

Histological Changes in the albino rats Ovary after Exposure of the Mothers to the Drug Tetracycline during Pregnancy

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Abstract: Many of the pregnant women and nursing mothers sometimes used different types of antibiotics for treatment of some diseases. The most important of these antibiotics is the tetracycline drug, which is used to treat many diseases. This displays fetuses and infants at risk of this drug, therefore, the objective of this study was to investigate the collateral damage on histology of the ovary. To achieve this, Pregnant female rats were administered orally a standard therapeutic dose of tetracycline (0.7mg \ kg b. wt.), as the chronic dose. The acute single dose (1.5 mg/kg b, wt) was administered to females on the tenth day of pregnancy. Another female group fed orally with a double single dose. Females at the age of 60 days after birth were examined. The results showed a lack of significant increase in weights and lengths. Also administration of drug at all tested doses led to the histological changes sharply in the ovaries such as decomposition of tissues and bleeding, sore and breadth of the distances because of swelling and the occurrence of necrosis and fibrosis were noticed. These observations make the pregnant women stay away from taking the drug during pregnancy, and try to determine the lowest possible dose to minimize side effects of the drug.

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1. Introduction

Tetracyclines are very effective against a wide range of aerobic and anaerobic, gram-positive and gram-negative bacteria. (Zhu, *et al.*, 2002), protein in both bacteria. Graham *et al* (1999), suggests that the drug damage molecules causing red blood cell hemolysis and damage the cell membrane and oxidation of fatty acids by the production of free radicals. Tetracycline was narrated by inhibiting protein synthesis of bacterial Attached to the below unit 30 S ribosomes in bacteria, thereby preventing the arrival of DNA carrier tRNA for amino acids and thus prevents its association with the future in the position A on the DNA sender mRNA on the ribosome (Zakeri and Wright, 2008)

Most of the tetracycline is absorbed in the stomach and upper small intestine by 80% - 60% and increase the intensity of absorption when the stomach is empty of food. This drug has a half-life in the range of 6-12 hours with a peak concentration in plasma between 2-4 hours after a single dose, distributed in all tissues including the uterus and gland prostate and in accumulates in the cells of the inner lining of the liver, spleen and bone marrow penetrate the rapid and sharp tissues, also has the ability to cross the placenta after half an hour of eating and also secreted in the milk. The college is considered the main route for most of the tetracycline excretion from the body, in addition to its accumulation in the liver where it is excreted through the bile into the intestines and then partially re-absorbed by the intestinal hepatic circulation (Wells, 1999, Huang *et al.*,2001).

According to Wu *et al.* (2004), during pregnancy, embryos were affected and principle of antibiotic treatment of microbial should be based on absolute necessity, and that cross the placenta and affect them.

Chabot *et al.*(1988) Explained that most of the antibiotics used to treat inflammatory diseases in pregnant women have been studied experimentally, including tetracycline, and had a toxic effect on the fetus in rats. Therefore, the researcher studied the effect of both of tetracycline and gentamicin during different stages of pregnancy, the use of both tetracycline and gentamicin led to a high number toxicity and death in utero, as well as occurrence of deformities and birth defects in some embryos, Bastos *et.al.*, (2012) Copeland *et al.*, (1990) found that gentamicin in the rat has the ability to penetrate the placenta when given to rats during pregnancy and discovered in the serum embryos, leading to poisoning

Copeland *et al.*(1990) confirmed that there are many drugs that have proven their impact that affect animals and humans together, such as tetracycline, which affects the composition of the bones of the fetus and the teeth.. There have been significant effort and resources expended in the past trying to achieve increased fertility in laboratory mice, (Bilezikjian, *et.al.* 2001) and farm animals by breeding for enhanced ovulation rate and/or uterine capacity (Clutter, *et.al.* 2004). Glaxo Smith Kline 2009, were studied reproduction in pregnant rats and mice when using Alamoxxa Doxycycline at doses of ten times bigger than the dose used in humans resulted in a twice increase in

the birth rate. The pregnant women and nursing mothers use types of antibiotics for treatment of some diseases. Most important of these antibiotics is the tetracycline drug, which is used to treat many diseases. This displays fetuses and infants at risk of this drug, but this was the objective of the research study of the collateral damage on histology of the ovary. To achieve this pregnant female rats administered oral therapeutic dose.

2. Material and Methods:

A total number of 280 of adult female albino rats, their weights (180- 200) grams were used in the present study. Pregnant female rats were administered orally a standard therapeutic dose of tetracycline (0.7mg \ kg b. wt.), as the chronic dose. The acute single dose (1.5 mg/kg b,wt) was administered to females on the tenth day of pregnancy. Another female group fed orally with a double single dose. Females at the age of 60 days after birth were examined, according to (Fainaru *et al.*, 2009) and divided dose. The ovaries of mice treated with or without drug were fixed in Bouin solution for ≥ 24 hrs, sectioned in wax, and stained with hemotoxylin and eosin under standard conditions. Each ovary was sectioned from beginning to end in groupings of four sections of 5 μ m thickness each. These groupings were interspersed with 50 μ m of tissue that was not sectioned. To avoid double counting, tertiary follicles was analyzed. The acute double dose was given to female rats, in two days. After that females were taken at the age of 60 days after delivery for examination in terms of external structure, taking their lengths and weights and then extract the ovary to study histological changes.

3. Results and Discussion:

a- Effect of the drug on female rats born at the age of 60 days:

Weight: Results demonstrated that there was a decrease in total body weights of acute and chronic single- dose treated groups. While a significant increase in the weights of the acute double treated groups of mentioned dose (Table 1).

Length: Results in table 2 indicated that there is a significant decrease in length of the treated groups of single-dose acute and chronic occurred, while there was an increase in the lengths of the group treatment of acute double dose

This may be due to its effects on bone as Geisser *et al.* (1988) mentioned, where the noticed that the deposition of tetracycline hydrochloride in the bones of rat embryos causes a lack of size. Su *et al.* (2005) concluded that giving Oxytetracycline to pigs did not cause a significant difference in either body weight or in total mortality rate in pigs born compared to controls. Also, supported by Bastos *et al.* (2012), in the use of doxycycline for treatment in pregnant women as

it leads to change the color of baby teeth and permanent lack of bone growth in fetuses and children.

The ovary of the control group female albino rats at the age of 60, a lobular shape and is surrounded from the outside with capsule consists of connective tissue padded with a layer of squamous epithelium.. Primary, secondary and tertiary follicles were defined as described previously, Winters *et al.* (2004). Care was taken not to double-count follicles. Tertiary follicles were counted and measured with an eyepiece micrometer in sections. The ovaries for this study were taken from the same mice analyzed for ovulation in (Figures 1- 9) that had been treated with tetracycline, show that there was no evidence for changes in the numbers of primary, secondary, or tertiary follicles, nor was there any difference in the average size of tertiary follicles. Furthermore, careful inspection of the ovaries showed no evidence of damage to the ovaries such as cyst formation, obvious depletion of primordial follicles, or any malformation in that were fed tetracycline over 60 day period. In fact, the ovaries of mice were not different from those of control mice (Fig. 2) except they contained more Corpora lutea

The ovary vacuum (periovarian space) lining the ovary with a single layer of fat cells. Long nucleus immersed in connective tissue called the tunica albuginea. The ovary is also characterized by the tunica albuginea which is divided into two parts, the cortex and the medulla. The cortex is characterized by the, appearance of all types of follicles in various stages of development. The follicles which were appeared composed of primary oocytes, surrounded by a single layer of fat cells, while emerged follicles surrounded by the transparent zona (pellucida ZP). There were more than a layer of granule cells, which appear between the cavities of follicle surrounded by a basal membrane. The basal membrane is separated from the follicle cover, Graf mature follicle that appeared close to the surface free of the ovary in the outer shell, which is made up of oocyte, circular in shape. A central position surrounded by a region of transparent and several layers of granule cells. The ovarian cytoplasm and nucleus also shows objects yellow corpus luteum at this age. Lutein cells punctuated by a network of capillaries and composed the core of connective tissue to connect many blood vessels as they appear also channels of different sections of oviduct. Deposited eggs in the ovary and be one of the three layers from the inside to the outside layer composed of mucous layer. the mucous layer composed of a cavity lined from 2-3 layers of epithelial cells based on the membrane base also contains the secretory ciliated and non ciliated cell. The layer consists of external longitudinal muscle fibers and an internal circular muscle layer. The rats did not show any apparent abnormalities. Chrast *et al.* (2004) give Oxy-tetracycline during the formation than oral therapeutic

dose for humans is causing toxicity to the mother and fetus with no significant abnormalities in the embryos, when studying reproduction in pregnant rats using doxycycline, doses ten times greater than the dose used in humans, a decrease in the number of births or serious damage in the embryos resulting from doxycycline. **Wu, (2004)**. We have also supported (**Pursel et al., 2006**), who advised the use of the proxy drug doxycycline in the prevention of anthrax in pregnant women. As well as researchers (**Sasaki et al., 2003**) concluded that the use of oral tetracycline during the second month of pregnancy can cause serious deformities in fetuses.

b- Acute treatment (GTAC1):

Single-dose acute GTAC1 Group treatment:

Dissolved and flat cell layers of the ovary from the outside to inside is separated. Different sections of the eggs channels are deposited. Abdominal hemorrhagicum were also observed. The ovary with a small number of follicles, especially Graf follicles were appeared. Initial oocytes decomposed with granular cells. Spindle cell consists of 1-2 layers were seen. Graf follicle close to the surface free of the ovary in the outer surface Cells aggregates in the back cavity, follicular sac in large and some of the others are small and divided into cavities as the back cover follicle. Two layers Increased in thickness for the control group. Bleeding around the follicle as necrosis and atrophy of the cells constituting the yellow body hemorrhage were appeared.

c- Acute double dose group (GTAC2) treatment.

Also distort the cover follicle with a few thick basement membrane did not reach the Graf vesicle and contains the egg cell with enclosed area granular.

d- Treatment of chronic dose group, show lake of the ovary which looks smaller than control group. Epithelium composed of a single layer of thick cells with emerged blood vessels, especially Graf follicles and the several follicles. Also emerged egg channels were observed. Presence of fibers around the follicle, Graf vesicle cannot be easily distinguished and is surrounded by cells, granules dissolved and the increase of fiber in the follicle. The presence of necrosis and bleeding also appeared. Blocks of glandular masses numerous cells of large multi-surfaces polyhedral discovered. Histology of the oviduct in the groups treated for control indicates swelling and decomposition, the appeared pulp consists of connective tissue, thick walls and by many of the blood cells distorted, we concluded that the drug has side effects on the fabric muscles of the ovary and the severity of these effects depends on the dose and giving time. It is well documented that tetracycline causes ovotoxicity by acceleration of follicular atresia (apoptosis) in primary follicles (**Guzman et al., 2002**,

Miller et al., 2003, Gossen et al., 2004). It appears that the effect of the drug on the ovary involves disruption in follicular survival signaling. Previous studies demonstrated that exogenous KITL (50–400 ng/ml) is able to attenuate induced ovotoxicity (**Jorgensen, 2004**). Our results indicate that Dox had no effect on the qualitative nature of any of the three types of follicles even after 30–39 days of treatment,

A variety of ovarian signaling pathways have been shown to be important in folliculogenesis and follicular survival, and these growth factors are released by and have their effects on a variety of follicular compartments. (**Huang, et al., 2001, Ford, et al., 2001**) explain growth factors that has been reported to promote primordial follicle survival (**Bilezikjian et al., 2004**) The other growth factors are thought to be involved in primordial-to-primary follicle transition. The present study was initiated to investigate tetracycline was able to attenuate induced ovotoxicity. This finding further indicates that drug is acting directly rather than by nonspecifically compromising multiple signaling pathways involved in follicle viability or growth. Because tetracycline had an effect on primordial follicles but decreased small primary follicle numbers suggests that they both retard primordial follicle activation and recruitment. However, drug provided a substantial blockage of primordial follicle recruitment, as reflected by the diminished pool of large primary follicles.

In summary, the present study further underscores the role of tetracycline in follicle survival, activation, and recruitment. Furthermore, these results also provide strong evidence for direct drug targeting in induced ovotoxicity. Currently, studies are underway to identify the specific downstream signaling pathways within the oocyte that are involved in primordial follicle survival/recruitment as they are impacted by tetracycline. Taken together, these studies support the hypothesis that drug induces ovotoxicity by directly targeting the oocytes via inhibition of KIT-mediated signaling components.

A variety of ovarian signaling pathways have been shown to be important in folliculogenesis and follicular survival, and these growth factors are released by and have their effects on a variety of follicular compartments. KITLG and LIF are produced by ovarian granulosa cells (**Kumar et al., 2000, 2001**) while GDNF, PDGFB, and FGF2 are produced by the oocyte (**McGee et al. 2000, Dmarak et al., 2003**). On the other hand, FGF7 is produced by ovarian theca cells (**Klerman et al., 2003**). Previously, whereas exogenous KIT attenuated VCD-induced ovotoxicity, GDF-9 and BMP4 were ineffective at protecting cells from this follicle loss (**Jin, et al., 2005**) BMP4 is the only one of those growth factors that has been reported to promote primordial follicle survival (**Greenwald et al., 2006**). The other growth factors are thought to be

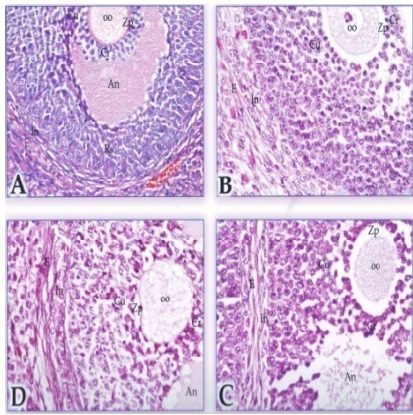
involved in primordial-to-primary follicle transition. The present study was initiated to investigate whether other known relevant growth factors play a role in tetracycline induced ovotoxicity. Of all growth factors tested, only ovary was unable to tolerate tetracycline induced ovotoxicity. This finding further indicates that the drug is acting directly on the ovary development, rather than by nonspecifically compromising multiple signaling pathways involved in follicle viability or growth.

TABLE 1: Weight of the studied groups at age of 60days old

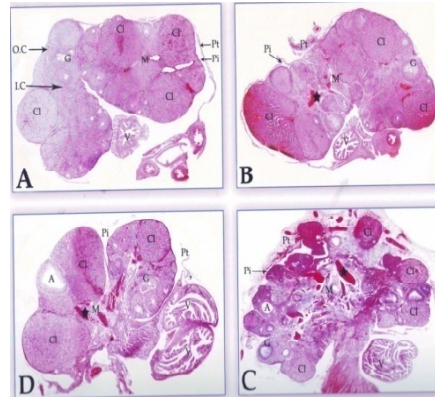
Groups	Mean	±SD	95%Confidence interval for mean
GC	162.53	±5.47	159.49-165.56
GTAC1	161.93	±3.6	141.39-145.4
GTAC2	175.86	±0.86	175.38-176.34
GTCR	143.4	±2.11	160.76-163.10

TABLE 2 Height of the studied groups at age of 60days old

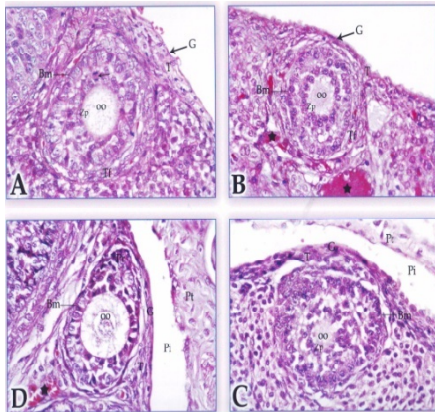
Groups	Mean	±SD	95%Confidence interval for mean
GC	21	±0.84	20.53-21.46
GTAC1	20.23	±0.42	20.26-20.73
GTAC2	21.5	±0.42	21.26-21.73
GTCR	20.5	±0.21	20.11-20.35



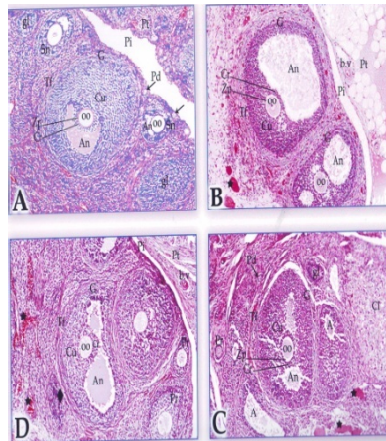
Picture (1): Showing the location and installation of the ovary in the control and treated groups A, B group of single-dose treatment, C- group treated with acute double dose, D- group treated chronic dose.



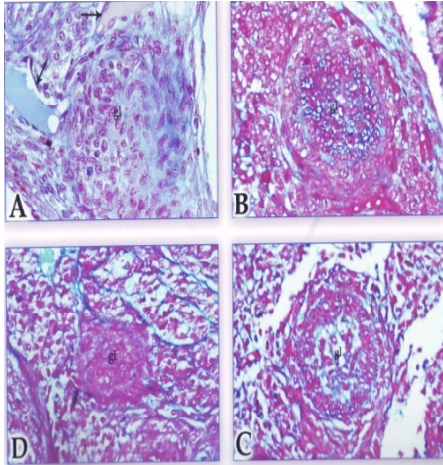
Picture (2): The sectors shows the lining of the ovary and the cover and white primary follicle in the control and treated A, B group of single-dose treatment, C- group treatment of acute -double dose, D- group chronic dose treatment.



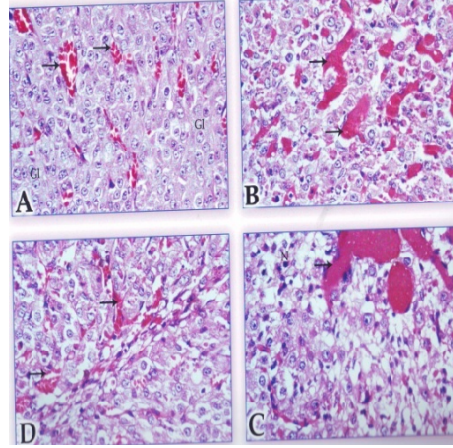
Picture (3): The sectors of primary and secondary follicles and Graf vesicle in the control and treated A, B group of single-dose, C- group treated of acute double dose-, D-group chronic dose treatment.



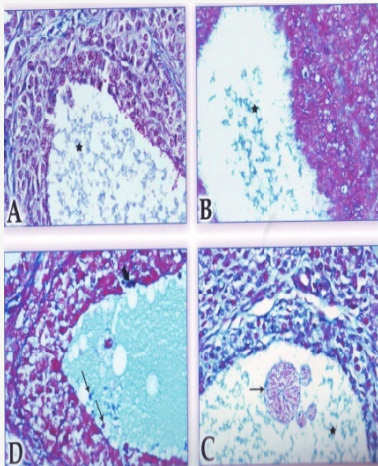
Picture (4): illustrates the sectors in the enlarged Graf vesicle show the control and treatment A, B group of single-dose treatment, C- group treatment of acute double-, D-group chronic dose treatment.



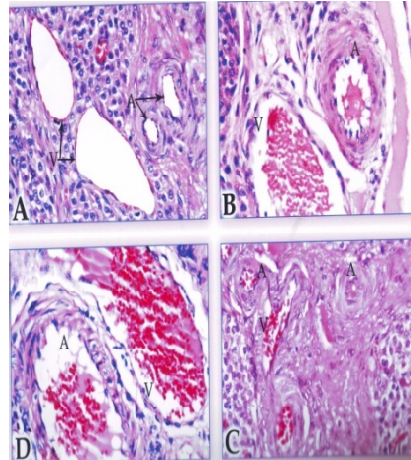
Picture (5): Shows the segments of body composition in the Yellow body in the control and treated A, B group of single-dose ,C - group treatment of acute dose-double C, D – group chronic dose treatment The religion of chronic dose-D.



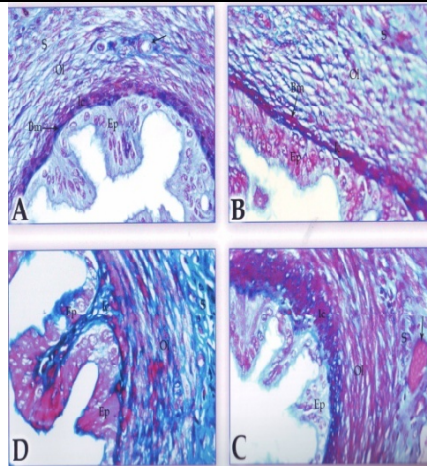
Picture (6): Sector shows the blocks in the glandular masses in the control and treated A, B- group of single-dose treatment, C- group treatment of acute dose-double, D- group, chronic dose treatment



Picture (7): Illustrates the sectors Graf follicles in four tested groups.



Picture (8): Shows the sectors of the pulp in four tested groups



Picture (9) :Sectors in the oviduct shows installed in four tested groups.

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