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Effect of Clarithromycin Administration during Late Gestational Period on The Pregnant Albino Rats and Their Fetuses

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ABSTRACT

Background:

Clarithromycin, a new macrolide antibiotic, is effective in the management of a wide range of clinical problems including outpatient treatment of community-acquired pneumonia, shortening the course of peptic ulcer disease associated with *Helicobacter pylori* infection and curing previously resistant respiratory infections in immune-compromised patients.

The present study is planned to study the effect of clarithromycin on the pregnant female rats and their fetuses during the last gestational period stage. This study includes the effect of clarithromycin on therate of abortion, malformation of fetuses, skeletal, histological changes and DNA fragmentation of liver cells of pregnant rats and their fetuses. In the present study two groups of pregnant animals were used. The first group received distilled water from 15^{th} to 19^{th} days of gestation and used as control and sacrificed at 20^{th} day of gestation. The other group is orally administered with 45mg/kgclarithromycin from 15^{th} to 19^{th} days and sacrifices at 20^{th} day of gestation (the therapeutic dose).

The obtained results showed a significant decrease in maternal body weight gain and increase in the rate of abortion, resorption and growth retardation of fetuses.Fetuses of the treated group showed severe lack of ossification on the skull bones, phalanges and sternum bone as well as shortness in the ulna and radius bones. Histological studies of pregnant rats revealed congestion and dilatation of the central vein of the liver lobules and fatty degeneration of the hepatocytes with severe DNA fragmentation.In 20 day-fetuses, there were a marked increase of necrotic hepatocytes associated with increased average of megakaryocytes and periportal leukocytic infiltration.

Key words: Macrolides, clarithromycin,fetogenesis, teratology, skeletal malformation, rat and liver histopathology, DNA fragmentation.

INTRODUCTION

In general, wide applications of the antibiotics such as penicillins, cephalosporins and macrolides, such as erythromycin revealed that they are safe.[1]However,less of works are concerned with the clarithromycin. The treatment with clarithromycin was found toexhibits no cytotoxicity in the non-small cell lungcancer (NSCLC) cell lines. [1]

Macrolide antibioticshave been usedfrequentlyto treat mild to-moderately severeupper and lower respiratory tractinfections and selected genitourinaryinfections. It showed potent effects on gram –negative bacteria.[2]

According to **Klein (1997)**[3], the macrolideantibiotics are often subdivided intoerythromycinand non-erythromycin drugs. The erythromycin is the first-introduced macrolidedrugs, whilethe non-erythromycin includes clarithromycin and azithromycin, which have fewer effects on gastrointestinalmotility than erythromycin.Clarithromycin is generally well tolerated, producing fewer gastrointestinal complaints than its parent compound, erythromycin. So, from 1994 through 2008, there was a decreasing trend in erythromycin use and an increasing trend in use of non-erythromycin macrolides during pregnancy

There are relatively limited data concerning the safety of azithromycin, clarithromycin and roxithromycin during gestation.[4]The relatively new macrolide antibiotics (clarithromycin, azithromycin and roxithrimycin) are better tolerated than erythromycin. [5]Clarithromycin and azithromycin have also been helpful in the treatment of toxoplasmosis during pregnancy. [5].

Bar-Oz B*et al.*, (2008)[4]reported that the new macrolides during the first trimester of pregnancy does not represent an increased risk for congenital malformations but strongenough to induce abortion after such an exposure. Elective terminations of pregnancy because of

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early exposure to these medications should be reconsidered.

Therefore, the present work is designed to study the effect of clarithromycin on the pregnant dams and their fetuses when it administrated during the late gestational period.

MATERIALS AND METHODS

I-Materials:

1-Administration of the drug:

Clarithromycin modified release tablets were used in the present study. The therapeutic dose in adult human is 500mg daily**.** [6] The tablets were grinded and suspended in distilled water and used after shaking in the dose 45mg/Kg drenched to the female rat which is equivalent to the therapeutic dose of human according to **Paget and Barnes, 1964**.[7]

2- Experimental animals and design:

Males and females of 11-13 weeks old were used in the present study. Zero day of gestation was determined by the presence of sperms in the vaginal smear at estrus phase. [8]

Pregnant female animals were divided into two main groups, each consisting of (6-8 rats):

Control group (C):-The first group received distilled water from 15^{th} to 19^{th} day and sacrificed on the 20^{th} day of gestation (served as control group).

Treated group (T):-The second group received 45mg/Kg of clarithromycin from15^{th} to 19^{th} day and sacrificed on the 20^{th} day of gestation.

II- Methods:

1-Sings of toxicity:

Different parameters were measured as: mothers weights, percentage of abortion, uterine weight, number of resorption sites, number of alive and dead fetuses, placental weights (gm), fetal growth parameters including total body weight (gm), body length (cm) and tail length (cm).

2-Morphological examination:

The fetuses were examined for the occurrence of any malformation using the dissecting microscope.

3-Endoskeleton staining and examination:

Fetuses were skimmed and carefully eviscerated to permit satisfactory penetration by the stain. Fixation was done in 95% ethyl alcohol for hardening of the specimens.Staining of fetal skeleton was done by using a double staining technique with alcian blue for cartilages and alizarin red S for bones according to the method described by**Peters (1977).** [9]After staining the specimens were kept in glycerin. The skeleton was examined under the dissecting binocular microscope to study any malformation and shortening in the bones of fetuses.

4-Histological observations:

The livers of mothers and fetuses as well as the 8^{th} day embryos were fixed in 10% neutral formalin buffer for one week, dehydrated in series of ethanol, cleared in xylene, embedded in several changes of paraffin wax and blocks were sectioned at 5µm, mounted on clean studies, and stained by haematoxylen and eosin stains and then examined using an optic microscope (Leica).

5-Molecular biology study

DNA fragmentation was determined via agarose gel electrophoresis**;** genomic DNA was isolated from the rat livers tissue and their fetal liversaccording to **Miller*et al.*, 1988** [10] using DNA Kite of Promega Corporation, USA. Agarose gel electrophoresis of DNA was done according to the method of **Sealey and Southern 1982.** [11]

6- Statistical analysis:

Results have been analyzed by prism version (5) programs. Comparison between the studied groups was carried out using the unpaired t-test[12], where $P<0.05$ was considered significant**.**All the values were presented as means ± standard errors of the means (S.E.M.)**.**

RESULTS

I-Morphological studies:

1- Effects of Clarithromycin on Maternal rats:

1.1- The body weight gain:

The maternal body weight was followed all over the period of gestation for the control and experimental groups.Pregnant dams of both control and clarithromycin-drenched groups showed a steady increase in weight gain during the gestation period. The rate of increase in maternal body weight (weight gain) of the treated dams was significantly decreased by 39% as compared to that of the control group. (Table 1 & Fig. 1)

1.2- Rate of abortion:

Abortion was indicated by a sudden decrease in the maternal body weight and presence of drops of blood at the vaginal opening. The percentage of abortion (completely or partially) was calculate

1.3 -The intrauterine growth of fetuses and placenta:

The placental weightof dams received clarithromycinand the weight of their fetuses were significantly decreasedthan that of the control group by 34.96 % and 9.29% respectively (Table 1 and Fig.2). The placental index (which is the placental weight divided by the fetal weight) showed a significant increase of the treated group compared to that of the control (Table 1 and Fig. 3).

2- Effects of clarithromycin on the developing fetuses:

2.1- Fetal mortality:

Total mortality rate including resorbed and dead fetuses at 20^{th} day of gestation were recorded for control and treated groups in table (2) and Fig. (4). Unlike the uteri of the control pregnant dams, received dist. H_2O during the last gestational period, the uteri of treated groups showed asymmetrical distribution of fetuses on both horns (Fig. 4). Meanwhile, the uteri of pregnant rats received clarithromycin had some resorped fetuses (Fig. 4) but with insignificant rate as compared to the control group (Table 2).

2.2- Growth retardation:

The morphological examination of the fetuses showed that clarithromycincauses growth retardation represented by significant decrease in fetal body weight, body length and tail length (Fig. 5) when it administrated during fetogenesis period of gestation with the percentages 9.29%, 13% and 18.11% respectively (Fig. 5).

2.3-External anomalies:

Fetuses maternally treated with clarithromycin in the period from the $15^{th}$to 19^{th} days of gestation (fetogenesis period) showed several hematoma on different parts of the body like hind-limb and, hemorrhagein the abdomen and back of fetuses, contraction and shortness in fore limb and their fingers (Brachydactyly), odema in the hind limb and bending in the tail as well as congestion in all the blood vessels of the body (Table 3 & Figs. 6&7).

2.4- Skeletal anomalies:

Fetuses of the treated group showed severe lack of ossification on the skull bone (frontal, parietal, inter-parietal and squamosal). Also, there are lack of ossification and shortness in the ulna and radius bones compared to the control group (Table 4, Figs. 8&9).

There is a great curvature in the vertebral column of some fetuses, a lack of ossification in the sacral, caudal vertebrae and metatarsal bones of the hind limb. Also, the tibia, fibula is shorter than that of the control group (Table 4, Figs. 8&9).

II-Histological examination:

Histological examination of the sections of liver of control pregnant rat and their fetuses showed normal histological structure of the central vein with the surrounding hepatocytes (Fig.10 A&B). However, examination of sections of the maternal livers treated with clarithromycin during the foetogenesis period of pregnancy showed dilatation and congestion of the central vein, with some degeneration of hepatocytes and vacuoles in surrounding sinusoids (Fig 10, $A_{1\&2}$).

Liver of fetuses maternally treated with clarithromycin showed severe congestion in hepatic portal vein., lymphocytic infiltration and with some degeneration and vacuoles in surrounding hepatocytes (Fig. 10, $B_{1\&2}$).

III-Molecular biology study:

The maternal and fetal genomic DNA of treated group showed marked fragmentation compared to the control group (Fig 11 A&B).

Discussion:

These results showed that clarithromycin administration caused several teratogenic effects as decrease in maternal body weight gain, placental weight and uterine weight during pregnancy as well as it increased the percentage of abortion and resorption comparing to the control group. The present results can be explained by that clarithromycin is a pregnancy category C drug and has the higher placental passage rate than other macrolide antibiotics**.** The mean transplacental transfer of clarithromycin was 6.1%[13], the obtained abortion may be attributed to the drug interference with fetal mitochondriogenesis[14] or to higher drug concentration in umbilical cord or neonatal serum than in maternal serum.[15] Besides that clarithromycin is practically partially soluble in water [16 , 17]and as the passage of pharmacologic agents across the placenta is influenced by solubility as lipid soluble agents readily cross the placenta,but water soluble compoundsare less readily transported. The results of our study contradict the prospective studies of **Drinkard *et al.* (2000)** [18] who stated that no increased risk of congenital malformations byclarithromycin in pregnancy**.** However, some studies stated a higher rate of spontaneous abortions than in the unexposed group.[18]

The pharmacokinetic profile of clarithromycin is similar to that of

erythromycin. Exposure to erythromycin in earlypregnancy has been associated with an increased risk of congenital heart defects**.** [17, 19]It is known that pregnancy, due to the mitochondria rich placenta, is a condition that favors oxidative stress [20]. In addition, pregnancy itself is a stressful condition in which many physiological and metabolic functions are altered to a considerable extent [21].Approximately 20–30% of pregnancies are complicated, either directly or indirectly, by placental pathologies associated with metabolic, oxidative or inflammatory stress. [22] **Sekhon *et al.*,(2010)**[23] demonstrated that oxidative stress influences multiple physiological processes, from oocyte maturation to fertilization, embryo development and pregnancy.Increase in reactive oxygen species (ROS) is also involved in defective embryo development and retardation of embryo growth, which is attributed to induced cell-membrane damage, DNA damage, and apoptosis.[24]

A number of pharmaceuticals inhibit ABC (ATP-binding cassette) activity including macrolide antibiotics (azithromycin, erythromycin and clarithromycin).[25] ABC transporters are involved in the protection of cells from the damaging effects of oxidative stress [26] and this may explain that in our study the oxidative stress increase and its bad effects also increase.

It is known that, the marked decrease in fetus body weight is an extremely sensitive indicator for fetal toxicity**.** [27]Our results revealed that clarithromycincauses growth retardation which is represented by the decrease in fetal body weight, body length and tail length.These may be explained due to the presence of clarithromycin in the fetal tissue as erythromycin is found in fetal tissues after maternal administration.[28]On the other hands, **Jang*et al.*(2001)** [29], reported that clarithromycin (category C drug) were insignificantly associated with increased risks for preterm birth or low birth weight, although, in monkeys, double oral doses of clarithromycin retarded fetal growth.

In the present study, the clarithromycin-treated ratsshowed lack of ossification in most components of skeleton including the skull, fore-limbs and hind-limbs, shortness of bones of limbs as well as missed ossification of vertebrae. The present results were in agreement with the previous study which reported that clarithromycin has high concentration in bone **[30]**. Also**, Arai *et al.* (2007)** [31] revealed that oxidative stress affected the mineralization of bone by removal of calcium and phosphorus from bone tissue also it had been reported that clarithromycin induces renal artersia and renal tubular degeneration decreases the Ca reabsorption in the nephron, thus resulting in hypercalciuria and low bone mineral density (BMD) and hence increased fracture risk [32]. These may explain the lack of ossification in the treated group.

Hepatoxicity, which had been appeared as pyknotic nuclei either maternal or fetal liver may agree with **Mirodzhev *et al.*, 2007** [33] who statedthat hepatotoxicity occurred in all species tested with clarithromycin (dog, rat, and monkey), but with high doses.

McEvoy, 1994 [32]**,** stated that**,**elevated liver function tests and hepatomegaly have been reported only in about one percent of patients who receive clarithromycin.On the other hand, Azithromycin and clarithromycin can be safely administered without regard to hepatic status.[33, 34]

CONCLUSION

Collectively, clarithromycin was developmentally toxic to pregnant rats and their fetuses as evidenced by increased abortion and resorption, intrauterine growth retardation of the fetuses, delayed ossification of bones with rib anomalies, hepatic histopathological changes in the pregnant rats and their fetuses and DNA fragmentation.

Care should be taken if clarithromycin was administrated during pregnancy.

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Table (1):- Effect of Clarithromycin on the body weight gain (BWG), uteri, placenta, fetus's weight and placental index:

Where treated group received clarithromycin (45mg/kg) during fetogenesis period of gestation

Parameters / Groups	No. of sacrificed rats	BWG	No. of abortion	No. of uteri without resorption	No. of uteri with partial resorption	Mean placental weigh (gm)	Mean fetal weight (gm)	Mean placental index
Control	6	65.33± 5.024	0	6	0	0.6155± 0.0364	3.963±. 0.05167	0.1548± 0.008697
Treated	7	40± 3.864*	1	7	1(12.5%)	0.4003± 0.0167*	3.595± 0.06263*	0.23085± 0.1226*

Table (2): Effect of Clarithromycin administration on Mortality rate of Fetuses Maternally Receiving Clarithromycin

Parameters / Groups	No. of sacrificed dams	No. of implantation sites (average/ mother)	No. of resorbed fetuses	No. of live fetuses	No. of dead fetuses	Total mortality rate
Control	6	45 (7.5)	0	45 (100%)	0	0
Treated	8	55 (6.8)	1	52 (94.5%)	2	5.5%

Where treated group received clarithromycin (45mg/kg) during late gestational period

Table (3):- Effects of Clarithromycin on external anomalies in the fetuses at 20th day of gestation:

Groups	No. of examined fetuses	hematoma	Anomalies of Limbs				Anomalies in tail
			Brachydactly	Paralysis	Contraction	Odema	
Control	45	0 (0%)	0	0	0	0	0
Treated	55	9 (16.36%)	1 (1.8%)	1 (1.8%)	8 (14.54%)	2 (3.6%)	2 (3.6%)

Table (4):- Effects of Clarithromycin on incidence of skeletal congenital malformation:

Axial Skeleton						
Group	No. of examined fetuses	skull	Vertebral column	Ribs		Sternum
				Shortness in rib No. 13	Missed Ossification	
Control	24	0	0	0	0	0
Treated	24	12	12	0	4	4

Appendicular skeleton					
Group	No. of examined fetuses	Pectoral Girdle	Fore limbs	Pelvic girdle	Hind limb
Control	24	0	0	0	0
Treated	24	8	8	8	8

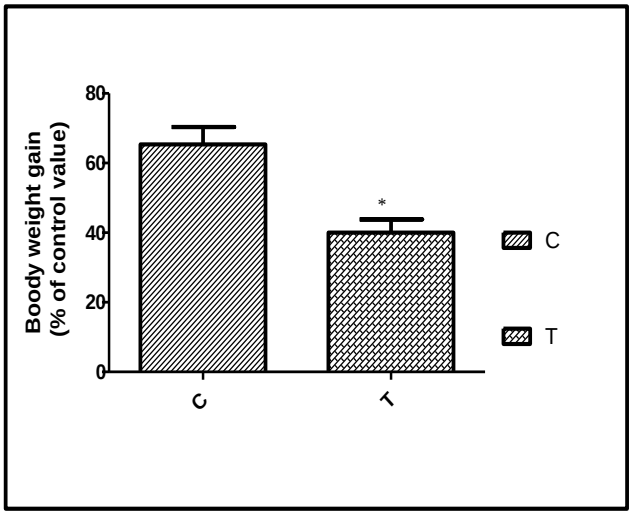

Figure (1): Effect of Clarithromycin on Body Weight Gain of Pregnant Rats: C; controlgroup. T; treated group

*significantly different from normal control group at **P** < 0.05.

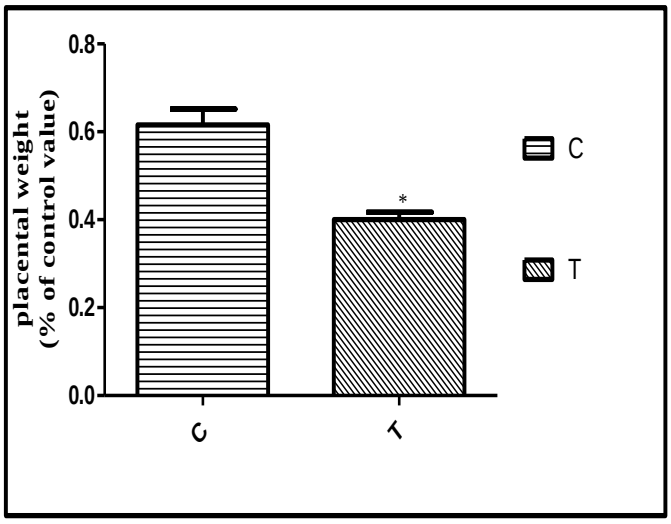

Figure (2): Effect of Clarithromycin on placental weight

*significantly different from normal control group at **P** < 0.05.

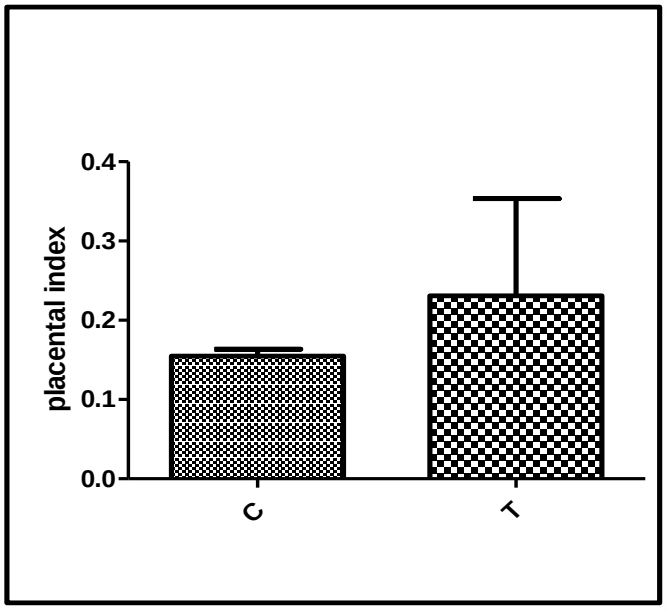

Figure (3): Effect of Clarithromycin on placental index

*significantly different from normal control group at P < 0.05.

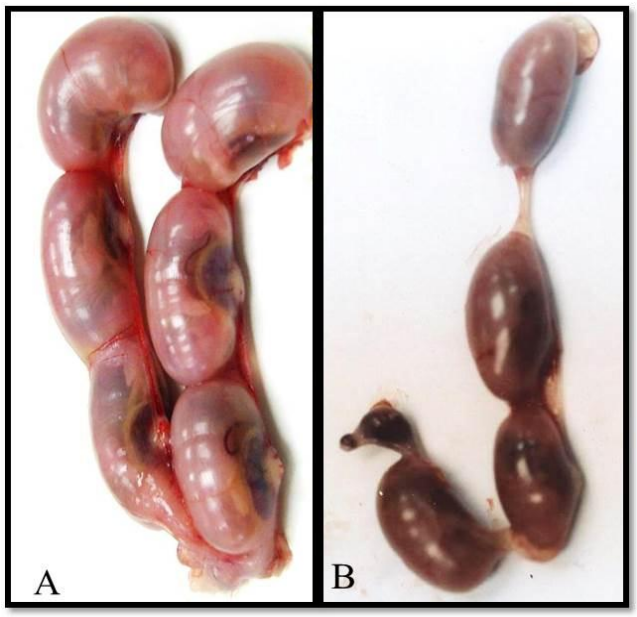

Figure (4): Photomacrographs of Uteri of Pregnant Rats on the 20th Day of Gestation receiving: (a) Dist. H2O (control) showing symmetrical distribution of fetuses on both horns, (b) Clarithromycin, showing a symmetrical distribution of fetuses on both horns and resorbed fetuses.

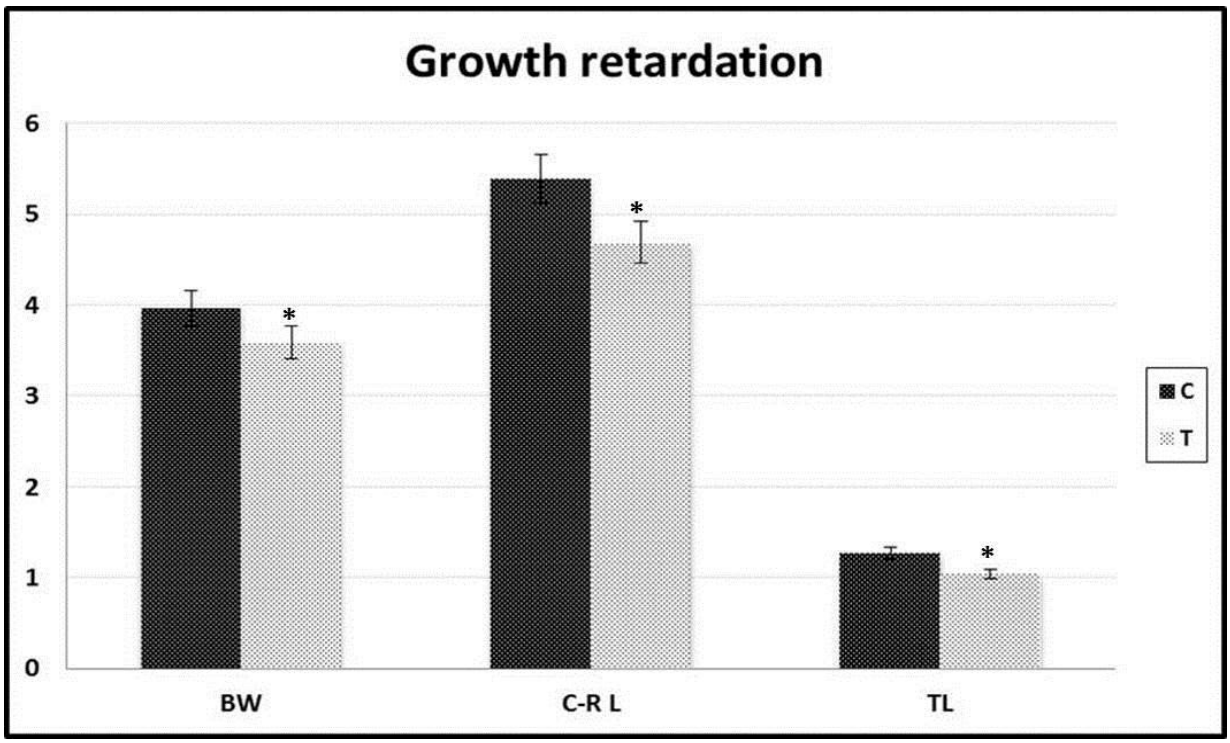

Figure (5): Effect of Clarithromycin on fetal body weight (BW), Crown-Rump length (C-RL) and tail length (TL) of the fetuses of the treated (T) and control (C) groups.

*significantly different from normal control group at $P < 0.05$.

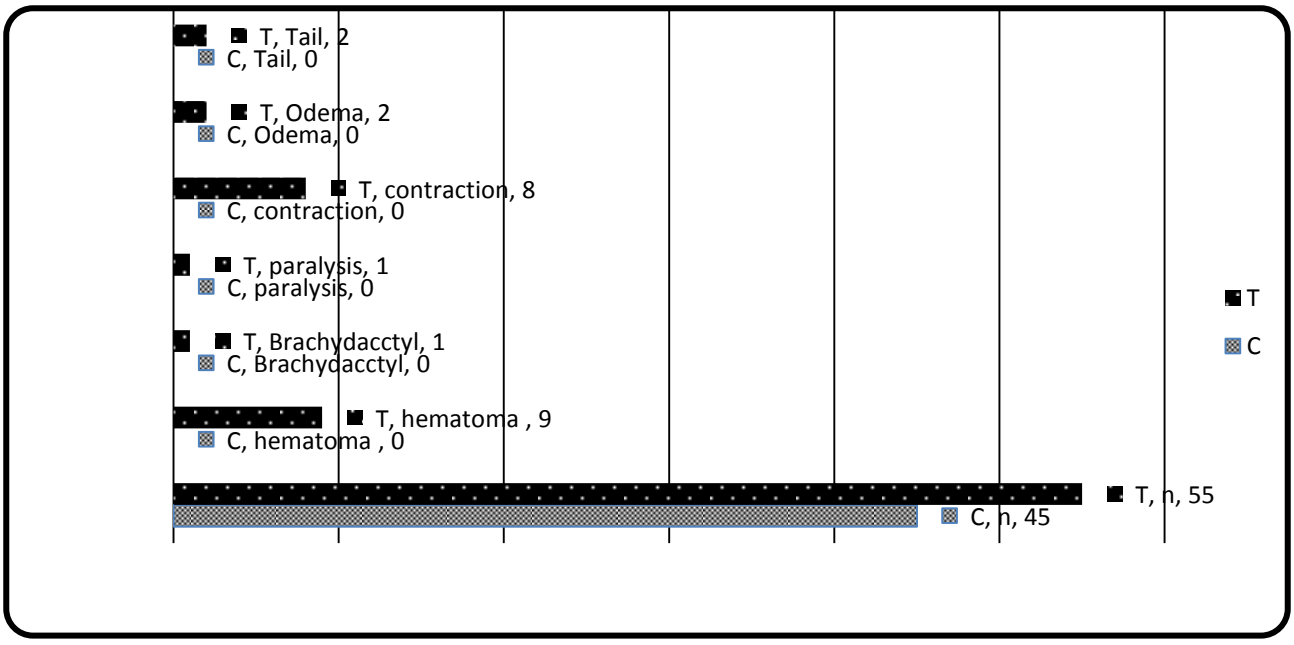

Figure (6): Histogram showing the incidence of external anomalies

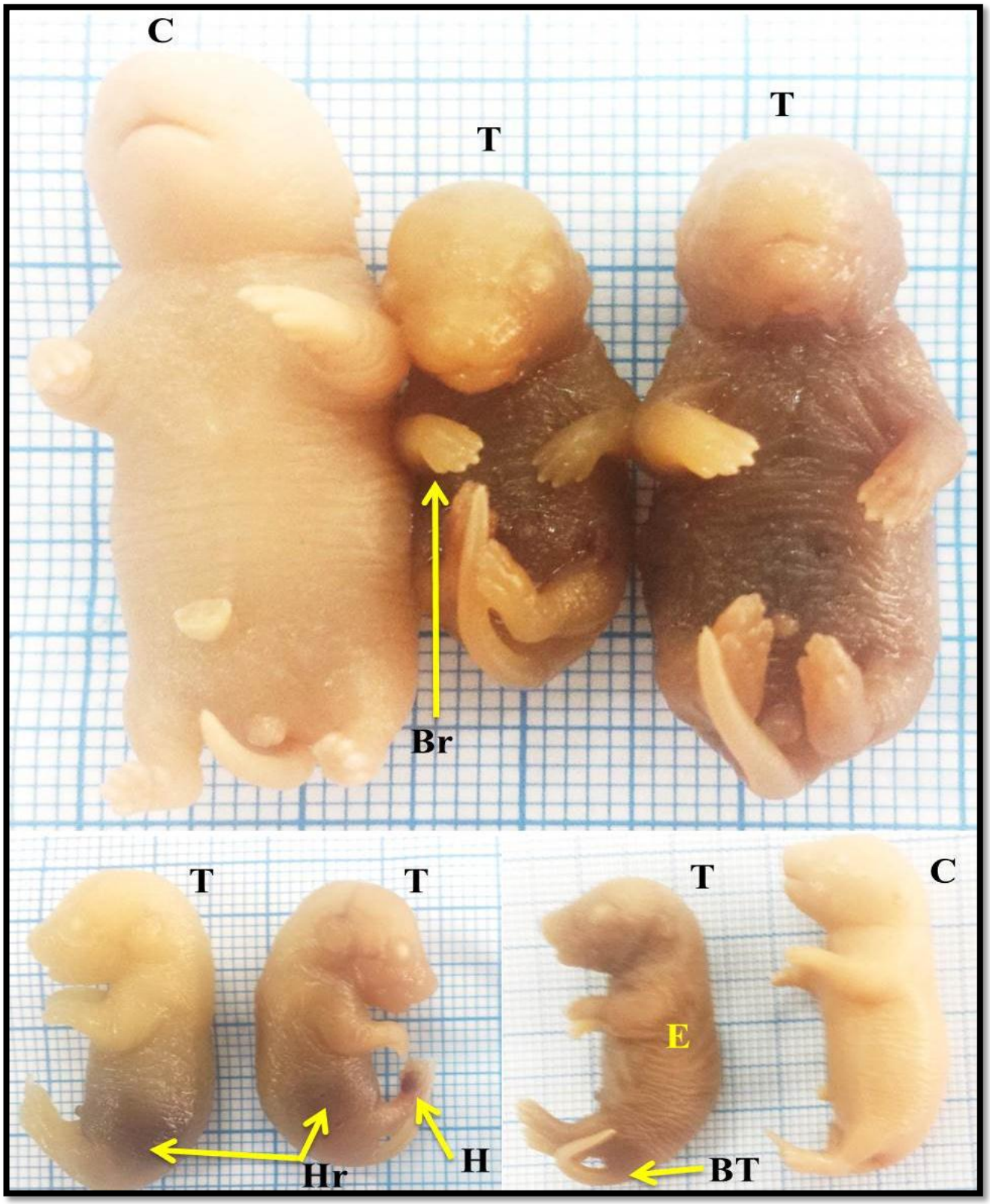

Figure (7): Photomacrograph of Fetuses at the 20thday of gestation maternally treated from 15thto 19th with Dist. H2O(C) as control, or with clarithromycin (T) showing Brachydactyly (Br) in the fore-limb, bent tail (BT), hemorrhage in the abdomen and back (Hr), haematoma in the hind limb (H)and edema in the fore-limb (E).

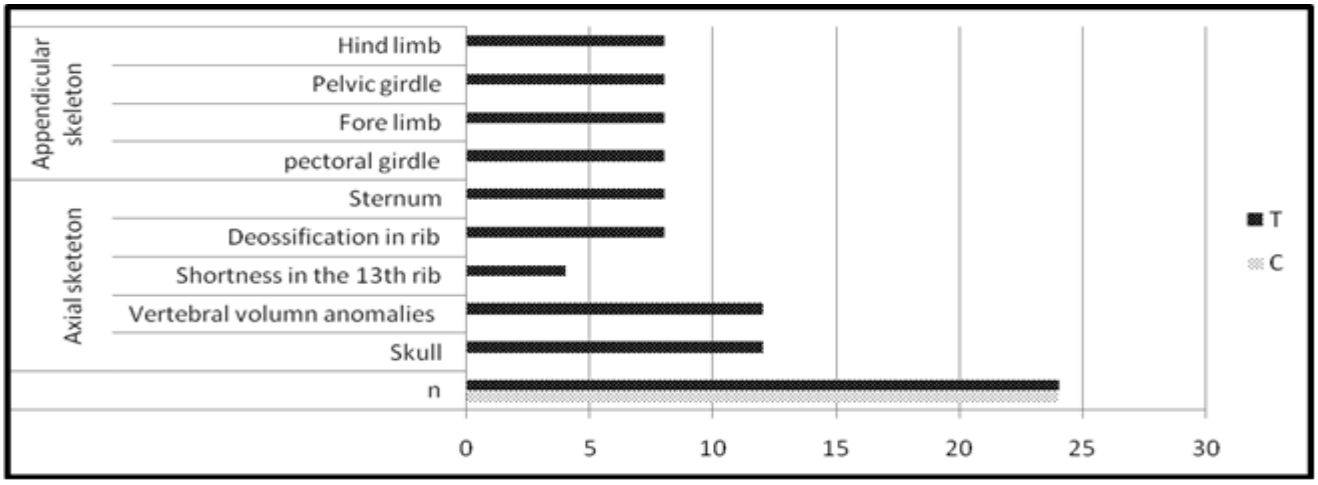

Figure (8): Histogram showing the incidence of congenital malformation of skeleton

Figure (9): Photomacrographs of skeleton of control fetus at the 20th day of gestation (C)and treated fetuses (T) showinglack of ossification of skull (**yellow arrow**), lack of ossification and short radius and ulna (**arrow head**) concave vertebral column (**black arrow**) and severe lack of ossification of sacral vertebrae and tail **(red arrows**).

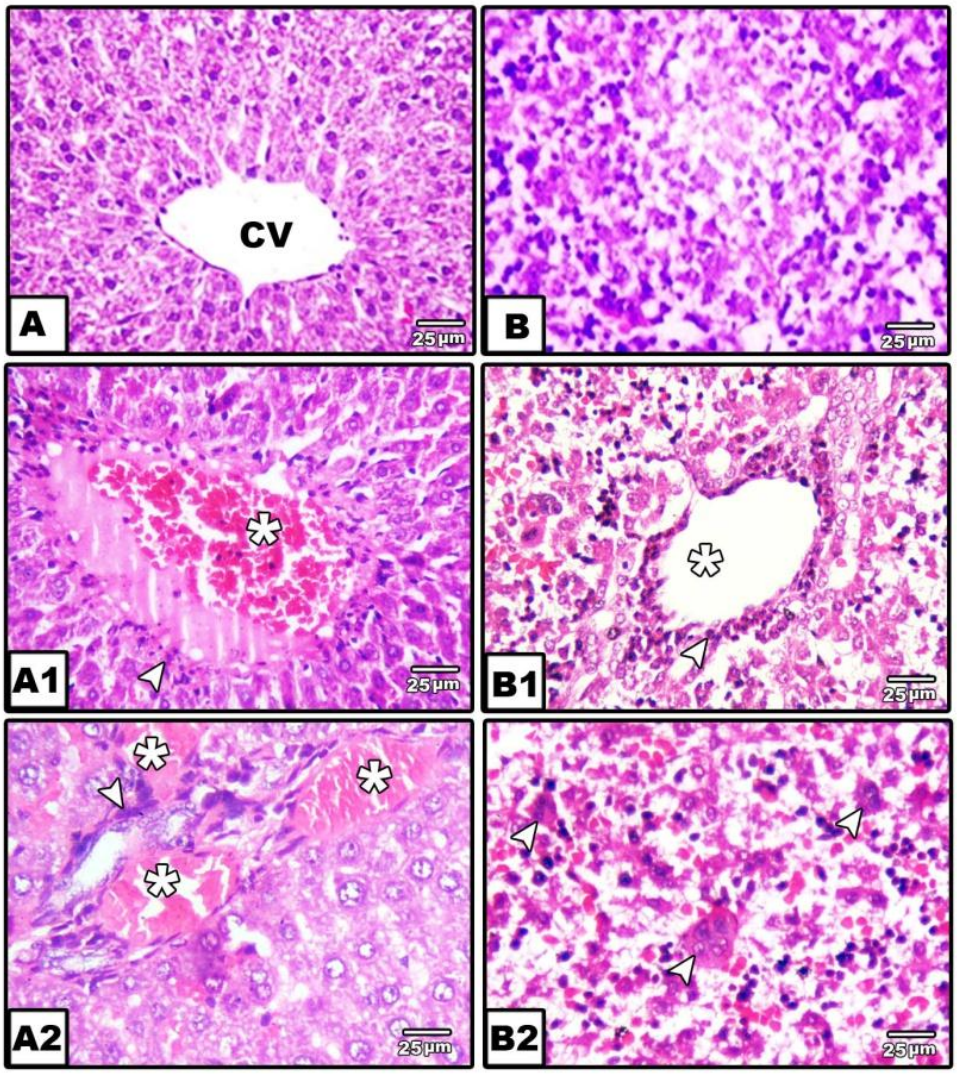

Figure (10): Photomicrograph of control maternal (A) and fetal (C)liver tissue section shows normal architecture.Treated pregnant dam liver has congested central vein (B). Treated fetal liver has congestion in the hepatic portal vein, lymphocetic infiltration and with some degeneration and vacuoles in surrounding hepatocytes (D).

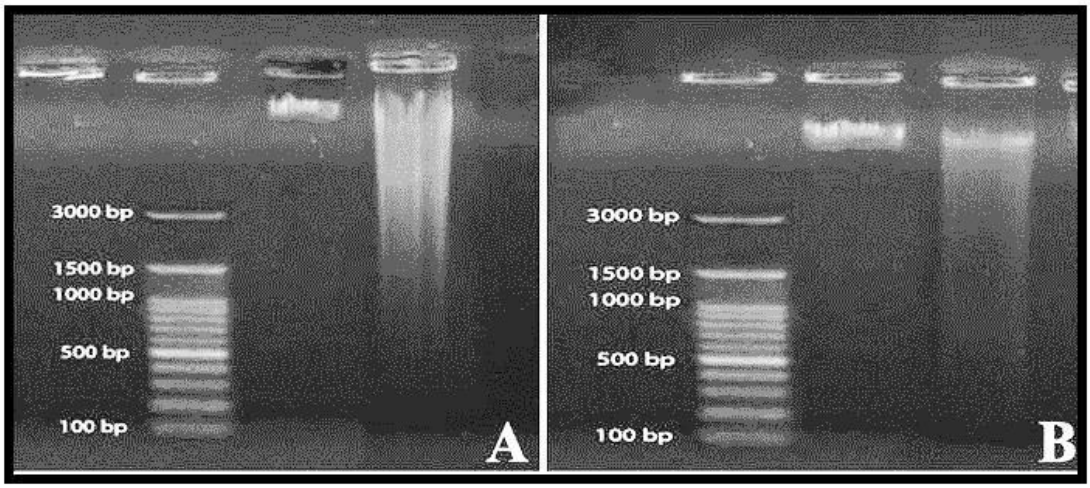

Figure (11): Agarose Gel Electrophoresis of Genomic maternal (A) and fetal (B) DNA at 20th day of gestation (C) control and (T) treated group.