Biological and Histopathological Studies on the Effect of the Antibiotic Clindamycin on Albino Rat Fetuses

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ABSTRACT--- ***Dalacin-C (clindamycin palmitatehydrochoride) is indicated in the treatment of serious infections due to sensetiveanaerobic bacteria and gram- positive aerobic organisms. The aim of this study was to evaluate the teratogenic effects of Dalacin-C on pregnant rats and their fetuses. Pregnant albino rats (Rattusnorvegicus) were administrated daily oral doses of 185mg/kg of Dalacin-C from the5th- 19thday of gestation. The animals were sacrificed at the 20th day of gestation. Fetuses were removed from the uterus and evaluated for mortality rate, growth parameters, morphological and skeletal malformation as well as histological study of liver, kidney and brain. Results showed significant reduction in weight gain of pregnant rats and decreased placental weight of pregnant rats treated with Dalacin-C. Fetal growth retardation during gestational period was recorded. Hematomas and anomalies of limbswere noticed morphologically in the recorded. These abnormalities included weak ossification of the skull bones roof. Histopathological studies of fetuses during gestation revealed Changes in liver histology such as degeneration of the cytoplasm of hepatocytes and increases in the number of megakaryocytes were seen, revealed degeneration in the tubular lining epithelium with swelling in the endothelial cells lining the tufts of the glomeruli within the Bowman's capsule while in the brain tissue no histopathological alterations as recorded following Dalacin-Cadministration. Our findings suggest the need for great caution to handle Dalacin-Cespecially during pregnancy.***

Keywords--- Dalacin-C, Teratogenicity, Gestation

1. INTRODUCTION

Teratology the study of abnormal prenatal development and congenital malformations induced by exogenous chemical or physical agents.A birth defect or a congenital malformation is astructural abnormality of any type present at birth. It may be macroscopic or microscopic, on the surface or within the body (Moore, 1988). Major structural anomalies occur in 2-3% of live born infants, and an additional 2-3% is recognized in children by age 5 years making a total of 5-6% (Sadler, 2000).The most vulnerable period for malformation to take place is the period of organogenesis. Agents given duringthis period are more likely to cause birth defects. This critical time of fetal development in rats and mice is from 6-12 days of their gestation (Somer, 1962; Farris, 1967).

The transplacental transfer of drugs from the maternal to thefetal blood and tissues, leading to potential effects on thefetus, is a major concern. Thus, both mother and fetus mustbe included in the risk/benefit assessment to ensure a rationaldecision, weighing the therapeutic benefits of the treatmentto the mother against its potential harm to the fetus(Marcela and Mar´ıa, 2010).

During pregnancy, untreated sexually transmitted or urinary tract infections are associated with significant morbidity, including low birth weight, preterm birth, and spontaneous abortion. Approximately one in four women will be prescribed an antibiotic during pregnancy, accounting for nearly 80% of prescription medications in pregnant women. Antibiotic exposures during pregnancy have been associated with both short-term (e.g., congenital abnormalities)(Brandon, 2015).

Antibiotics are among the most commonly prescribed prescription medications for pregnant and lactating women (Nahum *et al.*, 2006).

A recent study showed that after adjusting for a number of factors, prenatal exposure of the infant to antimicrobials resulted in a lowerbirth weight of approximately 138 g (Vidal *et al.*, 2013). Antimicrobialexposure during pregnancy has recentlybeen linked to childhood obesity (Mueller *et al.*, 2015). Prenatal antibioticuse and the risk of neurologic disease, includingcerebral palsy and epilepsy, and atopic disease,including atopic dermatitis and asthma (Thomas and Price, 2003, Stensballe*et al.*, 2013 and Lapin *et al.*, 2015).

Clindamycin palmitate hydrochloride is a water soluble palmitic acid ester of clindamycin.Clindamycin base exerts its antibacterial effect by causing cessation of protein synthesis and also by causing a reduction in the rate of synthesis of nucleic acids.Clindamycin crosses the human placenta readily.

The present study was carried out to evaluate the teratogenic potential and fetal toxicity of the antibiotic drug (Dalacin-C) administrated orally to pregnant albino rat during the gestation (5th -19th GD) with 185mg/kg.

2. MATERIALS AND METHODS

Experimental animals

The present experimental study is carried out on the albino rat (*Rattusnorvegicus*). The standard guidelines of the Institutional Animal Care and Use Committee (IACUC) were used in handling animals.
Twenty females of 11-13 weeks old were selected for the present study and vaginal smears were prepared every morning and examined under light microscope (according to the method of Snell (1956)) for 5 days to select the female with regular estrus. Two females with regular estrus cycle were selected in the pro-estrus stage and caged together with one male overnight under controlled environmental conditions of temperature, humidity and light. The first day of gestation was determined by the presence of sperms in the vaginal smear (McClain & Becker, 1975).

Experimental design

Dalacin-C Drug was manufactured by Pfizer Company.
The Route of administration was Oral. The time of administration was scheduled from the 5th day of gestation, daily until the end of gestation.

Experimental groups

Group A: Control group received distilled water from 5th day of gestation to 19th day of gestation.
Group B: Treated group received 185 mg/kg of Dalacin-Cfrom the 5th day of gestation, daily until the end of gestation.

Developmental observations

On the 20th day of gestation, all pregnant rats of groups A and B were sacrificed and total implantation sites, fetal mortality rate (resorped or still birth) and living fetuses were recorded. Fetal body weight, body length, tail length and external malformation were also recorded.
Skeletal examination
Fetuses were preserved in 95% ethyl alcohol and were stained with double staining of fetal skeletons for cartilage (blue) and bone (red) according to the method described by Deinz*et al.*, (1995).

Histological examination
Autopsy samples were taken from liver, kidney and brain of fetuses in different groups at the 20th day of gestation. They were fixed in 10% formal saline for twenty four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin& eosin stain for routine examination then examination was done through the light electric microscope (Bancroft*et al*., 2002).

3. RESULTS

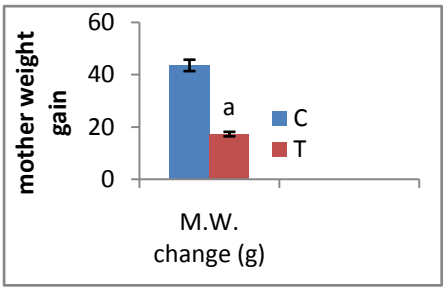
A. Morphological studies

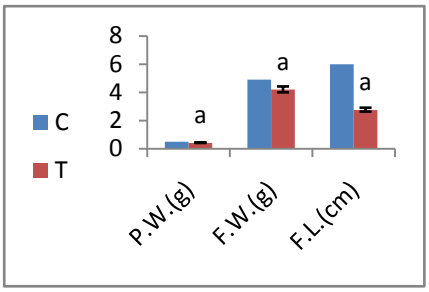
The average weight of placenta of all treated pregnant rats groups was decreased as compared to control (Table 1& Fig. 1). There was significant ($P \leq 0.05$) reduction in placenta weight of animals that received Dalacin-C.

Fig. 1:

A- Histogram showing effect of Dalacin-C on mother weight gain (M.W).

B- Histogram showing effect of Dalacin-C on placenta weight (P.W), fetus weight (F.W) and fetus length (F.L) at 20th day of gestation.





Values are expressed as Mean. The statistical differences were analyzed by Duncan's test. a= P ≤ 0.05 compared with control.

The uterus of control pregnant rats on day 20 of gestation showed normal distribution of the implanted fetuses between the two horns (Fig. 2). The uterus of pregnant rats treated with 0.3 mg/Kg showed normal shape and sometimes with asymmetrical distribution of fetuses in the two uteri, completely resorbed uterus also revealed (Fig. 3).

Fig. 2: A photograph of uterus of control pregnant rat at the 20th day of gestation.

Showing normal symmetrical distribution of fetuses in the two uteri horns. U= Uterus, V= Vagina.

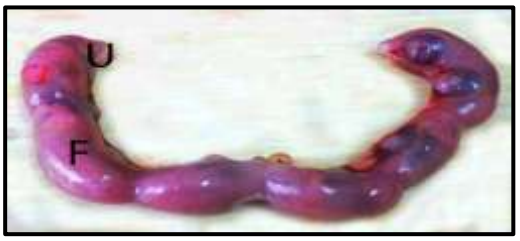
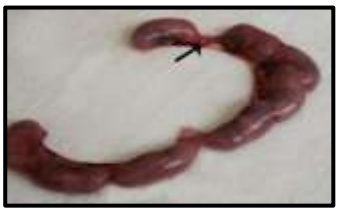


Fig. 3: Photographs of uterus of pregnant rat treated with 0.3 mg/Kg of Dalacin-C at the 20th day of gestation.



The morphological examination of the fetuses showed that the Dalacin-C caused growth retardation represented by adecrease in fetal body weight and body length (Table 1 &Fig. 1). There was a significant (P≤ 0.05) reduction in fetus weight and fetus length in treated groupwhen compared with the control group (A).

The fetus from control animals appeared with normal shape, correct weight and length (Fig. 4), also appeared straight dorsally. The malformations are not recorded in fetuses from treated group except one or two cases represented in hind limb shortness and hematoma that detected in the neck region (Fig. 5).

Fig. 4: A Photograph of fetus of control mother at 20th day of gestation.Fetus exhibited normal morphology and normal length.

Fig. 5: Photographs of fetuses of maternally treated with 185 mg/Kg of Dalacin-C at 20th day of gestation. Showing:

Fetuses showed deformed hind limbs (arrow) and hematoma (Thick arrow)

Table 1: Showing effect of Dalacin-C on fetus weight, fetus length, placenta weight and mother weight gain at 20th day of gestation.

Group	Fetus weight (F.WT)	Fetus length (F.L)	Placenta weight (P.WT)	Mother weight gain (M.WT)
Control (A)	4.91±0.067	6.00±0.449	0.511±0.006	43.58±3.34
Group (B)-185 mg/Kg	4.19±0.101[a]	2.75±0.293[a]	0.418±0.025[a]	17.33±4.02[a]

Fetal mortality:

Total mortality rate included resorbed fetuses and stillbirth (dead fetuses at birth) and was recorded for both control and treated groups in table 2.

Table 2: Effect of Dalacin-C on fetal mortality at the 20th day of gestation.

Groups	Total no. of pregnant rats	No. of implantation sites	No. of live fetuses		No. of resorbed fetuses		No. of dead fetuses	
Control (A)	6	54		100%	0	0 %	0	0 %
Treated Groups (5th-20th) mg/Kg (B)	7	64	54	84.4%	10	15.6%	0	0%

Values are expressed as Mean ± SEM. The statistical differences were analyzed by independent samples T test. a= $P \leq 0.05$ compared with control.

B. Skeletal anomalies

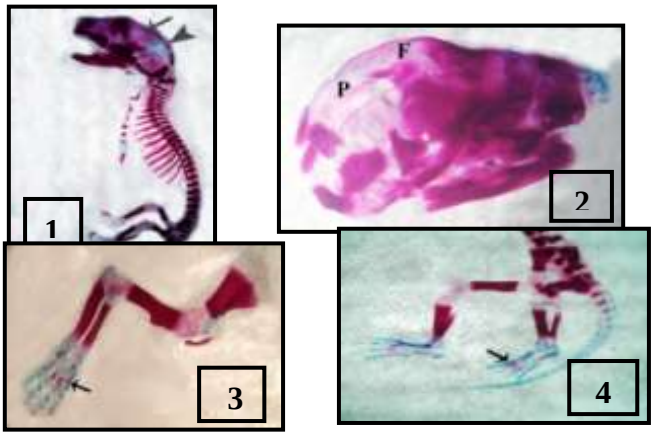
At the 20th day of gestation, the cleared cartilage and bone preparations of control rat fetuses have designated that in all parts of the axial skeleton skull, vertebrae and ribs as well as appendicular skeleton comprising the fore and hind limbs, pectoral and pelvic girdles, both chondrification and ossification processes have been obviously completed. The cartilaginous parts of the skull included the nasal region (Fig. 6). On the other hand, fetuses maternally treated with 185mg/Kg of Dalacin-C showed lack of ossificationof the skull roof (frontal and parietal), dalaye ossification of metacarpus, metatarsus and phalanges (Fig. 7).

Fig. 6: A Photomicrograph of a skeleton of a fetus from control mother. H&E stain.

Fig. 7: A Photomicrograph of a skeleton of a fetus from treated mother. H&E stain. Showing:

1) Incomplete ossification parietal (arrow) and interpareital (head arrow).
2) Delay of frontal ossification (F) and un-ossified parietal bone.

3-4) Weak ossified metacarpus (arrow) and metatarsus (arrow) respectively.



C. Histological studies of fetuses

Liver

Light microscopic examination of sections of the fetal livers of controlgroup using H&E stain showed ill-defined demarcation of hepatic lobules andthe interlobular connective tissue was poorly developed. In each hepatic lobule, hepatocytes were arranged as irregular, branching and interconnected cords originating from a central vein and goes peripherally. Blood sinusoids were seen between the hepatic cords. The cytoplasm was abundant, granular and stained acidophilic. The nucleus was euchromatic, located centrally, rounded, and contained one or more nucleoli (Fig. 8).
Changes in liver histology such as degeneration of the cytoplasm of hepatocytes and increases in the number of megakaryocytes were seen in the groups receiving Dalacin-C during the pregnancy (Fig. 9).

Fig. 8: A Photomicrograph of a section of liver of a fetus from control mother. H&E stain.Showing normal architecture of the liver tissue.The hepatic lobules that can be only distinguished by their central veins (CV) and hepatocytes (H) and numerous erythroblasts (arrow).100X

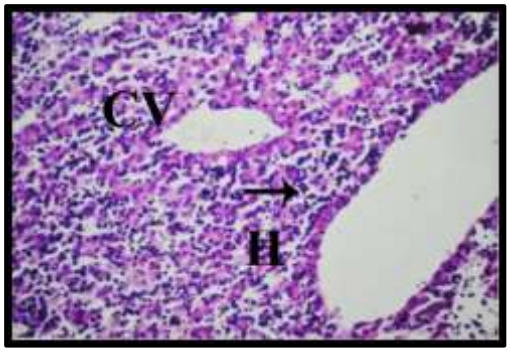
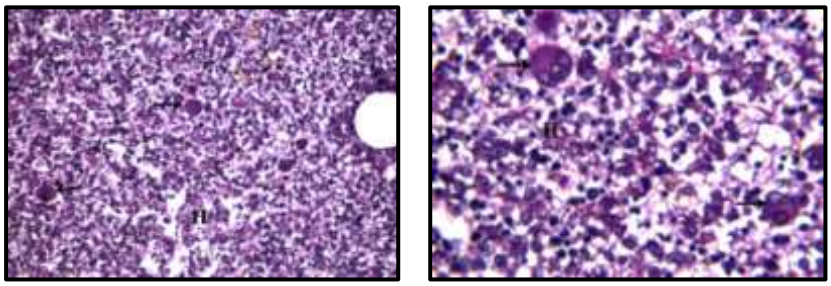


Fig. 9:

A- A Photomicrograph of a section of liver of a fetus from treated mother. H&E stain. Megakaryoblasts (arrow) were detected in between the degenerated hepatocytes (H) in diffuse manner all over the parenchyma.100X

B- A Photomicrograph showing the magnification of Fig. 9A. 400X



Kidney

Examination of the kidney of control fetus revealed that it is differentiated in to outer cortex and aninner medulla, which is formed of conicalpyramids. Each medullary pyramidwith the corresponding part of the cortexrepresents a renal lobe which consists of theuriniferous tubules and stromal tissue.The uriniferous tubule is composed of thenephron which is formed of the Malpighiancorpuscle, the proximal convoluted tubule, thedescending and the ascending limbs of Henle'sloop and the distal convoluted tubule. Eachcorpuscle consists mainly of a tuft of bloodcapillaries, or glomerulus and a Bowman'scapsule which is a double walled cup formedof two layers, an outer parietal layer and aninner visceral layer separated by a clear, distinctcapsular space (urinary space) (Fig. 10).

Examination of the kidney of fetus maternally treated with 185 mg/Kg, revealed degeneration in the tubular lining epithelium with swelling in the endothelial cells lining the tufts of the glomeruli within the Bowman's capsule as recorded in Fig. 11.

Fig. 10: Photomicrograph of a section of kidney of a fetus from control mother. H&E stain.Showing a part of the cortical region containing, a glomeruli (G) within Bowman's capsule (BC) tubules (T).100X

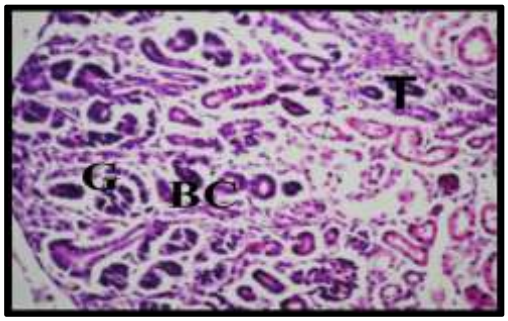
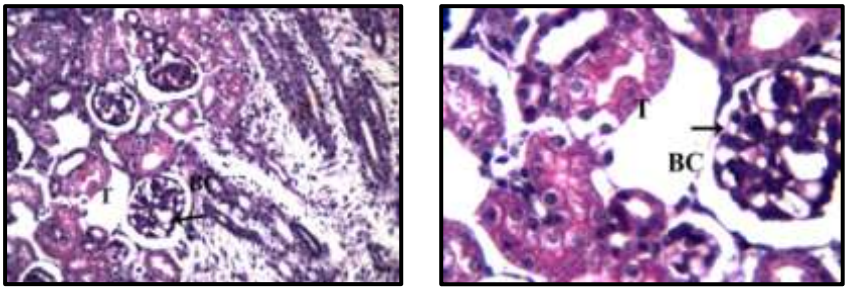


Fig. 11:

A- A Photomicrograph of a section of kidney of a fetus from treated mother. H&E stain. There was degeneration in the tubular lining epithelium (T) with swelling in the endothelial cells lining the tufts of the glomeruli (arrow) within the Bowman's capsule (BC).

B- A Photomicrograph showing the magnification of Fig. 9A.400X



Brain

The brain tissues of fetuses from control pregnant rats showed, showed normal features under microscopic observation (Fig. 12).

Examination of the brain of fetus maternally treated with 185 mg/Kg, revealed no histopathological alterations as recorded in Fig. 13.

Fig. 12: A Photomicrograph of a section of brain of a fetus from control mother. H&E stain.Showing normal structure of brain tissue, CC= cerebral cortex.

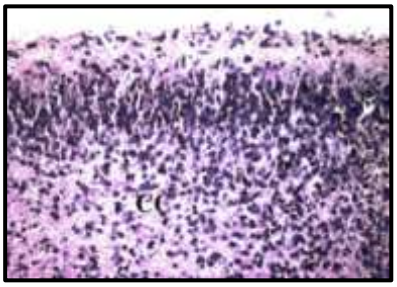
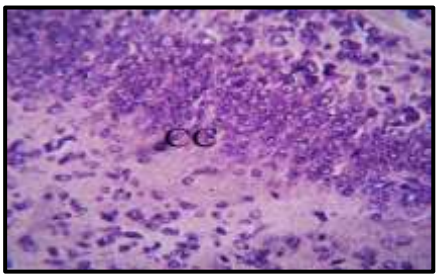


Fig. 13: A Photomicrograph of a section of brain of a fetus from treated mother. H&E stain.There was no histopathological alteration.100X



4. DISCUSSION

The present study is carried out to evaluate the teratogenic potential of Digoxin on the fetuses maternally treated with 0.3 mg/Kg of the used drug during the gestation. However, many of the cardiovascular drugs that are prescribed for a pregnant woman have the potential to cross the placenta and exert a pharmacologic or even teratogenic effect upon the fetus.

Teratology, the study of abnormal prenatal development and congenital malformations induced by exogenous chemical or physical agents, is a growing area of medical research in the quest for the eradication of preventable birth defects. Birth defects are known to occur in huge numbers; roughly 7~10% of all children require extensive medical care to diagnose or treat a birth defect; this compromises the quality of life of millions of people worldwide (O'Rahilly, 2001). Almost all therapeutic agents cross placental barrier and enter fetal circulation. Every agent given during pregnancy therefore has a tendency to produce some sort of structural abnormality in the neonate at birth until proved otherwise (Schlegel *et al.,* 1991). A birth defect or a congenital malformation is a structural abnormality of any type present at

birth. It may be macroscopic or microscopic, on the surface or within the body (Moore, 1988). During the past few decades, it has become increasingly evident that human and animal embryos are subjected to the toxic effects of many drugs, such as the use of some antibiotics in the treatment of serious diseases occurring during pregnancy.

There are many physiologic changes that occur during pregnancy, including increased demands on the cardiovascular system. Physiologic changes in the cardiovascular system include peripheral vasodilation resulting in decreased systemic vascular resistance, requiring increased cardiac output. This increase in cardiac output is accomplished by an increase in ventricular end-diastolic volume, wall mass, and contractility, which creates an increase in stroke volume and heart rate. Due to these alterations, pregnant women are placed at higher risk for developing comorbidities such as cardiac arrhythmias that range from benign to life threatening (Burt and Durbridge, 2009 and Joglar and Page, 2014).
In the present study, oral treatment of pregnant rats with Dalacin-C during the gestation led to a significant reduction in the weight, length and tail length of fetuses. The observed fetal growth retardation may be arising from the direct action of the used drug on embryos and fetal tissues.

The current work revealed that Dalacin-C treatment induced complete fetal resorption when rats were given 185 mg/kg/day of Dalacin-C at gestational days during organogenesis period.

The present work showed few cases of external malformations and skeletal abnormalities. The most repeated anomalies observed were hematoma, hind limb defects, unossified skull bones and Weakossification of phalanges.
The results obtained from the present study showed that administration of the therapeutic doses in organogenesis period induced various changes in liver and kidney of fetuses maternally treated with 185 mg/kg of Dalacin-C from 5th day to 20th day of gestation. Liver showed degeneration of the cytoplasm of hepatocytes and increases in the number of megakaryocytes. The fetal liver assumes the primary role of blood cell development at mid- and late-gestation.20 The histopathological changes in liver that were observed with alcohol treatment may affect haematopoietic cell trafficking from the liver to other sites. Kidney revealed degeneration in the tubular lining epithelium with swelling in the endothelial cells lining the tufts of the glomeruli within the Bowman's capsule.

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