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A Study of Histopathological Effects of Gentamicin Drug on Placenta of Pregnant Rats

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Abstract

The study has been conducted in Animal House/Faculty of Sciences/University of Kufa between December 2022 and February 2022, fifteen female Albino Rats are used. Gentamicin is aminoglycoside antibitics widely used during pregnancy for treatment the infections. The present study has been intended to show the histolopathologiacl effects of gentamic in on placenta in female Albino Rats. The females Rats are randomly divided into three main groups, comprising five rats for each group. The control group is given inta peritoneal injection of physiological normal saline and the second and third group are given inta peritoneal injection of gentamicin doses twenty, forty mg/kg/day respectively for 20 days from the first day to the end of experimental allocated for each female.The rats are sacrificed in 20 th (dpc) to study the hitopathological effects of gentamicin on placenta. The histopathlogical study of female rats embryos treated with twenty, forty mg/kg/day of gentamicin have shown degeneration of spongiotrophoblast and congestions in placental labyrinth compare to control group.The scanning electron microscope study show deformation of architecture structure of placental parenchyma. In conclusion; gentamicin caused hitopathological effects on placenta in 20 th (dpc).

Keywords: Rats, Pregnant, Gentamicin, Placenta

1. Introduction

During pregnancy many pregnants are on medications for acute infections related with pregnancy (e.g., heart burn & morning sickness), and also utilized for chronic illness management, predating or developing during pregnancy (e.g., epilepsy, asthma, depression, psychosis, chronic hypertension and genitourinary bacterial infections), sometime a treatment is endorsed to the mother to treat fetal diseases (e.g., fetal dysrhythmia) (Yates and Thomas, 2016).

Little is thought about the impacts of human fetal exposure when another medication is approved unless it is specifically prescribed for use in pregnancy, since numerous factors may add to adverse fetal impacts, having comprehensive information about in utero exposures will enhance our capacity to make correct determinations about causality (Clemow *et al*., 2015).

The pregnancy duration represent critical periods in which the pregnant mother is exposed to external impacts and the most likely effects are the period called the embryonic period, which extends in the human fertilized stage until the end of the eighth week of pregnancy, during this period the three germinal layers are formed which included ectoderm, mesoderm and endoderm and also neural crest cells, it is known that different body organs originate from these germinal layers (Deye *et al*., 2016).

It has been confirmed that antibiotics can damage developing fetal organs during pregnancy (Romero *et al*., 2007; Sachdeva *et al*., 2009). Placenta is permeable to the most antibiotics, for example gentamicin and cephalotin preserve in amniotic fluid and embryonic tissues in high concentration, it is worthy to note that permeability of placenta to various antibiotics varies during pregnancy, as it is reduced to Gentamicin in the last days of the pregnancy but increase under Cephalotin effect (Schuenemann, *et al*., 2007).

Gentamicin is antibiotic an aminoglycoside derived from Micomono- sporapurpurea, it has a bactericidal effect on gram-negative microorganisms by preventing the production of protein in the bacterial cell therefore it is used in the treatment of a number of infections caused by a number of bacterial species such as respiratory infection, skin, gallbladder, bile ducts, urinary tracts, meningitis, septicemia diseases (Narayana, 2008).

Gentamicin is quickly absorbed after intra muscular injection and mainly diffused in extracellular fluid (peak serum levels usually reached within 30 to 90 minutes) and it accumulates in the renal proximal convoluted tubules (50 to 100 times more than that in serum). Although gentamicin passes through the placenta and is excreted in breast milk, it is not absorbed from the

gastrointestinal tract. Gentamicin concentration in umbilical cord blood and the placenta reaches 50% and 8% of mother's blood serum respectively, after 1-2 hours of intra muscular injection (Pacifici and Marchini, 2017).

In mammals, the peri-implantation is considered as a developmental period, implantation and signaling for pregnancy maintenance, which are a periods for floating time after zona pellucida hatch, but the cells growth and differentiation during embryonic development (Elmetwally *et al*., 2019). Implantation is defined as the process by which the embryo attaches to the endometrial surface of the uterus and invades the epithelium and then the maternal circulation to form the placenta (Mesiano, 2019).

2. Material and Methods

2.1 Animal Model

This study achieved on pregnant white rat *Rattus norvegicus* females (15) and males (5) for mating. All rat weights ranging from 200-250 g. They should be in good health. The rats are placed in plastic cages with metal covers, 48 cm wide, 15 cm wide and 7 cm wide. The sawdust, which should be replaced three times a week, is considered in its care to clean the hatching of the special diet and plastic bottles can be used to make a watering tough with a cork equipped with metal pipes. The animals are placed under suitable laboratory conditions in terms of temperature 18-26 C° and light/dark cycle 10/14 and ventilation rate time/hour 10-15 and also the relative humidity 30-70 (Tan & Tan, 2017) [7].

2.2 Drug Used

From the ampoule of gentamicin, required dosages can be administered to animals of varying body weights as illustrated in table below:

Standard dose	Stock solution	Animals body weight (g)	Calculated dose in mg	Equivalent dose in ml
Gentamicin, 80 mg/kg for Rats	80 mg/2ml =40 mg/ml	100 g	8 mg	0.20 ml
		150 g	12 mg	0.30 ml
		250 g	20 mg	0.5 ml

Single dose= 20mg /kg/day (equivalent 0.5 ml of gentamicin).
Double dose= 40 mg /kg/day (equivalent 1 ml of gentamicin).
The average weight of animals = 250 g equivalent 0.25 kg (Erhirhie *et al.*, 2004).

2.3 Experimental Groups

First: Control group: included five female rats injected by normal saline (Nacl 0.9%) intraperitoneally for seven days, the group sacrifice it in the end of experiment.,

Second: first treated group: included five female rats injected intraperitoneally by dose 20mg /kg/day of gentamicin for twenty days, the group sacrifice it in the end of experiment.

Third: second treated group: included five female rats injected intraperitoneally by dose 40mg /kg/day of gentamicin for twenty days, the group sacrifice it in the end of experiment.

2.4 Animals Sacrifice and Collection of Placenta

The experimental animals of all groups were sacrifice after general anesthesia by combination of Ketamine: Xylazine (90mg/ kg: 10mg/ kg intraperitoneal), used ketamine 0.5 ml & xylazine 0.1 ml to each 250 g of body weight for anesthesia when sacrifice the female rat from the control & treated croups, after the anesthesia the females rats put in anatomical dish and made linear incision by scissors in abdominal region for extraction the placenta that contains for collected, by anatomical tools. Saved in containers contains 10% formalin (AlTameemi, 2014) [1].

2.5 Histological Preparations

Done samples saved after remove them from animals in containers contains 10% formalin (38%100ml formalin in 900ml tap water) and then done series of operations depending on the method described in (Suvarna *et al*., 2018).

2.6 Staining and Mounting

Used the following special stains to colorize slides of different types of tissue:

2.6.1 Harris Hematoxylin Stain

A general base color used to color the nucleus in dark blue color**.**

2.6.2 Eosin Stain

A general acidic color used to color the cytoplasm in dark red color. (Suvarna *et al*., 2018).

2.7 Scanning Electron Microscope

Procedure:

A. Glutelaldehyde Fixation

1. Prepare fixative solution and fill the scintillation vials with fixative.
2. Cut specimen tissue and place in tube with glutelaldehyde solution.
3. Place tube on its side to keep the air away from the tissue.
4. Incubate at 4 C° for 12-24 hours (Overnight is good).

B. Osmium tetroxide fixation

1. The solution can be stored in the cold room or freezer for about one month. If frozen, thaw at room temperature. This solution should be straw colored. If it is purple it is no longer good.
2. Dilute to a 1% solution in 25 mM phosphate buffer.
3. Pour off fixative and add the 1% osmium tetroxide solution.
4. Incubate in the cold room overnight to several days. The osmium turns black.

C. Dehydration of tissue

1. Pour off osmium solution and rinse 3 times with 25mM phosphate buffer.
2. Put tissue through an alcohol series. 15 to 30 minutes each step.

3. Results & Discussion

3.1 Histological Study of Placenta in Female Rats

Cross sections of placenta from pregnant rats 20 th dpc in control groups showed normal histological structures of decidual tissue, spongiotrophoblast, villus and placental labyrinth (Fig 1), while in treated by dose 20mg/kg/day of gentamicin show degeneration of spongiotrophoblast and congestions in placental labyrinth (Fig 2), but in treated by dose 40mg/kg/day of gentamicin show severely effects (Fig 3).

Cross sections of placenta from pregnant rats 20th dpc in control groups showed normal histological structures. The results have showed colored sections by H&E taken from

the placeta sites of treated group by gentamicin for 20th day post coitum results different to control group. Which affected due to histopathological effects of gentamicin in both doses 20, 40 mg/kg/day show spongiotrophoblast, villus and placental labyrinth, but in treated by dose 40mg/kg/day of gentamicin show severely histological effects.

The outer most layer of the chorionic villi where feto-maternal subcutaneous injections of BPA reduction of the spongiotrophoblast layer of the placenta (Gingrich *et al*., 2018) [9]. The placental villi histologically effects usually due to exposes aminoglycosides during the pregnancy (Gingrich *et al*., 2020) [8]. In the placentas of females treated with both restricted diet, such changes were much more severe, these alterations suggest a direct, toxic effect of aminoglycosides on the placental cells, so the phagocytic activity exhibited by trophoblast cells, may be playing a role in the removal of death cells from the maternal-placental interface and/or in a compensatory mechanism to maintain the uptake of maternal nutrients, following decreased metabolic exchange functions of the labyrinth due to the toxic effect (Levario-Carrillo *et al*., 2004) [10]. Clinical detection and management of chorioamnionitis is challenging given the gold-standard for diagnosis remains placental pathology, the results of which are only available after delivery, recommended diagnostic criteria for clinical chorioamnionitis have evolved over time. The goal of this study was to describe trends and differences in chorioamnionitis diagnostic and management practices in Canada that lead to effect on placenta (Charpentier *et al*., 2022) [11]. Aminoglycoside courses nephrotoxicity and placental toxicity due to accumulated effects of aminoglycosides (Gilbert and Benichou, 1990) [12].

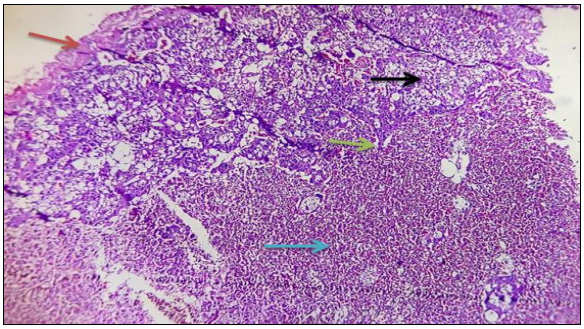

Fig 1: Cross section of normal placenta of pregnant rat (20th dpc) control group, show decidual tissue, spongiotrophoblast, Villus Placental Labyrinth, H&E, 40X

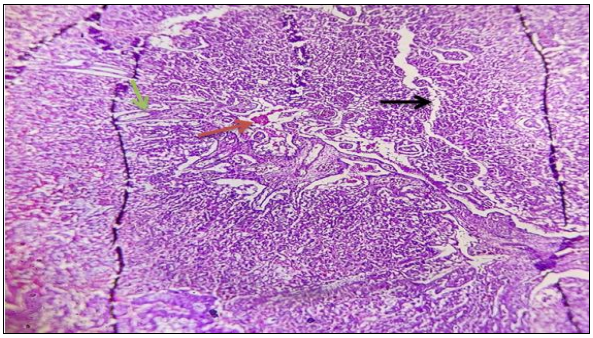

Fig 2: Cross section of placenta of pregnant rat (20th dpc) treated dose 20 m/kg/day of gentamicin show, degeneration of spongiotrophoblast, Villus, congestions in placental labyrinth, H&E, 40 X

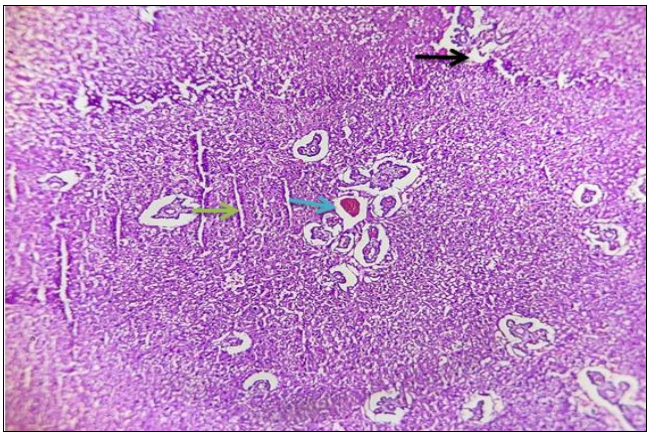

Fig 3: Photograph of placenta of pregnant rat (20th dpc) treated dose 40m/kg/day of gentamicin show, degeneration of spongiotrophoblast, Villus, congestions in placental labyrinth, H&E, 40X

3.2 Scanning Electron Micrographs Results of Placenta

Scanning electron micrographs of placenta from pregnant rats 20 th dpc in control groups showed more regular architecture of placental parenchyma (Fig 4), while in treated by dose 40mg/kg/day of gentamicin show deformation of architecture structure of placental parenchyma (Fig 5).

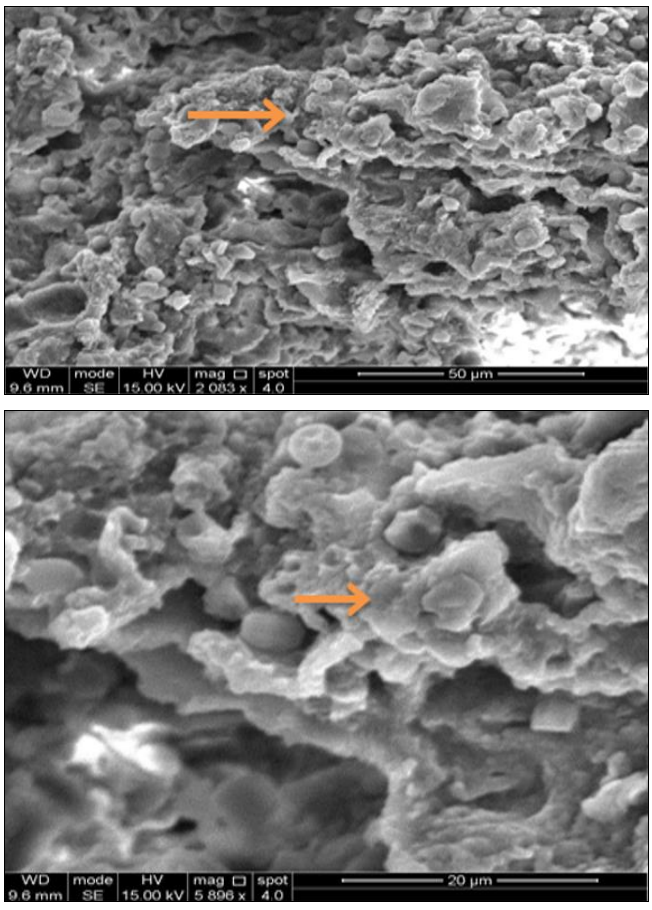

Fig 4: Scanning electron micrograph of placenta in pregnant rat (20th dpc) control group show a normal architecture of parenchyma

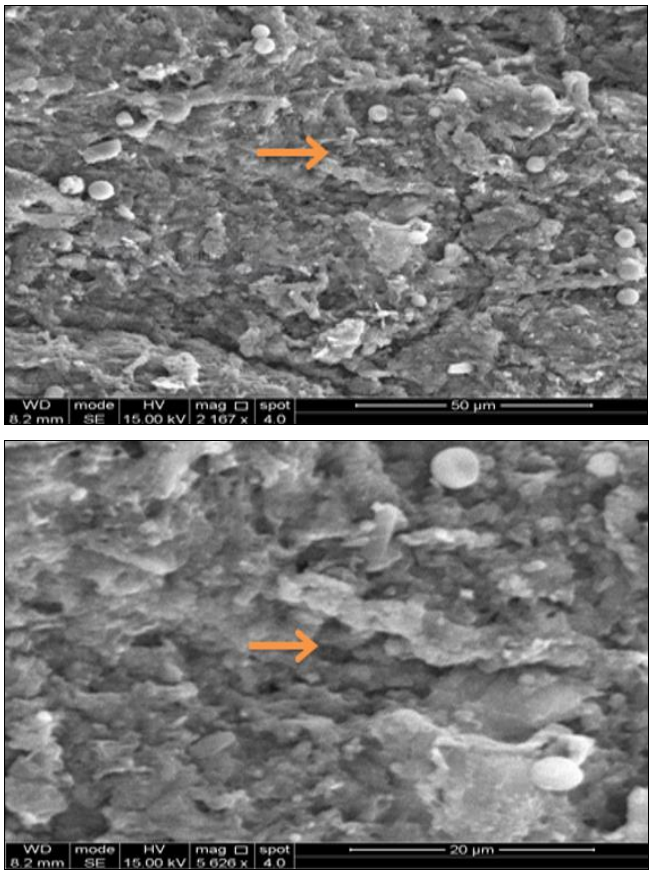

Fig 5: Scanning electron micrograph of placenta in pregnant rat (20th dpc) treated dose 40 mg/kg/day of gentamic in show deformation of architecture structure of placental parenchyma →

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