adult male overnight. The appearance of a vaginal plug confirmed pregnancy the next morning, pregnant females were kept in their own cages. A drop of vaginal fluid was produced in the absence of a vaginal plug, and it was tested for the presence of spermatozoa, which was interpreted as a sign of copulation. These females were thought to be pregnant on the 1st day. Weight and length were measured, and external malformations were examined morphologically.

**Animal Groups:**

*Rattus norvegicus domestica* virgin females and males from Theodor Bilharz research institute in El Nile Road, Warrak, Egypt, were chosen for the experiment. They were provided with food, water and housed in clean ventilated cages with a 12-hour light/dark cycle, controlled temperatures (22°C), and a 50% humidity level. Guidelines for the use of animals in research were prepared by the Regional Ethics Committee of the Faculty of Science, Benha University, Egypt (Grant No.:ZD/FSc/BU-IACUC/2022-8) and (BUFS-REC-2023-35Zoo). These restrictions follow worldwide recommendations and were developed by national health institutions for using animals in experimental research.

In this study, 35 rats (5 groups of 7 each) were used: the control group (C), orally received distilled water. G1; males were given (0.03 mg/kg) MTX for 10 days before mating with control females. G2; females were given (0.03 mg/kg) MTX for 10 days, then permitted to mate with control males. G3 males and females were allowed to mate after receiving MTX (0.03 mg/kg) for 10 days.

G4; a group of pregnant females was given (0.03mg/kg) of MTX from the 1st to the 20th day of pregnancy (Fig.1).

On day 20 of pregnancy, the uteri were removed via caesarean section. The number of surviving and dead foetuses, as well as early and late resorptions, were all recorded for each mother. The size of early and late resorptions was used to distinguish between them. For morphological examinations, foetuses were obtained from all of the above-mentioned groups. Living and dead foetuses were counted, their weight and length assessed, and their morphology studied for external malformations were detected. Foetuses were preserved in 95% ethyl alcohol and stained with alizarin red and alcian blue to study the abnormalities in their bones. Following soft tissue digestion with 2% potassium hydroxide, the tissue samples were preserved in different concentrations of glycerin that increased until the bones could be seen (Falkeholm *et al*., 2001).

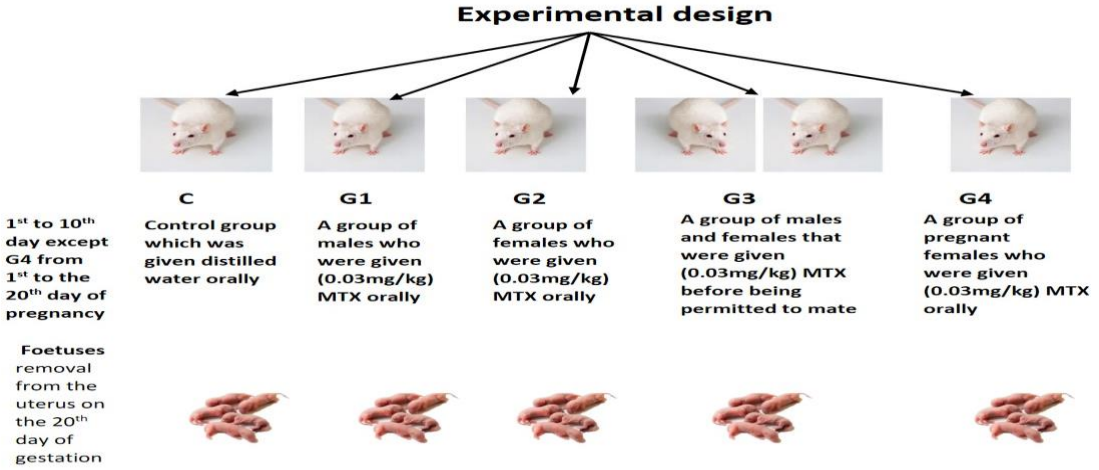


**Fig. 1:** Experimental design.

**Samples Preparation:**

On the 20th day of pregnancy, the heart and kidney were removed from the control and MTX groups. The heart and kidney tissues were fixed in 10% buffered formalin, and the tissues were then embedded in sections of paraffin that were 6 m thick for hematoxylin and eosin staining (Stojiljkovic *et al*., 2012).

**Statistical Analysis:**

The results were presented as mean ± SD. The Kruskal-Wallis H test was used to determine the statistically significant differences (Corder & Foreman, 2009). Followed by the post-hoc Dunn's test (Field, 2013). For the mortality rate, a pairwise comparison with Pearson's Chi-square test was used (Scott *et al*., 2013).

## RESULTS

**External Morphological Studies:**

According to the outer examination of the 20th-day foetuses in this study, MTX caused growth retardation in foetuses, resulting in a reduction in the length and weight of the foetus (Fig.2a, b). The Kruskal-Wallis H test indicated that there was a significant difference in the dependent variable between the different groups, $\chi^2(4) = 26.85, p < .001$, in foetuses weight with a mean rank score of 28.71for control group, 26.79 for G1, 19.5 for G2, 10.71 for G3 and 4.29 for G4 (Fig. 2a). Also, the mean rank score for the foetuses body length was 29.57 for the control group, 23.57, 21.57, 9.71, 5.57 for G1, G2, G3 and G4, respectively. The post-hoc Dunn's test using a Bonferroni corrected alpha of $p < 0.001$ for foetuses body weight indicated that the mean ranks were significantly different for C-G3, C-G4 and G1-G4. It also showed a significant difference, $p < .01$ between G1-G3 and G2-G4. Additionally, the post-Dunn's test for the body length showed significant differences for C-G3, C-G4, G1-G4 and G2-G4 (Fig. 2b). Analyzing the whole morphology of parentally administered

MTX foetuses revealed the existence of congenital abnormalities such as convexity of the body, elongation of the head region, partial development of limbs and tails and the occurrence of superficial haematomas in various body regions (Fig. 5).

**Foetuses Mortality Rate:**

Statistical analysis revealed a significant difference between C-G3 and C-G4 for $p < .001$. The results of mortality rates were illustrated in (Fig. 3). The percent changes in live foetuses compared to control live foetuses were 11.1%, 18.6%, 40.8%, and 48.1% for G1, G2, G3, and G4, respectively (Fig. 3). The Control group had a uterus that naturally encapsulated large embryos. The G1 and G2 groups showed a normal uterus that contained a large number of foetuses. In G3 and G4 fewer embryos were generated in a tiny uterus, and some were resorbed (Fig. 4).

**Endoskeleton Observations:**

**Axial Skeleton and Appendicular Skeleton:**

The skeletal structure of albino rats is divided into two parts: the axial and appendicular skeleton. The axial skeleton consists of the skull, spinal column, ribs, and sternum. The appendicular skeleton consists of a pectoral girdle & pelvic girdle & forelimbs and hind limbs (Figures 5-6C). MTX treatment to the parents generated many undesirable consequences ranging from mild to severe abnormalities in foetuses on the 20$^{th}$ day, according to osteological malformations (Figs. 5-6; G1-G4).

The control albino rat foetuses' skulls showed full ossification of their components on the 20$^{th}$ day of gestation (Figs. 5b, 5c and 6a). There is an abnormality in the skull of foetuses whose parents were treated with MTX, incomplete ossification of the nasal, supra-occipital, frontal, parietal, interparietal, zygomatic process of the squamosal, tympanic bulla, supra-occipital, periotic, palatine, pterygoid & ethmoid bones. As a result, the length and volume of parentally MTX-treated foetuses were significantly lower than those of the control group. The lower jaw bones in the MTX-treated groups' foetuses showed only slight with a progressive absence of ossification, as shown in (Fig. 5c).

Control foetuses had well-ossified vertebrae, with 7 cervical, 12 thoracic, 7 lumber, 4 sacral, and 10 caudal vertebrae (Figure 6b). The atlas and axis ossification were significantly reduced in the four groups. The majority of foetuses in the treated groups had ossification in their cervical, thoracic, lumbar, sacral, and caudal vertebrae to varying degrees. There are 13 pairs of ribs in the control group's foetuses, each of which is composed of a cartilaginous sternal region and a bony vertebral area. Except for the final three pairs, the sternum and the sterna portion of the ribs are connected (Fig. 5b). There were no differences in the number of ribs or their ossification in the MTX groups as compared to the control group. Six straight, rod-like, ossified sternebrae, the last of which is the Xiphi-sternum, make up the sternum of control foetuses (Fig. 6C). Compared to the control group, the sternebrae of foetuses whose parents administered MTX displayed more or fewer ossifications.

On the 20$^{th}$ day of pregnancy, the pectoral girdle of control foetuses consists of a cartilaginous supra-scapula stained alcian blue and an alizarin red-stained well-ossified scapula and clavicle. The forelimbs of control foetuses have cartilaginous carpalia and meta-carpalia, as well as the ossified humerus, radius, and ulna and phalanges with five digits (Fig. 7a). In comparison to the control group, the pectoral girdle and forelimb of foetuses born from parents who received 0.03mg/kg of MTX show a decrease in size, length (Fig. 7a and Table 2a) and ossification level.

Control foetuses have a pelvic girdle made of three well-ossified bones (ilium, ischium and pubis). In its natural state, the pubic symphysis is cartilaginous. The femur, tibia, fibula, tarsals, metatarsals, and phalanges make

up the hind limb of control foetuses (Fig. 7b). The pelvic girdle and hind limbs of MTX-parentally treated foetuses were significantly shorter and thinner, with incomplete and absent ossifications of their components when compared to control foetuses (Fig. 7b). In comparison to the control group, the administered foetal groups also showed deformation in the cartilage drafting of the metacarpal bones and phalanges.

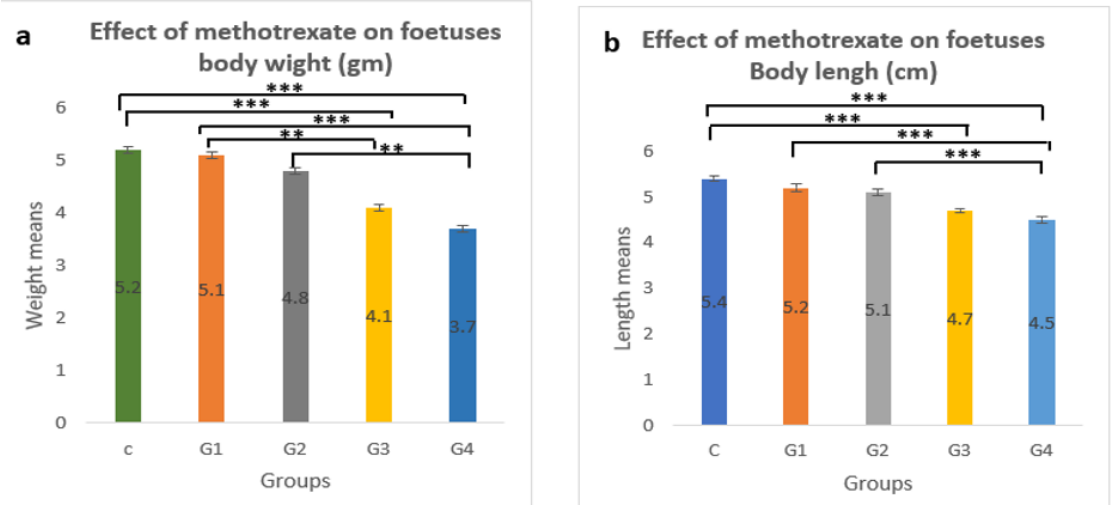


**Fig. 2:** The impact of a single dose of MTX (0.03 mg/kg) on the morphology of foetuses on day 20 of pregnancy in comparison to the control group (C). (A): displayed the mean body weight variation (gm). (B): displayed the average change in body length (cm),. Data are expressed as mean ± S.E.M. *p < 0.05, **p < 0.01 and ***p < 0.001.

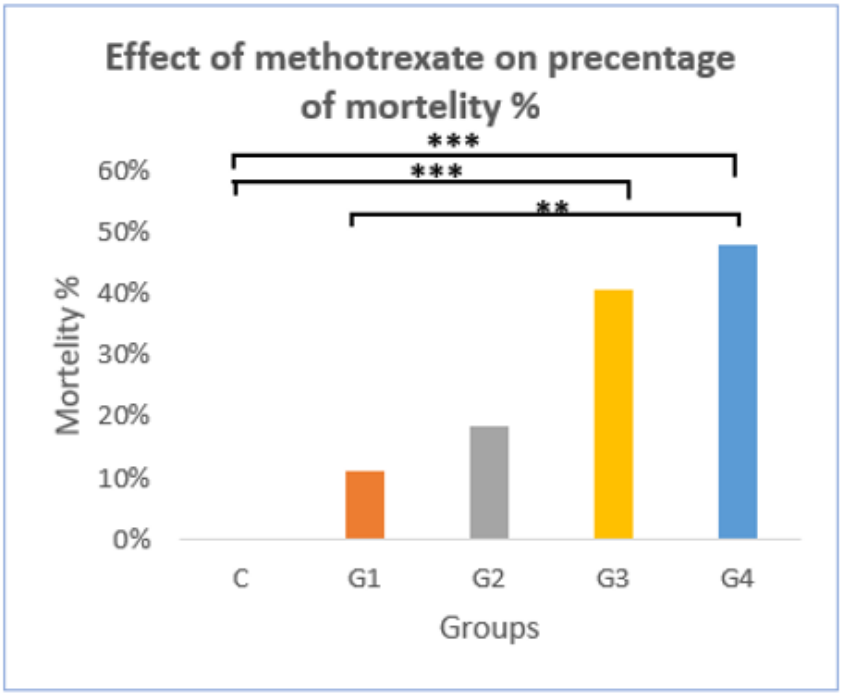


**Fig. 3:** Effect of a single dose of MTX (0.03 mg/kg) on the mortality percent of foetuses. on day 20 of pregnancy in comparison to the control group (C). where mortality was in Control= 0%, Group1= 11.10%, Group2= 18.60%, Group3= 40.89%, Group4= 48.10%. Data are expressed as mean ± S.E.M. * p < 0.05, ** p < 0.01 and ***p < 0.001.

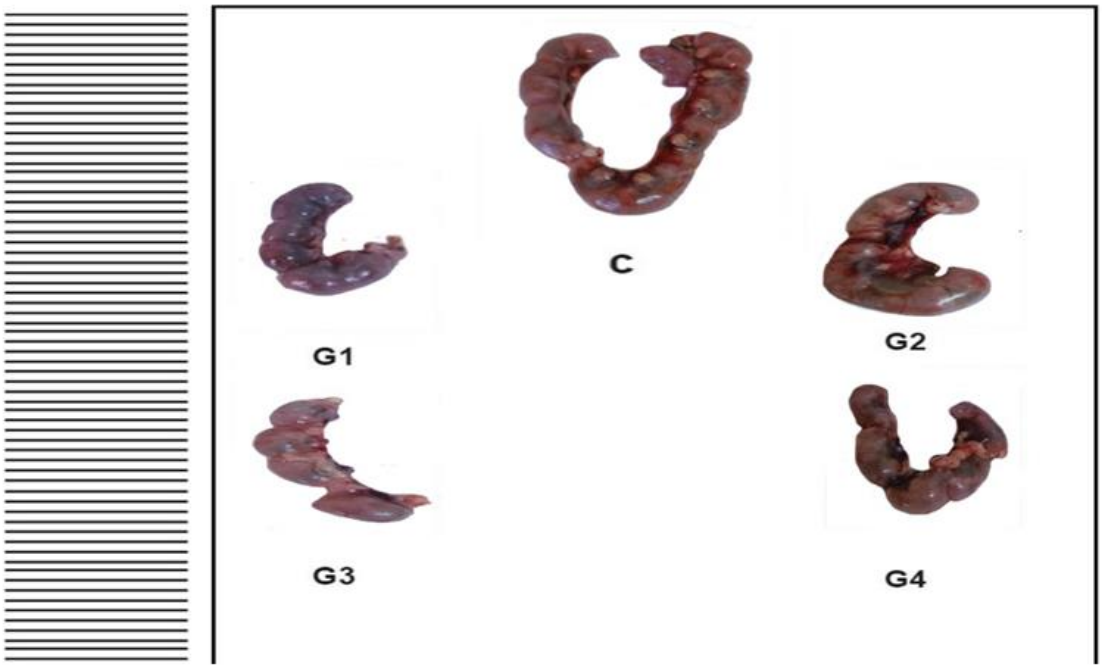


**Fig. 4:** Ventral view of the uteri shows the control and parentally treated groups with a single dose of MTX (0.03 mg/kg) on 20th day of pregnancy.

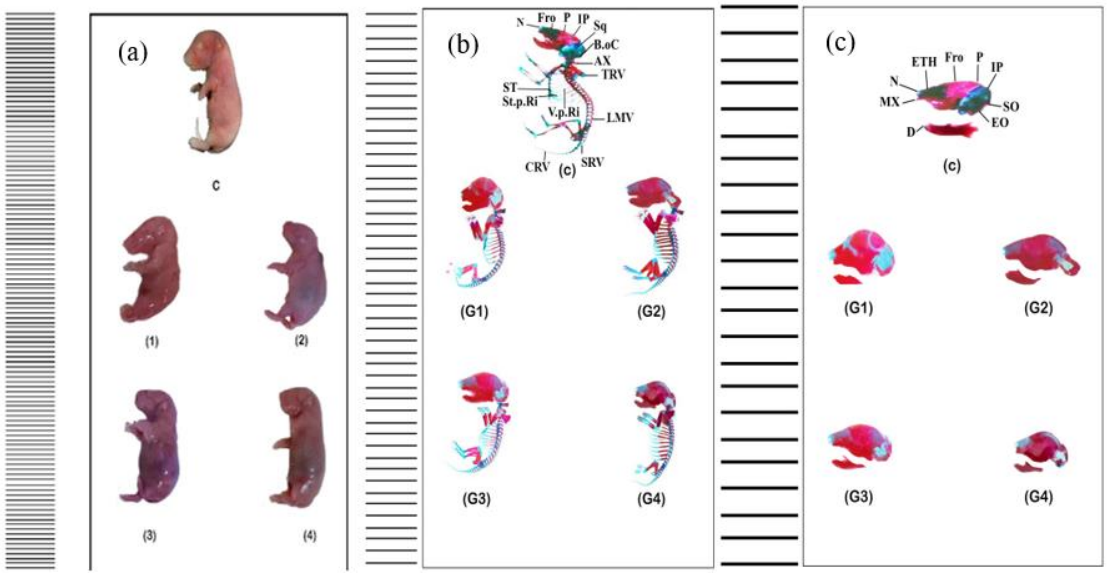


**Fig. 5:** Photograph of lateral view (a) showed external morphology; (b) Skeletal System and (c) skull of rat foetuses at the 20th day of gestation; the Control (C) group and four parentally treated groups with 0.03 mg/kg body wt of MTX (G1, G2, G3& G4).

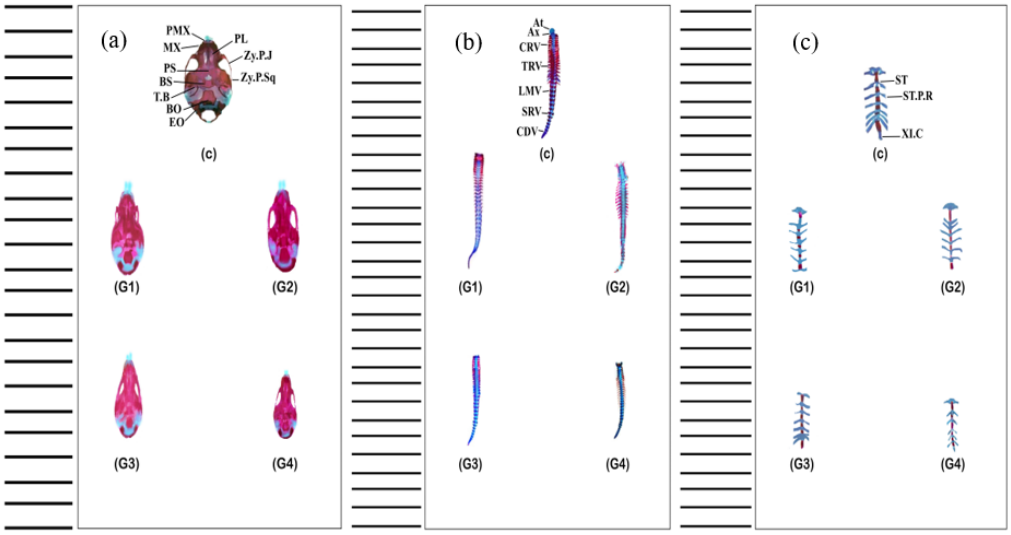


**Fig. 6:** Ventral view (a) showed skull; (b) vertebral column and (c) sternum of rat foetuses on the 20th day of pregnancy.

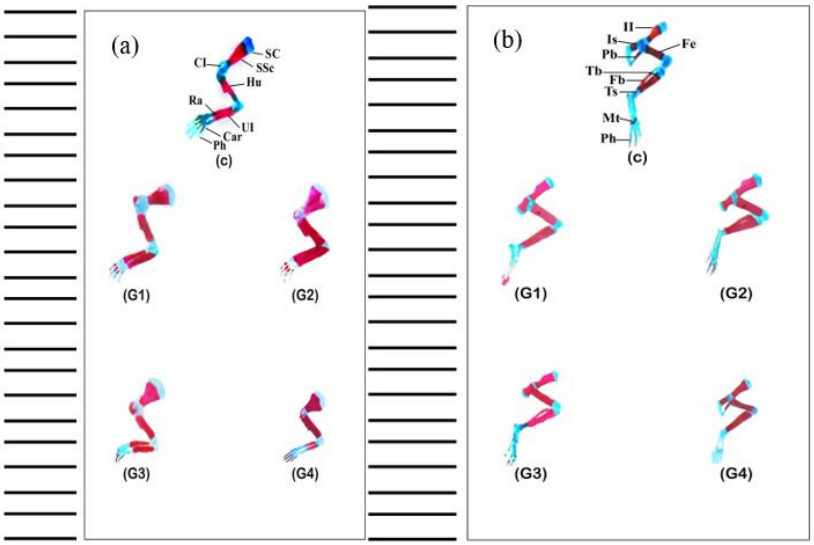


**Fig. 7:** Lateral view (a) showed pectoral girdle and fore limb, (b) pelvic girdle and hind limb of albino rat foetuses at the 20th day of gestation.

| Abbreviations | | | | | |
|---|---|---|---|---|---|
| AT | Atlas | ETH | Ethmoid | P | Parietal |
| AX | Axis | Fb | Fibula | Pb | Pubis |
| B.OC | Basioccipital | Fe | Femur | Ph | Phalanges |
| BO | Basioccipital | Fro | Frontal | PL | Palatine |
| BS | Basisphenoid | Hu | Humerus | PMX | Premaxilla |
| Car | Carpales | IP | Interparietal | PS | Presphenoid |
| CDV | Caudal vertebrae | IS | Ischium | Ra | Radius |
| CL | Clavicle | LMV | Lumber vertebrae | SC | Scapula |
| CRV | Cervical vertebrae | Mt | Metacarpalia | SO | Supraoccipital |
| D | Dentery | MX | Maxilla | Sq | Squamosal |
| EO | Exooccipital | N | Nasal | SRV | Sacral vertebrae |
| IL | Ilium | T.B | Tympanic bulla | SSc | Supra-scapula |
| UI | Ulna | Tb | Tibia | ST | Sternebrae |
| Ts | Tarsalia | TRV | Thorathic vertebrae | St.p.Ri | Sternal portion of ribs |
| V.P.Ri | Vertebral portion of ribs | XI.C | Xiphoid cartilage | Zy.P.J | Zygomatic process of jugal |
| Zy.P.Sq | Zygomatic process of squamosal | | | | |

**Histopathological Observation:**

In our study, the light focuses on two important organs; the kidneys and the heart. The kidneys of control foetuses are made up of tightly packed uriniferous tubules, with each tubule made up of an initial unbranched nephron and a branched collecting tubule. The nephron develops from a renal corpuscle, which is made up of a double-walled Bowman's capsule that partially surrounds the glomerulus. Bowman's capsule is made up of two simple epithelial layers: an inner or visceral layer that covers the glomerulus, and an outer or parietal layer that is not. The two layers merge at the rim of the renal corpuscle opening (Fig. 8C). The tubular part of the nephron is made up of two parts: the proximal tubule and the distal tubule. A single layer of low columnar or pyramidal cells with round nuclei and an acidophilic granular cytoplasm line the proximal convoluted tubule. The distal tubule is lined with cuboidal cells that are lower and narrower than the proximal tubule. Simple cuboidal epithelium lines the collecting tubules, which have relatively wide lumina (Fig. 8C).

In comparison to controls, parenterally administered MTX caused significant changes in foetal kidneys. G1 showed low histopathological alterations. Malpighian corpuscles and renal tubules missed their normal pattern due to degenerated epithelium. Glomerular degeneration with an atrophic appearance was observed. As a sign of necrosis, the proximal and distal convoluted tubule epithelial cells had pyknotic nuclei and hydropic degeneration. The lining epithelial cells are swollen, and the cytoplasm appears foamy with pyknotic nuclei (Fig. 8G1). G2 showed that the alternation was more pronounced than that of the previous group. Fresh haemorrhage and edoema were found in the interstitium. The lumen of the tubules was dilated, and the lining epithelium showed vacuolar degeneration (Fig. 8G2). G3 Showed histopathological changes that were more pronounced than G1 and G2. Epithelial cells had pyknotic nuclei, hydropic degeneration, and necrosis. Interstitial edema with focal mononuclear cell infiltration into renal tubules has been reported. The lumen of renal tubules was filled with red blood cells (Fig. 8G3). G4 showed severe histopathological changes. Many epithelial cells lining the renal tubules become swollen and project inward attaining a conical-shaped appearance, therefore the lumina of these tubules becomes relatively small. Glomerular congestion and haemorrhage. Epithelial cells have pyknotic nuclei, hydropic degeneration, and necrosis. Bowman's space has been expanded. There was an enlarged space between the parietal and visceral layers of Bowman's capsule with glomerular atrophy. The stroma showed edema with multiple foci of tubular ischemic necrosis (Fig. 8G4).

The heart of control foetuses displayed normal cardiac myocytes with prominent striations in the longitudinal section. There were no striations in the perinuclear region. They showed a single nucleus in the center. Myocytes and capillary endothelial cells were invested by the connective tissue stroma. Each muscle fiber was surrounded by a delicate connective tissue endomysium with a dense capillary network. Fibroblast nuclei are more flattened and darker stained than those of cardiac muscle cells, and they are in the periphery. Cross sections of cardiac muscle were found to be irregular. The nuclei of cardiac fibers were discovered near the center. Occasionally, brighter perinuclear regions were seen. Connective tissue was seen between bundles of muscle cells. Fibroblast nuclei were observed in connective tissue or on the periphery of muscle fibers. Among the cardiac muscle fibers, there were numerous capillaries. (Fig. 9C).

G1 showed no significant change from controls in centrally located myocardial fibers. (Fig. 9 G1). G2

Showed Congestion in myocardial muscle fibers with an irregular arrangement and cardiomyocyte degeneration in the form of necrotic areas (Fig. 9 G1). G3 revealed histopathological alteration. In some areas with less obvious striation, there is interstitial oedema and degeneration. Some fibers had pyxcnosised which caused disarrangement. Myocytes have been vacuolated with a necrotic area. (Fig. 8G3). G4 showed more histopathological changes than G1. Signs of toxicity were massive, degeneration of myocardial fibers with pyknotic nuclei and vacuoles, congestion of blood vessels, leukocyte infiltration and necrosis (Fig. 8G4).

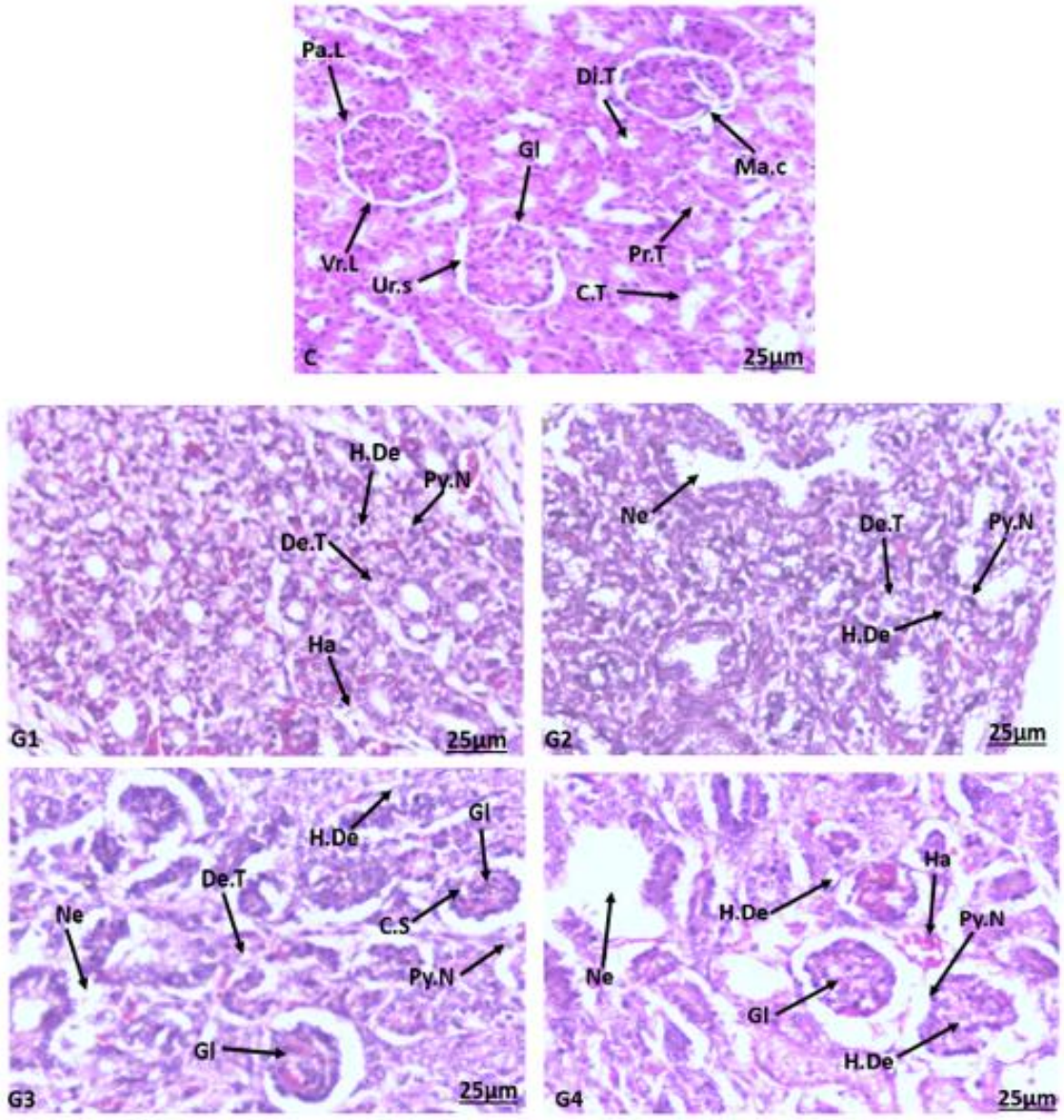


**Fig. 8:** A photomicrograph of a transverse section in the kidney of the 20$^{th}$-day foetus (C) showing: normal structure kidney in the control group; Collecting tubules, C.T; Distal tubule, Di.T. glomerules, Gl; Malpighian corpuscle, Ma.c; Parietal layer, Pr.L; Proximal tubules, Pr.T and Visceral layer,Vr.L. (G1): showing degenerated tubule, De.T; Pyknotic nuclei, Py.N; hydrobic degeneration, H.De and Haemorrhage, Ha. (G2): showing Degenerated tubule, De.T; Hydropic degeneration, H.De; Nerosis, Ne and Pyknotic nuclei, Py.N . (G3): showing: Degenerated glomeruli, Gl; Degenerated tubule, De.T and Pyknotic nuclei, Py.N. (G4): showing Degenerated tubule, De.T; Degenerated glomeruli, Gl; Haemorrhage, Ha; hydropic degeneration, H.De; Necrosis, Ne and Pyknotic nuclei, Py.N.

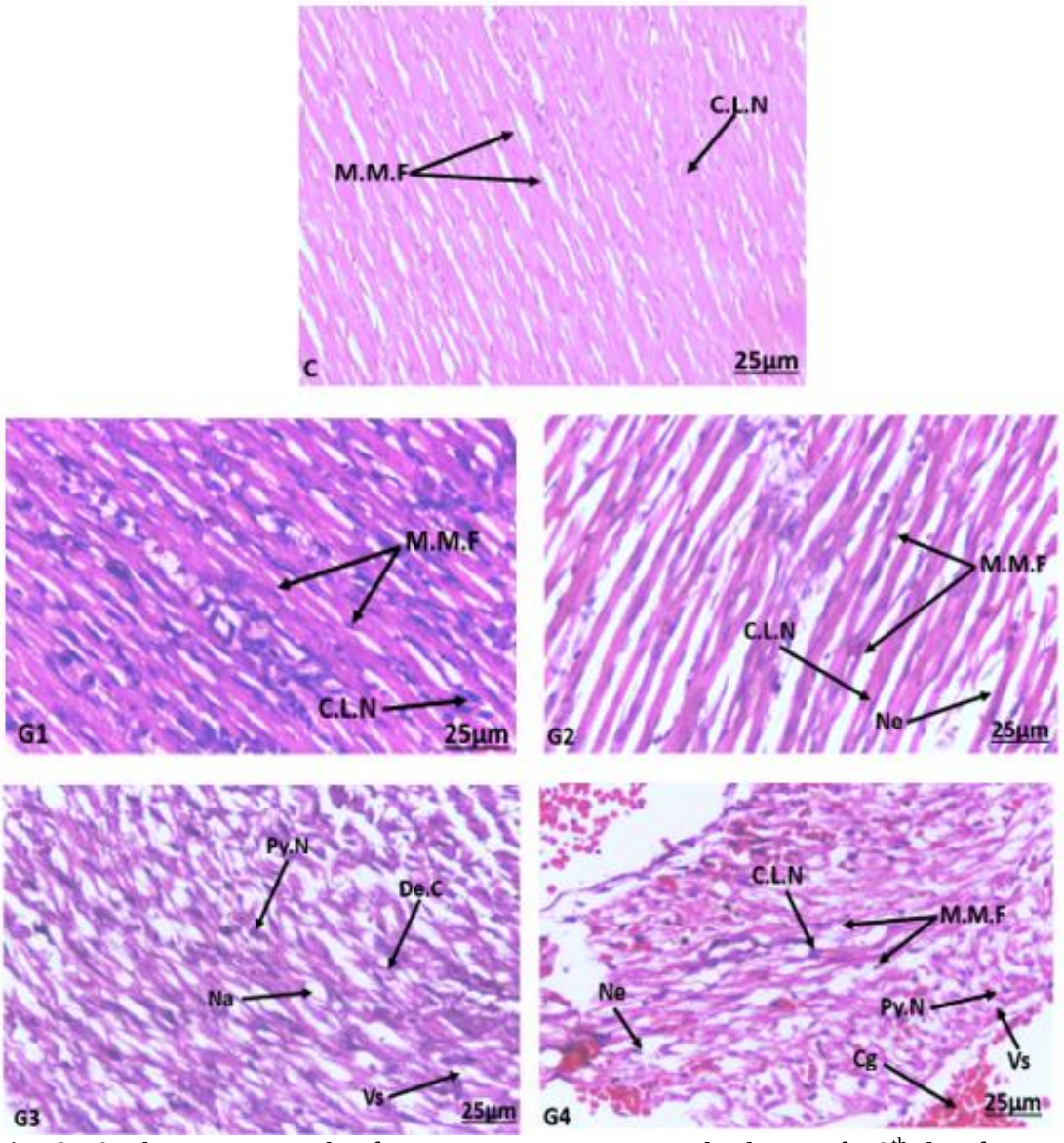


**Fig. 9:** A photomicrograph of a transverse section in the heart of 20$^{th}$-day foetuses showing: (C): normal structure heart in the control group. (G1): showing myocardial muscle fibers, M.M.F and centrally located nuclei, C.L.N. (G2): showing myocardial muscle fibers, M.M.F; centrally located nuclei, C.L.N and Necrosis, Ne (G3): showing degeneration of cardiomyocytes, De.C; necrotic areas, Na; pyknotic nuclei, Py.N and widely spread vacuolated spaces, Vs. (G4): showing congestion in myocardial muscle fibers with lack of regular arrangement, Cg; centrally located nuclei, C.L.N; myocardial muscle fibers, M.M.F; Necrosis, Ne; pyknotic nuclei, Py.N and widely spread vacuolated spaces, Vs.

## DISCUSSION

Methotrexate (MTX) is an antimetabolite and antifolate that is used to treat rheumatoid arthritis and psoriasis, among other autoimmune diseases. It is also used as a chemotherapeutic agent for a variety of cancers, including lymphoma and breast cancer (Abdel-Raheem &Khedr, 2014).

In our research, according to the external morphological examination of the 20$^{th}$-day foetuses, MTX caused growth retardation in foetuses, resulting in a reduction in foetal body weight, length, and congenital abnormalities. Similar results have been reported by Lewden *et al*., 2004.

In our study, parentally treated foetuses with MTX using a single dose resulted in a variety of skeletal deformities as well as the absence of ossification in some skeleton

components and abnormality in the skull. These results are consistent with the results of (Van Leeuwen *et al*., 2003) who had reported that MTX-induced growth retardation in trabecular bone volume rather than changes in the growth plate. Abnormal skull development due to MTX embryopathy (Lewden *et al*., 2004 and Hyoun *et al*., 2012. Also, MTX was discovered to cause apoptotic cell death in growth plate regions, hypertrophic zones and proliferative zones (Xian *et al*., 2008 and Lee *et al*., 2012). By using MTX, the proportion of dead foetuses increased while the number of live foetuses decreased. Similarly, (Mitchell *et al*., 2010 and Nancy, 2022) results observed that parental MTX exposure increased the rate of spontaneous abortion and foetal death.

In our work, the sternebrae of foetuses who were given MTX by their parents had more/fewer ossifications than the control group. This was parallel to the results of (Davies *et al*., 2002; Van Leeuwen *et al*., 2003 and Fan *et al*., 2009) who found that MTX reduced the number of osteoblast-like cells and osteoprogenitor cells, inhibited osteoblast proliferation, and increased bone resorption. Additionally, the components of the girdles and limbs of foetuses whose parents were treated with MTX showed a reduction in size and degree of ossification. Similar works of (Lewden *et al*., 2004 and Hyoun *et al*., 2012) confirm our observations, as losing bones in the feet and limb defects by maternal exposure to MTX during pregnancy. Other authors (Seidahmed *et al*., 2006) have also reported that MTX caused limb abnormalities in foetuses that are known to be associated with aminopterin syndrome.

Histopathological results in the current study revealed that MTX caused kidney damage in parentally administered foetuses, and also showed severe glomerulus deterioration, nuclei pyknosis, haemorrhage, inter-tubular capillary congestion, glomerulus atrophy, cytoplasm vacuolation, cloudy swelling, hydropic degeneration and necrosis of renal tubule cells. These results were consistent with different studies which showed that MTX causes a decrease in glomeruli size, an increase in blood cells, Bowman capsule cavity enlargement, infiltration of lymphocytes and kidney tubule degeneration (Ulusoy *et al*., 2016; Al-Rashidy *et al*., 2018 and Jalili *et al*., 2020). Also, MTX can cause a variety of side effects, including mild tubular degeneration and glomerulosclerosis (Asci *et al*., 2017; Saka *et al*., 2017 and Mahmoud *et al*., 2018). In a similar study, the histological structure of the kidneys showed expansion of the capsular space, defects in the renal corpuscles, shrunken glomeruli, and necrosis of the renal tubules in MTX-treated rats (Ahmed *et al.*, 2021). High MTX doses harm the kidneys in two ways: by precipitating in the kidney tubules and reducing the glomerular filtration rate (Tian & Cronstein., 2007). Also, MTX metabolites were found to precipitate in renal tubules resulting in severe nephrotoxicity in rats (Ulusoy *et al*., 2016).

The heart of the foetuses with parentally administered MTX demonstrated significant changes in comparison to the control. Congestion, cardiomyocyte degeneration, necrotic areas, infiltration of inflammatory leucocytes, and a lack of regular arrangement were observed. Similar results of (Abdel-Daim *et al*., 2017, Mohamed *et al*., 2018 and Mahmoud *et al*., 2021) who reported toxicity symptoms, like massive myocardial fiber degeneration, blood vessel congestion, leukocytic infiltration, necrosis and apoptosis, dark eosinophilic cardiac muscle cells with small pyknotic nuclei as well as interstitial oedema in cardiac muscle fibers. In addition, the use of MTX caused histopathological cardiac tissue damage (Tousson *et al*., 2016).

## CONCLUSIONS

The abovementioned results showed that methotrexate (MTX) treatment caused developmental

abnormalities in parentally treated foetuses, as evidenced by significant weight and length reductions, an increase in mortality rate, partial ossification, and a significant decrease in the skeletal bones. Histopathological alternation in the kidney; The presence of atrophic glomerular and tubule degeneration. The heart showed histopathological changes; marked degenerative changes, Cardiomyocyte failure with pyknotic nuclei and lack of regular arrangement.

**Ethical Approval:**

All experimental procedures were approved by Faculty of Science, Benha University, Egypt with code number (Grant No.:ZD/FSc/BU-IACUC/2022-8) and (BUFS-REC-2023-35 Zoo).

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