

Histological Study and DNA Changes in the Kidneys of Rat Fetuses Maternally Treated with Clarithromycin

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ABSTRACT

Introduction: macrolide antibiotics are a class of potent and well established antimicrobials that also possess anti-inflammatory and/or immunomodulatory properties. Because of their size, lower levels of macrolides are able to reach the developing fetuses.

Materials and method: the pregnant rats were orally administered with clarithromycin at early and late gestational periods. The 20 day-old fetuses were dissected for excision of the kidney. Half of the kidney was processed and stained with H & E, PAS, Masson's trichrome and Feulgen techniques then followed by morphometric measurements and statistical study. The other half of the kidney was preserved for DNA fragmentation assay. **Results:** This study revealed that clarithromycin administration to pregnant rats showed different histopathological, histochemical and DNA changes in the kidneys of their fetuses.

Conclusion: Administration of the antimicrobial agent; clarithromycin at early and late gestational periods exhibits nephrotoxicity in the developing fetuses.

Key words: Clarithromycin, Antimicrobial drug, Rat fetuses, Kidney.

INTRODUCTION

The correct choice of an antimicrobial agent to treat urinary tract infections during pregnancy is complex because it requires full attention to maternal and fetal safety^[1].

Antibiotic" earlier referred to a compound or a substance produced by a microorganism (i.e., a bacterial or a fungal metabolite), which inhibits the growth of other microorganisms. Today this designation relates to any compound, natural, semisynthetic or synthetic which exhibits such inhibitory effect^[2].

Antibiotics can be classified based on the cellular component or system they affect, in addition to whether they induce cell death (bactericidal drugs) or merely inhibit cell growth (bacteriostatic drugs)^[3] Clarithromycin is a semisynthetic macrolide antibiotic which exhibits broad-spectrum activity against gram-positive and gram-negative aerobes. It is known to have better oral bioavailability and tissue penetration^[4]. Clarithromycin acts by binding to the peptidyl transferase region of 23S rRNA and inhibits bacterial protein synthesis^[5].

Macrolides are bacteriostatic antibiotics that inhibit protein biosynthesis via reversible binding to the bacterial 50S ribosomal subunit. Because of their size, lower levels of macrolides are able to reach the fetus^[6]. The antibacterial spectrum of macrolides includes

predominantly gram-positive *cocci*, but also *chlamydia*, *mycoplasma* and *legionella*, *campylobacter* as well as *coxiella*, *bartonella*, *corynebacteria* and several *mycobacterium* species. Macrolides are suitable alternatives for patients who are allergic to penicillin^[7].

MATERIALS AND METHODS:

1- Drug and dosage:

Clarithromycin is an antibacterial agent, C₃₈H₆₉NO₁₃, provided by Kahira Pharmaceuticals and Chemical, Egypt, in a commercial product called Klacid XL as 14 film coated tablets. Each tablet contains 500 mg of clarithromycin. The drug was orally given once daily to pregnant rats at different periods of gestation by gastric tube at a dose of 45 mg/kg/day. The dose was equivalent to therapeutic dose of human (500 mg/day) and calculated according to interspecies dosage conversion scheme of *Paget and Barnes*^[8]. The tablet was crushed to powder and was dissolved in distilled water. The given volume was adjusted so that each 100g of the animal body weight received 1ml solution containing the required dose.

2- Experimental animals:

The present experimental study was carried out on the adult female and male

albino rats (*Rattus norvegicus*); weighing 140–200 grams and they were obtained from the National Organization for Drug Control and Research (NODCAR, Cairo, Egypt). Male and female rats were housed separately in metal cages with wire-grid floors. The animals were kept at standard housing facilities (25±2 °C, 45 ± 5% humidity and 12 hrs. light/dark cycles). The animals were fed on a standard laboratory chow and water *ad libitum*. The standard guidelines of National Organization for Drug Control and Research (NODCAR) were used in handling animals.

3- Housing and mating:

Adult female rats in proestrus cycle were caged overnight with males of proven fertility. The day on which the sperm were found in the vagina was designated as zero day of gestation (GD) (Fig.1). A daily record of the weight of the pregnant females was made. The abortion was determined by the presence of blood drops and sudden drop in weight of the pregnant females^[9].

4- Experimental design:

The pregnant females were randomly divided into four equal groups (n= 6, each) as follow:

Group C1f : The pregnant rats were orally received distilled water from the 1st to the 7th day of gestation.

Group T1f : The pregnant rats were orally administered with clarithromycin at a dose of 45 mg/kg/day from the 1st to the 7th day of gestation.

Group C2f : The pregnant rats were orally received distilled water from the 15th to the 19th day of gestation.

Group T2f : The pregnant rats were orally administered with clarithromycin (45 mg/kg/day) from the 15th to the 19th day of gestation.

5- Tissues sampling:

At 20th day of gestation, the pregnant females were sacrificed by decapitation, the peritoneal cavity was opened and fetuses were separated. Kidneys of the fetuses were rapidly excised then, half of them stored at -80°C for isolation the genomic DNA and the other half fixed immediately for the histopathological, histochemical and histomorphometrical investigations.

For histological and histochemical examination:

The excised organ was fixed in 10% neutral buffered formalin solution for about 24

hours, washed in running water, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and impregnated in parablax for blocking, serial sections of 5 µm thickness were prepared and stained with: hematoxylin and eosin, periodic acid schiff's, Masson's trichrome and Feulgen reaction.

DNA fragmentation assay:

DNA fragmentation determined via agarose gel electrophoresis; genomic DNA, was isolated from the fetuses' kidney tissue according to Miller *et al.*^[10] using DNA Purification Kite Promega, Promega Corporation, USA. Agarose gel electrophoresis of DNA was done according to the method of Sealey and Southern^[11].

Morphometric measurements:

Histomorphometric measurements were performed using Leica Qwin 500 Image Analyzer (LEICA Imaging systems Ltd, Cambridge, London) in Pathology Department, National Research Centre, Cairo. The measurements included the glomerular diameter in H&E stained sections and nuclear area of the convoluted tubular cells in Feulgen stained sections, the values were measured in fifty randomly parameters per group at magnification X 200 and X 800, respectively. The mean grey of carbohydrate content and DNA content and the mean area percent of collagen content slides were measured within 10 non-overlapping fields/ section for each animal, at X400 magnification.

Statistical analysis:

The morphometric results were expressed as mean ± standard errors (SE). Statistical analysis was carried out using the “prism version 5” statistical software. Comparison between different groups was done using one way analysis of variance (ANOVA) followed by Tukey test^[12]. The results were considered statistically significant when value was $P < 0.05$.

RESULTS

The kidney of 20-day old fetuses:

I. Histopathological investigations:

I.1. H&E stain:

a- Control fetuses:

Normal renal architecture of kidney cortex of control fetuses showed: renal corpuscles made up of glomeruli, Bowman's capsules and urinary spaces, proximal and distal convoluted tubules (Fig. 2).

b-Treated fetuses:

Sections in the kidneys of 20-day old fetuses maternally treated with clarithromycin (group T1_f) showed some histopathological changes included enlarged glomeruli, some necrotic tubules and other tubules with some pyknotic nuclei, some cytoplasmic vacuolations was also seen (Fig. 3). Some convoluted tubules showed cloudy swelling of their lining cells with complete obstruction of their lumen (Fig. 4). Congested blood capillaries of the glomerular tufts and hemorrhage in the interstitial space were shown (Fig. 5).

Sections in the kidneys of 20-day old fetuses maternally treated with clarithromycin (group T2_f) showed enlarged glomeruli and degenerative changes in the epithelial cells lining the renal tubules with cloudy swelling, vacuolations, pyknosis, karyolysis and cells detachment (Fig. 6). In addition, hemorrhagic areas in the interstitial space and congestion of blood capillaries of the glomerular tufts were reported in the kidney of clarithromycin-treated fetuses (Fig. 7).

I.2. Masson's trichrome stain:

a. Control fetuses:

Examination of sections in the renal cortex of 20 day-old fetuses yielded from control rats stained with Masson's trichrome showed scanty collagen fibers in the capsular wall, peritubular and intraglomerular capillaries (Fig. 8).

b. Treated fetuses:

Sections in the kidneys of 20-day old fetuses obtained from clarithromycin-treated dams (group T1_f) showed moderate increase in the collagenous fibers in glomerular capillaries, around the capsular wall and in the interstitial tissues (Fig. 9).

Masson's trichrome-stained sections in the kidneys of 20-day old fetuses obtained from clarithromycin-treated dams (group T2_f) showed severe increment in the collagenous fibers in the tubulointerstitial tissues (Fig. 10).

II. Histochemical results:

II.1. Carbohydrate Content:

a. Control fetuses:

Examination of sections in the renal cortex of 20 day-old fetuses yielded from the control group and stained with PAS reaction shows a positive PAS reaction in the brush borders of the proximal convoluted tubules, Bowman's capsule and the basement membranes of the glomerular capillaries.

However, the tubular basement membranes as well as the nuclei show PAS negative reaction (Fig. 11).

b. Treated fetuses:

Section of the kidney of 20-day old fetuses maternally treated with clarithromycin (group T1_f) showed a weak PAS reaction in the capsular wall. Destructed brush borders of the proximal convoluted tubules were shown (Fig. 12).

Section of kidney of 20-day old treated fetuses (group T2_f) displayed weak PAS reactivity of the brush borders of the tubular cells (Fig. 13).

II.2. DNA Content:

a. Control fetuses:

The sections of kidneys of 20-day old fetuses yielded from control group (Fig. 14) showed normal distribution of DNA molecules. Most of the nuclei are in the same size and strongly stained magenta by using Feulgen reaction. The cytoplasm of these cells showed a negative staining reaction.

b. Treated fetuses:

DNA of sections of the kidneys of 20-day old fetuses maternally treated with clarithromycin (group T1_f) showed a weak stain reaction in the karyolytic nuclei of tubular cells. In addition, numerous pyknotic nuclei were reported (Fig. 15).

The sections of kidneys of 20-day old treated fetuses (group T2_f) showed a marked decrease in DNA content in the karyolytic nuclei. Moreover, numerous pyknotic nuclei were densely stained (Fig. 16).

III. Histomorphometric analyses:

The renal sections of 20-day old fetuses of different groups stained with Masson's trichrome were subjected to image analysis for determination of mean area percent of collagen content. Moreover, renal sections stained with PAS and Feulgen were subjected to image analysis for determination of mean grey of carbohydrate content and DNA content, respectively. All data were represented in Table 1 and Figures 17, 18 and 19.

Sections of the kidneys of 20-day old fetuses maternally treated with clarithromycin (group T1_f) showed a significant increase at $P < 0.05$ in mean area % of collagen contents and the mean grey of carbohydrate content and DNA content as compared to control group (group C1_f) (87.86, 13.78 and 4.3, respectively).

A significant increase $P < 0.05$ was shown in the mean area % of collagen contents and the mean grey of carbohydrate content and DNA content in the kidneys of 20-day old treated fetuses (group T2_f) comparing to its control (group C2_f) (121.3, 14.43 and 6.22, respectively).

The kidney sections of 20-day old fetuses of different groups were subjected to histomorphometric measurements of the glomerular diameter and the convoluted tubules nuclear area. These data were represented in Table 2 and Figures 20 and 21.

Sections of the kidneys of 20-day old treated fetuses (group T1_f) showed a non-significant change of glomerular diameter and significant decrease at $P < 0.05$ in mean values of nuclear area of CT, as compared to control group (group C1_f) and were recorded as -2.4 and -18.2 as a percentage change respectively.

A non-significant change in the mean values of glomerular diameter (1.5) and a significant decrease in the mean values of the nuclear area of CT -18.6 were reported in the kidneys of 20-day old fetuses maternally treated with clarithromycin (group T2f) as compared to the control group (group C2_f) at $P < 0.05$.

IV. **Molecular investigations:**

IV.1. Determination of DNA purity:

The total genomic DNA isolated from kidneys of 20-day old fetuses was evaluated for purity and the results were summarized in Table (3) and they showed the absence of contamination in all samples.

IV.2- Agarose gel electrophoresis of DNA:

As shown in Figure (22), the renal cells of control 20-day old fetuses showed that, no smear (lane 2) was observed and the fragmentation of the DNA remained negligible while, DNA of the treated groups showed some degree of DNA damage of smear pattern.

The kidneys of 20-day old fetuses maternally treated with clarithromycin (group T1_f) showed a moderate DNA fragmentation (lane 3). Moreover, the kidneys of the other treated group (T2_f) showed a strong DNA damage of smear pattern (lane 4) compared to the control group.

DISCUSSION

The kidney plays an important role in the elimination of numerous hydrophilic

xenobiotics, including drugs, toxins, and endogenous compounds [13].

Rat is commonly used as a model in studies on embryology and reproduction toxicology [14]. In the present study, the histopathological examination showed that the oral administration of the therapeutic dose of clarithromycin extended release tablets once daily in the different periods during pregnancy in rats induced a nephrotoxicity in the developing fetuses. This is in agreement with the results of *Chapelsky et al.* [15] who found that usual doses of clarithromycin (500 mg orally every 12h for 13 doses) had significant potential for causing nephrotoxicity and also, *Guay et al.* [16] reported that clarithromycin had low potential for ototoxicity, hepatotoxicity and nephrotoxicity in studies involving rats, dogs and primates.

The nephrotoxicity of clarithromycin might be happened due to the induction of the oxidative stress in the renal tissue. *Olayinka and Ore* [15][17] found that oral administration of clarithromycin has adverse effects on both enzymic and non-enzymic antioxidant status and induced oxidative stress, the macrolides were extensively distributed in tissues and obtain higher concentrations than erythromycin in the bronchus, tonsils, gastrointestinal tract, liver, kidney, spleen, and bone [18], and also 30 to 40% of an oral dose of clarithromycin was excreted in the urine via glomerular filtration of the kidney [19]. Also the sensitivity for toxic compounds is increased during pregnancy [20]. Pregnancy significantly alters the expression and activity of drug metabolizing enzymes and these changes are likely to have toxicological and therapeutic implications [21].

Moreover, the macrolides accumulate in the lysosomes of eucaryotic cells [22], [23], causing metabolic alterations that can lead to cell toxicity [24].

Clarithromycin is insoluble in water [25] 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 compounds are less readily transported. Drug exposure is responsible for 2% of birth defects and is a potentially avoidable risk [26].

Clarithromycin, a pregnancy category C drug, has a higher placental passage rate than other macrolide antibiotics and the mean trans-placental transfer of clarithromycin was

6.1 %^[27], thus causing nephrotoxicity to the fetuses. Furthermore, during kidney development, a large-scale proliferation has been observed^[28]. So, the renal development is influenced by any insult disturbing the fine balance in the interactions that form the kidney^[29].

Oxidative stress is a common pathogenetic mechanism contributing to initiation and progression of cell damage^[30]. The oxidative stress refers to a cell's state characterized by excessive production of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) and/or a reduction in antioxidant defenses responsible for their metabolism^{[31], [32]}.

Damage induced by ROS includes alterations of cellular macromolecules such as membrane lipid, DNA, and/or protein. The damage may alter cell function through changes in intracellular calcium or intracellular pH, and eventually can lead to cell death^{[33], [34]}.

Premature neonates have an immature antioxidant defense system and therefore are highly susceptible to the deleterious effects of reactive oxygen species (ROS)^[35]. *Tsunoda et al.*^[36] demonstrated that oxidative stress influences multiple physiological processes, from oocyte maturation to fertilization and embryo development. Increase in ROS is also involved in defective embryo development and retardation of embryo growth^[37], which is attributed to induced cell-membrane damage and DNA damage^[38].

Most drugs found to cause nephrotoxicity and exert toxic effects by one or more common pathogenic mechanisms. These include altered intraglomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy^[39]. The histopathological examination of kidney of 20-day old fetuses maternally treated with 45 mg/kg of clarithromycin in present study showed that clarithromycin induced nephrotoxicity as manifested by the appearance of enlarged or shrunken glomeruli with a wide urinary space and some necrotic tubules with high vacuolations, cells detachment, pyknotic and karyolytic nuclei. Some convoluted tubules showed cloudy swelling of their lining cells with complete obstruction of their lumen. Congested blood capillaries of the glomerular

tufts and hemorrhage in the interstitial space were shown also. Similar results were introduced by *Lameire and Vanholder*^[40] who reported that the histopathological examination of kidneys suffering from toxicological insult, the major changes appear to be localized in the renal tubules, including the degeneration or necrosis of tubular cells, swelling of the epithelium and the detachment of tubular epithelial cells from the basement membrane. Renal tubular cells, in particular proximal tubule cells, are vulnerable to the toxic effects of drugs because their role in concentrating and reabsorbing glomerular filtrate exposes them to high levels of circulating toxins^[41]. Drugs that cause tubular cell toxicity do so by impairing mitochondrial function, interfering with tubular transport, increasing oxidative stress, or forming free radicals^[42].

A significant increase in the mean value of glomerular diameter of the kidneys of the clarithromycin-treated fetuses at $P < 0.05$ in comparison with the control group was showed in the present study. Also, clarithromycin treatment induced inflammatory infiltration in the interstitium and this result does not agree with the result of *Özdemir et al.*^[43] who stated that clarithromycin had a protective effect on bowel injury owing to anti-inflammatory effects. Tubulointerstitial inflammation may result from leakage of filtered urine to the interstitial space through capsular adhesions^[44]. The works of *Kriz and LeHir*^[45] have exquisitely shown the formation of bridges between podocytes and the Bowman's capsule in sites of capsular adhesions and a remaining narrow urinary space allows leakage of ultrafiltrate to tubulointerstitial areas. According to *Cotran et al.*^[46] inflammation is fundamentally a protective response whose ultimate goal is to rid the organism of both the initial cause of cell injury and the consequences of such injury, the necrotic cells and tissues. Inflammation serves destroy, dilute, or wall off the injury agent.

In this study, the mean values of lumen area of the convoluted tubules of the kidneys of the clarithromycin-treated fetuses showed significant decrease ($P < 0.05$) in comparison with the control group. This result appeared in H&E stain as cloudy swelling of their lining cells with small or complete obstruction of their lumen. The morphology of necrosis is

characterized by swelling and degeneration of the entire cytoplasm^[47]. *de Wardener*^[48] described the cloudy swelling mechanism and stated that many disease processes may directly inhibit the sodium pump of the cell. Sodium then accumulates within the cell and subsequently the water is drawn into the cell causing this cloudy swelling^[49].

Vacuolations and deeply eosinophilic cells in the epithelium lining the renal tubules were seen in this work. Vacuolation and deeply eosinophilic cells, representing necrotic cells, in the epithelium lining the proximal renal tubules are classic features of acute tubular necrosis^{[50], [51]}. Necrosis may occur as a direct adverse effect of a metabolite or xenobiotic on the tubules^[52]. Necrotic cell death begins with swelling of the cell and mitochondrial contents, followed by rupture of the cell membrane^[53]. Necrosis can trigger an inflammatory reaction in the surrounding tissue as a result of the release of cytoplasmic contents, many of which are proteolytic enzymes^[54].

Vacuolation is recognized as clear, round spaces of variable size within the cytoplasm. Clear vacuolation of variable dimensions suggests hydropic change. Smaller, more uniform translucent vacuoles suggest fat or lipoprotein accumulation^[55].

Hemorrhage in the interstitium was shown in the present study in the kidneys of clarithromycin-treated fetuses. This result agrees with those of *Olayinka and Ore*^[56] who reported that foci of haemorrhages in the renal cortex were observed after administration of 17.6 mg kg⁻¹ clarithromycin. Hemorrhage often accompanies acute injury and can occur in the kidney as a primary lesion associated with nephrotoxicants. Hemorrhage can occur from inflammation, tubular necrosis, and vascular injury or from the presence of calculi or tumors^[55].

Using Masson's trichrome stain, sections of the kidneys of the treated fetuses showed fibrosis in glomeruli accompanied by areas of tubulointerstitial fibrosis and around the capsular wall. So, there is a significant increase at $P < 0.05$ in the mean area % of collagen contents in the kidneys of the treated fetuses as compared to the control group.

Tubulointerstitial fibrosis is a common feature of progressive renal injury in almost all forms of renal diseases^[57]. It has been shown that tubulointerstitial injury is a more

consistent predictor of functional impairment than glomerular damage^[58].

The cortical interstitium is normally composed of a network of fibroblasts and dendritic cells with only a small number of lymphocytes or macrophages^[59]. In chronic interstitial fibrosis, the interstitial cells have myofibroblast characteristics which are considered to be derived from the proliferation and differentiation of the residual fibroblasts^[60].

In the present study clarithromycin treatment induced glomerular degenerative changes. Once hemodynamic or degenerative changes in the glomerulus are initiated, a complex sequence of events within the mesangium and podocytes are initiated, mediated by TGF-beta and CTGF, which result in the stimulation of fibroblast proliferation and collagen formation with the eventual replacement of normal architecture^{[45], [61]}. Oxygen and nitrogen free radicals are highly reactive and are capable of damaging macromolecules like carbohydrates^[62]. So, there was a weak PAS reaction in the Bowman's capsular wall. Also, there were destruction and thinning of brush border of the proximal tubules. *Lameire and Vanholder*^[40] had reported that loss of the brush border was appeared in kidneys suffering from toxicological insult. A significant increase of the mean grey of carbohydrate content at $P < 0.05$ was shown in renal sections of dams treated with 45 mg/kg clarithromycin orally as compared to control which confirmed the decrement of polysaccharides content.

In this study, necrosis was assessed in the kidneys of the treated fetuses with the appearance of pyknosis or karyolysis. These results were confirmed with measuring the mean values of nuclear area of the convoluted tubules that showed a significant decrease ($P < 0.05$) in comparison with the control group. Also, the karyolysis was indicated by the significant increase at $P < 0.05$ in the mean grey of DNA content as compared to control group. The genomic DNA from renal cells of 20 day-old fetuses maternally treated with 45 mg/kg of clarithromycin showed a degree of DNA damage of smear pattern which indicates to the presence of necrosis. The degradation of DNA due to the increased ROS^[63] and RNS (e.g. Peroxynitrite) which interact with cellular macromolecules such as DNA and causes chemical cleavage of DNA^[64].

Pyknosis, or shrunken nuclei, is the irreversible condensation of chromatin in the nucleus of a cell undergoing necrosis^[65],^[66] or apoptosis^[67]. It is followed by karyorrhexis which is the destructive fragmentation of the nucleus of a dying cell^[68] involving loss of integrity of the nucleus. Karyolysis is the complete dissolution of the chromatin of a dying cell due to the enzymatic degradation by endonucleases. The whole cell will eventually stain uniformly with eosin after karyolysis in which a Feulgen reaction was faint, ghost-like image of the nuclei remained^[66]. It is usually preceded by karyorrhexis and occurs mainly as a result of necrosis, while in apoptosis after karyorrhexis the nucleus usually dissolves into apoptotic bodies^[69],^[70]. Finally, the above-stated findings lead to the conclusion that the presence of the histopathological changes revealed nephrotoxicity in the fetuses maternally treated with clarithromycin. So, clarithromycin is not safe for pregnant mothers and their fetuses and it should not be used except under strict conditions in medication.

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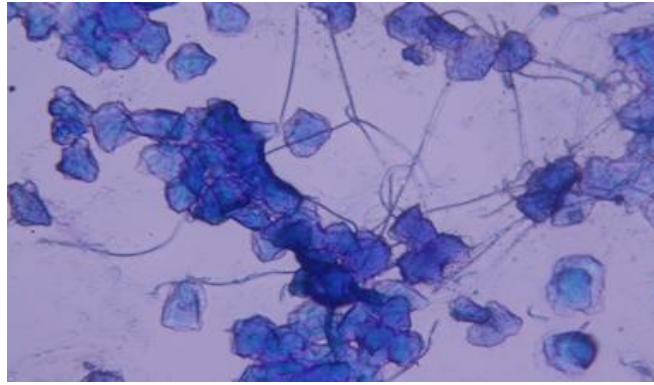


Fig. 1: Estrus stage of vaginal smear of rat with spermatozoa.

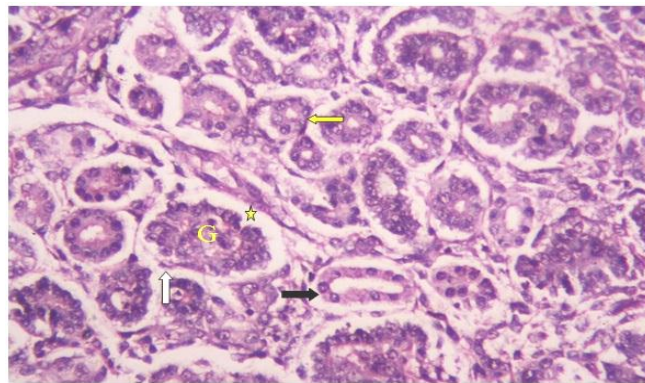


Fig. 2: Photomicrograph of a section in the cortex of kidney of 20 day-old fetus yielded from a control dam (C1f) showing spherical renal corpuscles with evident Bowman's spaces (asterisk), capillary tufts "glomerulus" (G) and Bowman's capsule (white arrow). Normal histological structures of proximal (yellow arrow) and distal (black arrow) convoluted tubules are recorded.(H&E, $\times 400$).

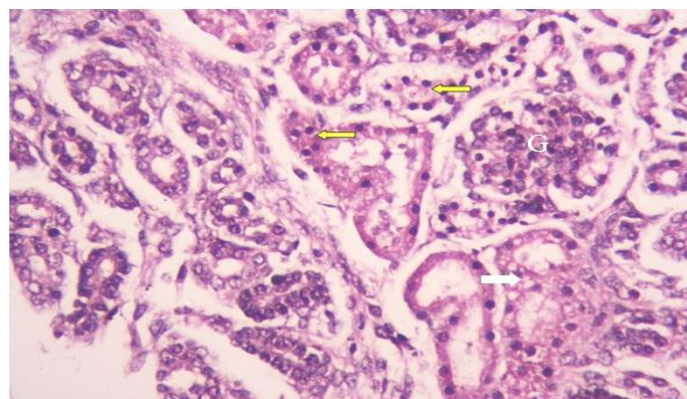


Fig. 3: Photomicrograph of a section in the cortex of kidney of 20 day-old fetus yielded from a clarithromycin-treated dam (T1f) showing enlarged glomerulus (G) and some necrotic tubules with high vacuolations (**white arrow**) and pyknotic nuclei (**yellow arrow**). (H&E, $\times 400$).

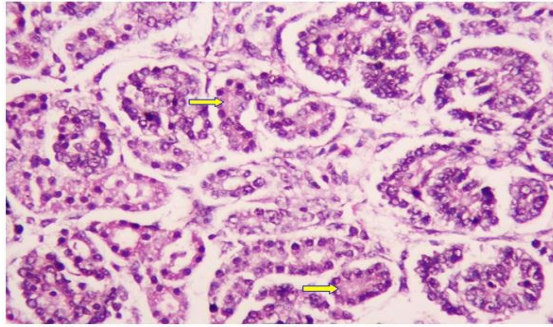


Fig. 4: Photomicrograph of a section in the kidney of 20 day-old fetus yielded from a clarithromycin-treated dam (T1f) showing cloudy swelling of convoluted tubule cells with complete obstruction of their lumen in some tubules (arrow). (H&E, $\times 400$)

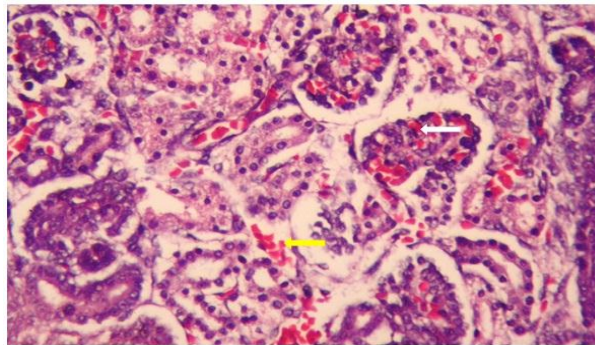


Fig. 5: Photomicrograph of a section in the kidney of 20 day-old fetus yielded from a clarithromycin-treated dam (T1f) showing congested blood capillaries of the glomerular tufts (white arrow) and hemorrhage in the interstitial space (yellow arrow). (H&E $\times 400$).

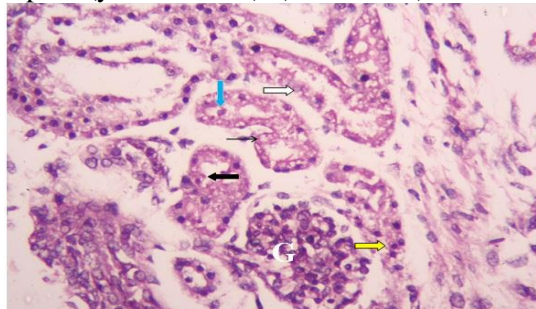


Fig. 6: Photomicrograph of a section in the kidney of 20 day-old fetus yielded from a clarithromycin-treated dam (T2f) showing enlarged glomerulus (G) and degenerative changes in the epithelial cells lining the renal tubules with cloudy swelling (black thick arrow), vacuolations (black thin arrow), pyknosis (yellow arrow), karyolysis (white arrow) and cell detachment (blue arrow). (H&E, $\times 400$).

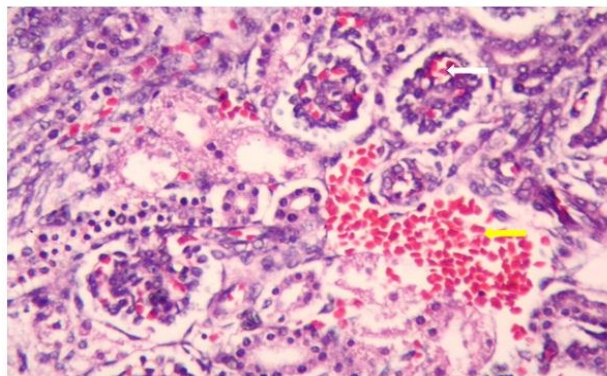


Fig. 7: Photomicrograph of a section in the kidney of 20 day-old fetus yielded from a clarithromycin-treated dam (T2f) showing congested blood capillaries of the glomerular tufts (white arrow) and hemorrhage in the interstitial space (yellow arrow). (H&E, $\times 400$).

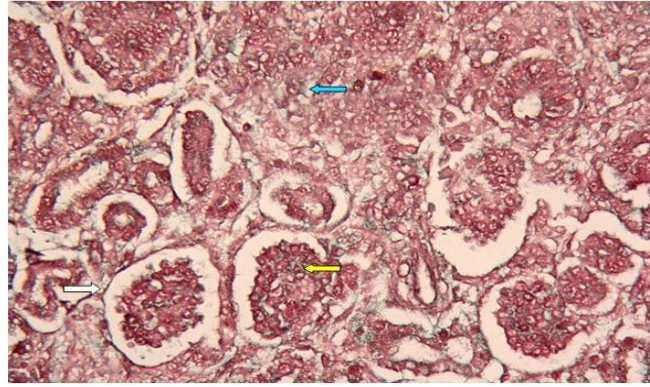


Fig. 8: Photomicrograph of a section in the cortex of the kidney of 20 day-old fetus yielded from a control dam (C1f) showing a scanty collagen fibers around capsular wall (white arrow), intraglomerular capillaries (yellow arrow) and in the interstitial tissues (blue arrow). (MT, $\times 400$)

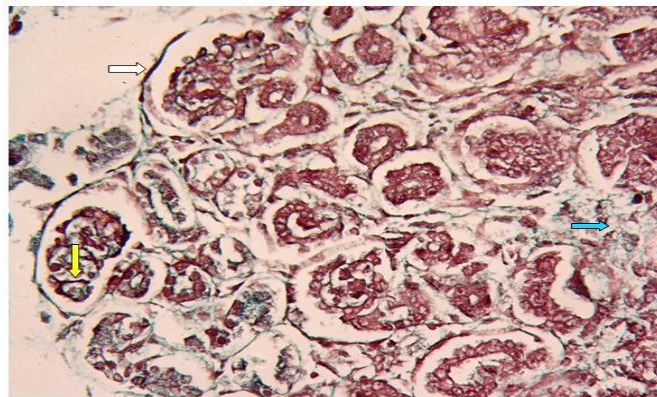


Fig. 9: Photomicrograph of a section in the kidney of 20 day-old fetus yielded from a clarithromycin-treated dam (T1f) showing a moderate increase of the collagen fibers distributed in glomerular capillaries (yellow arrow), around the capsular wall (white arrow) and in the interstitial tissues (blue arrow). (MT, $\times 400$)

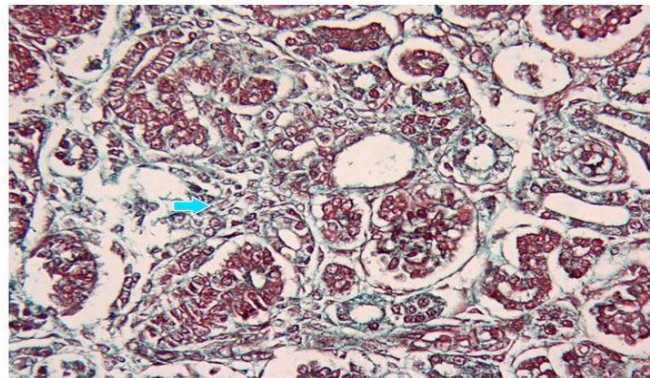


Fig. 10: Photomicrograph of a section in the kidney of 20 day-old fetus yielded from a clarithromycin-treated dam (T2f) showing a severe increment of collagenous fibers in the tubulointerstitial tissues (blue arrow). (MT, $\times 400$)

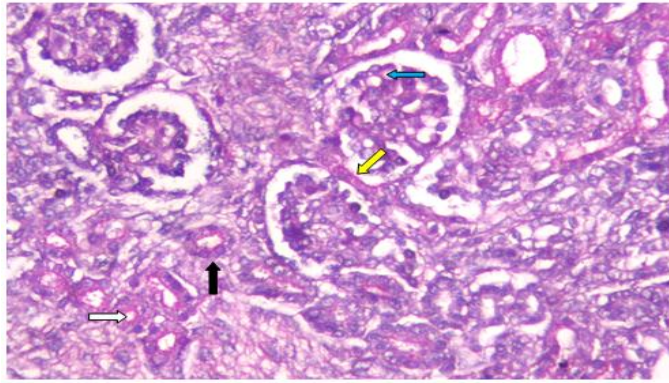


Fig. 11: Photomicrograph of a section in the cortex of kidney of 20 day-old fetus yielded from a control dam (C1f) showing positive PAS reaction in the brush borders (white arrow) of the proximal convoluted tubules, Bowman's capsule (yellow arrow) and the glomerular capillaries (blue arrow). The tubular basement membranes show a negative reaction (black thick arrow). (PAS & H \times 400)

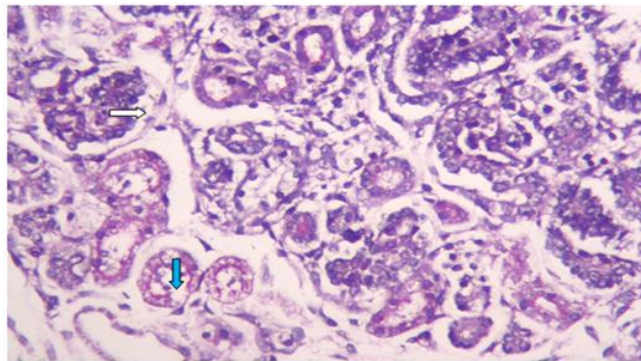


Fig. 12: Photomicrograph of a section in the kidney of 20 day-old fetus yielded from a clarithromycin-treated dam (T1f) showing a weak PAS reaction in the capsular wall (white arrow) and in the brush borders (blue arrow) are shown. (PAS & H, \times 400)

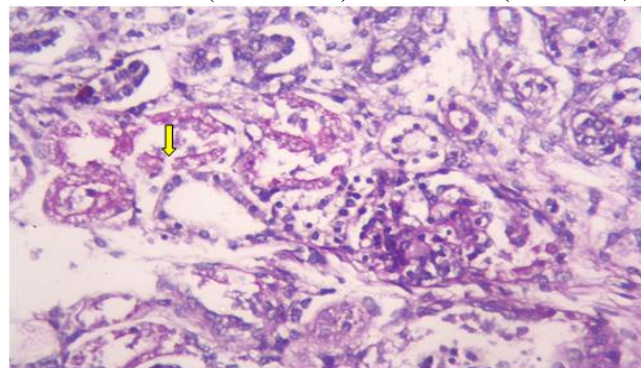


Fig. 13: Photomicrograph of a section in the kidney of 20 day-old fetus yielded from a clarithromycin-treated dam (T2f) showing a weak PAS reaction in the brush borders (yellow arrow) of the proximal convoluted tubules. (PAS & H, \times 400)

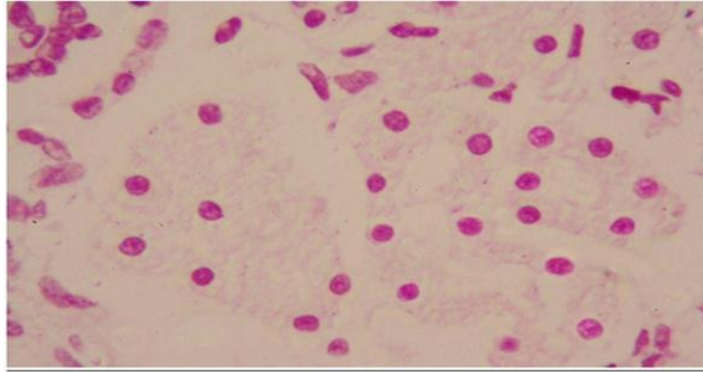


Fig. 14: Photomicrograph of a section in the cortex of kidney of 20 day-old fetus yielded from a control dam (C1f) showing the normal distribution of DNA content in the nuclei of renal tubules. (F, $\times 1000$)

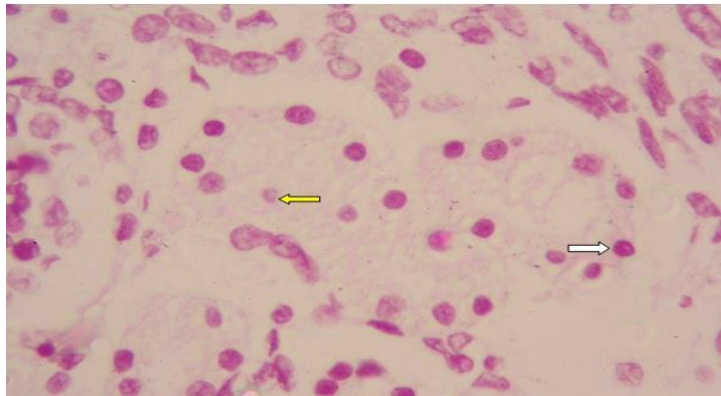


Fig. 15: A photomicrograph of a section in the kidney of 20 day-old fetus yielded from a clarithromycin-treated dam (T1f) showing a pyknotic (white arrow) and karyolytic nuclei (yellow arrow). (F, $\times 1000$)

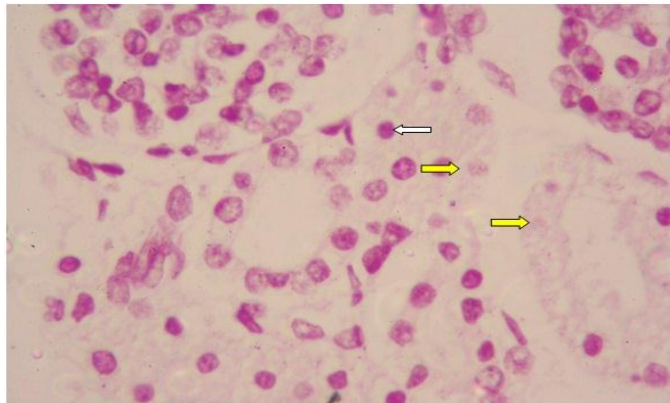


Fig. 16: A photomicrograph of a section in the kidney of 20 day-old fetus yielded from a clarithromycin-treated dam (T2f) displaying intensive stain in the pyknotic (white arrow) and weak stain in the karyolytic nuclei (yellow arrow). (F, $\times 1000$)

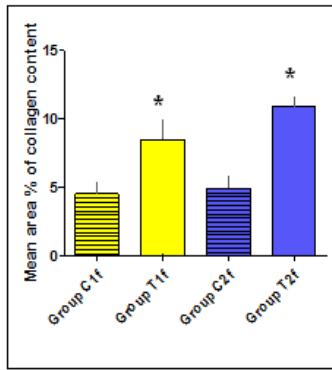


Fig. 17: Histogram showing the mean area % of collagen content in the kidney of 20-day old fetuses of different groups

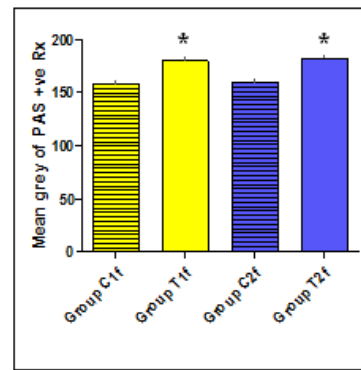


Fig. 18: Histogram showing the mean grey of PAS +ve reaction in the kidney of 20-day old fetuses of different groups.

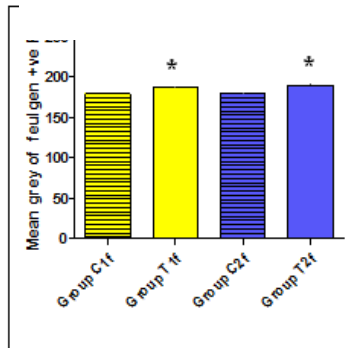


Fig. 19: Histogram showing the mean grey of Feulgen +ve reaction in the kidney of 20-day old fetuses of different groups.

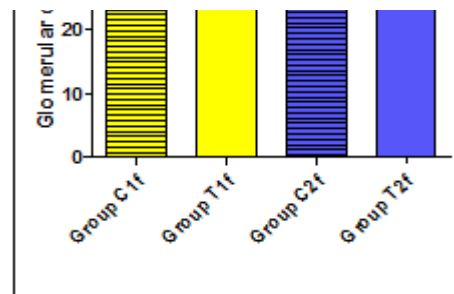


Fig. 20: Histogram showing glomerular diameter in the kidney of 20-day old fetuses of different groups.

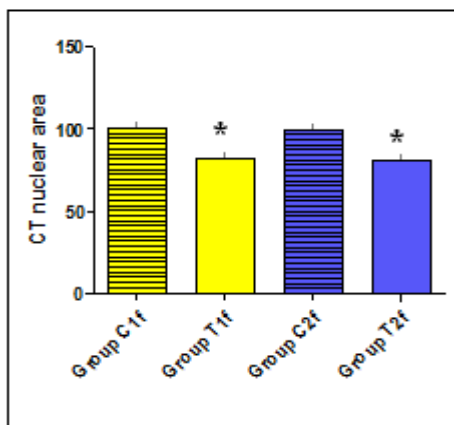


Fig. 21: Histogram showing the nuclear area of CT in the kidney of 20-day old fetuses of different groups.

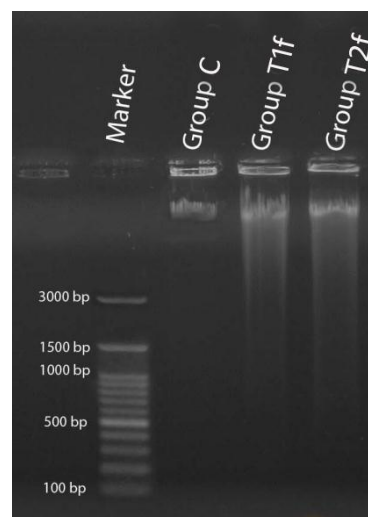


Fig. 22: Agarose gel electrophoresis of genomic DNA isolated from kidney of 20-day old fetuses of different groups

Table (1): Image analysis of mean area % of collagen content, and the mean grey of carbohydrate content and DNA content of kidneys of 20-day old fetuses of different groups.

| <i>Parameters</i> <i>Groups</i> | <i>Collagen content</i> | <i>PAS +ve Rx</i> | <i>Feulgen +ve Rx</i> |
|------------------------------------|---------------------------|---------------------------|-----------------------------|
| Group C1_f | 4.499±0.6485 | 158.1±4.088 | 179.1 ± 0.6843 |
| Group T1_f | 8.452±1.296* (87.86) | 179.9 ±4.596** (13.78) | 186.8 ± 1.014*** (4.3) |
| Group C2_f | 4.912±0.8863 | 159.4±3.993 | 178.6±0.8724 |
| Group T2_f | 10.87±0.7522** (121.3) | 182.4 ±3.532** (14.43) | 189.7 ± 0.8284*** (6.22) |

The number of animals were 6 in each group. Data are expressed as mean ± SE.

*: Significant change at $p < 0.05$ with respect to corresponding control group (Group C1_f or C2_f).

(): % difference with respect to control value.

Table (2): Histomorphometrical measurements of the glomerular diameter, and the nuclear area of convoluted tubules (CT) in kidneys of 20-day old fetuses of different groups.

| <i>Parameters</i> <i>Groups</i> | <i>Glomerular diameter</i> | <i>CT nuclear area</i> |
|------------------------------------|----------------------------|---------------------------|
| Group C1_f | 31.55± 0.71 | 100.5± 3.71 |
| Group T1_f | 30.78± 0.77 (-2.4) | 82.22± 3.84** (-18.2) |
| Group C2_f | 29.46±0.63 | 99.99±3.721 |
| Group T2_f | 29.89± 0.62 (1.5) | 81.38± 3.464** (-18.6) |

The number of animals were 6 in each group. Data are expressed as mean ± SE.

*: Significant change at $P < 0.05$ with respect to corresponding control group (Group C1_f or C2_f).

(): % difference with respect to control value.

Table (3): The purity of total genomic DNA isolated from kidneys of 20-day old fetuses of different groups.

| <i>parameters</i> <i>Groups</i> | <i>A₂₆₀</i> | <i>A₂₈₀</i> | <i>DNA Purity</i> <i>(A₂₆₀/A₂₈₀)</i> |
|------------------------------------|------------------------|------------------------|---|
| Group C | 0.17 | 0.185 | 1.8 |
| Group T1_f | 0.22 | 0.11 | 2 |
| Group T2_f | 0.13 | 0.07 | 1.83 |