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TERATOGENIC EFFECT OF METHOTREXATE ON HISTOGENESIS OF TESTIS IN NEWBORN RAT

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ABSTRACT

Background: Methotrexate (MTX) was one of the first drugs synthesized for a specific chemotherapeutic purpose used to inhibit folic acid (FA) for the treatment of acute lymphoblastic leukemia in children. Its history is closely related to the discovery and characterization of folic acid. It is used clinically in medicine to treat a range of cancerous and noncancerous conditions. MTX is currently used in gynecology to treat disorders arising from trophoblastic tissue, namely, ectopic pregnancy. MTX, the most frequently used disease-modifying anti-rheumatic drug (DMARD), suppresses disease activity and reduces joint damage. **The aim of study:** It is designed to demonstrate the effect of MTX (7.5 mg/wk.) on the histogenesis of gonad (testis) of newborn Albino rat. **Material & Methods:** Twenty pregnant female rats taken, classified equally to 10 control and 10 treated with MTX intramuscularly (first dose at day 15 of gestation and second dose at two day after birth). After normal vaginal delivery, newborns harvested at day seven. For histological study the newborn gonad (testis) were fixed in Bouin's fixative, paraffin infiltration, and then embedded sections stained with haematoxylin and eosin, the specimens independently read. **The result:** The histological investigation showing atrophy of seminiferous tubules, increase interstitial space with reduce interstitial connective tissue and leydig cell, destruction of basement membrane, complete detachment of spermatogenic cell from basement membrane, atrophy of spermatogenic cell, swelling spermatogenic cell, degeneration sertoli and leydig cell, cell death, congested blood vessels and hemorrhage. The statistical analysis at day seven shown significant decrease ($P<0.05$) in newborn weight compared with control group. Highly significant increase ($P<0.001$) in diameter of testes. Significant decrease ($P<0.01$) in diameter of seminiferous

tubules. Significant decrease (P<0.05) in numbers of spermatogenic cell in seminiferous tubules. **The conclusion:** The findings of the present study show that therapeutic dose of methotrexate is capable of producing histological changes in newborn's testis at day seven.

KEYWORDS: methotrexate, newborn's rat testis and hitogenesis.

INTRODUCTION

Methotrexate (MTX) was first synthesized for a specific chemotherapeutic purpose which was to inhibit folic acid synthesis in the cells of acute lymphoblastic leukemia in children, Nowadays the drug is being used at low dose to treat anti-inflammatory conditions and the treatment of various autoimmune diseases, including rheumatoid arthritis, lupus, psoriasis, juvenile idiopathic arthritis and ectopic pregnancy while high dosage is used to treat different types of malignancies.[1]

Inside the cells MTX is converted to MTX polyglutamates which are the active compound that may be retained in the tissue for months.[2] MTX is a structural analog of folic acid that can interfere with intracellular folate metabolism by binding to dihydrofolate reductase (DHFR) resulting in decreasing bioavailability of tetrahydrofolate which is an important cofactor in thymidylate synthesis and de novo purine synthesis, thereby MTX Inhibits deoxyribonucleic acid (DNA) synthesis and cause depletion of nucleotide which affects cells capability to carry out the excision repair of DNA damage.[3]

MTX has been proved to be transferred through the placenta by the presence of the drug in the umbilical blood of a woman who received the drug hence it causes chromosomal aberrations in the neonate.[4] Chemotherapeutic doses of MTX produces distinct pattern of central nervous system, craniofacial and skeletal defects while The risk of fetal abnormalities at rheumatological doses of MTX (5–25 mg weekly) is less clear, there have been several reports of increased rates of spontaneous abortion and major fetal anomalies, as well as successful pregnancies that result in a healthy infant.[5]

MATERIAL AND METHODS

The experiments were performed on 20 mature female albino rat (*Rattus rattus*), their ages ranged between 2-6 months with a body weight ranging between 150-250g. These rats were divided into two groups: 10 control group and 10 treated group. Vaginal smear were performed to all the adult female rats to diagnose the stages of estrus cycle, to detect heat

stage for mating. Females in the estrus phase were left with mature healthy males for mating. The occurrence of vaginal plug considered as the day zero of pregnancy.[6] The pregnant female was removed into separate cages. The treated group injected with MTX (7.5 mg/wk.), first dose at day 15 of gestation and second dose at two day after birth, after normal vaginal delivery, newborns harvested at day seven and their testes removed. For histological study 10 treated newborn's testes were fixed in Bouins fixative for 4 hrs, then dehydration, infiltration with paraffin and embedded sections were stained with haematoxylin and eosin.[7]

RESULTS

Histological study of newborn's testis day seven of control group show the normal structure of testis, which consist of circle or ovoid seminiferous tubules, spermatogenic cell arrangement on basement membrane inside seminiferous tubules, supporting cell whiche is sertoli cell, interstitial tissue contain leydig cell and multinucleated cell giant cell inside seminiferous tubules (Fig. 1). While treated group show atrophy of seminiferous tubules, increase interstitial space with reduce interstitial connective tissue and leydig cell, destruction of basement membrane, complete detachment of spermatogenic cell from basement membrane, atrophy of spermatogenic cell, swelling spermatogenic cell, degeneration sertoli and leydig cell, cell death, congested blood vessels and hemorrhage (Fig. 2, 3, 4).

The statistical analysis shown significant decrease ($P<0.05$) in newborn weight compared with control group, where the mean weight of control group at day seven (12.914±0.228) while treated group (6.648±0.14). Highly significant increase ($P<0.001$) in diameter of testes, where mean diameter of newborn's testis in day seven of control group (1537.986±8.235) while treated group (1660.226±5.17). Significant decrease ($P<0.01$) in diameter of seminiferous tubules, where mean diameter in day seven control group (62.875±1.503) while treated group (48.304±1.119). Significant decrease ($P<0.05$) in numbers of spermatogenic cell in seminiferous tubules, where mean numbers in day seven control group (15.80±0.416) while treated group (14.40±0.4) as shown in table (1).

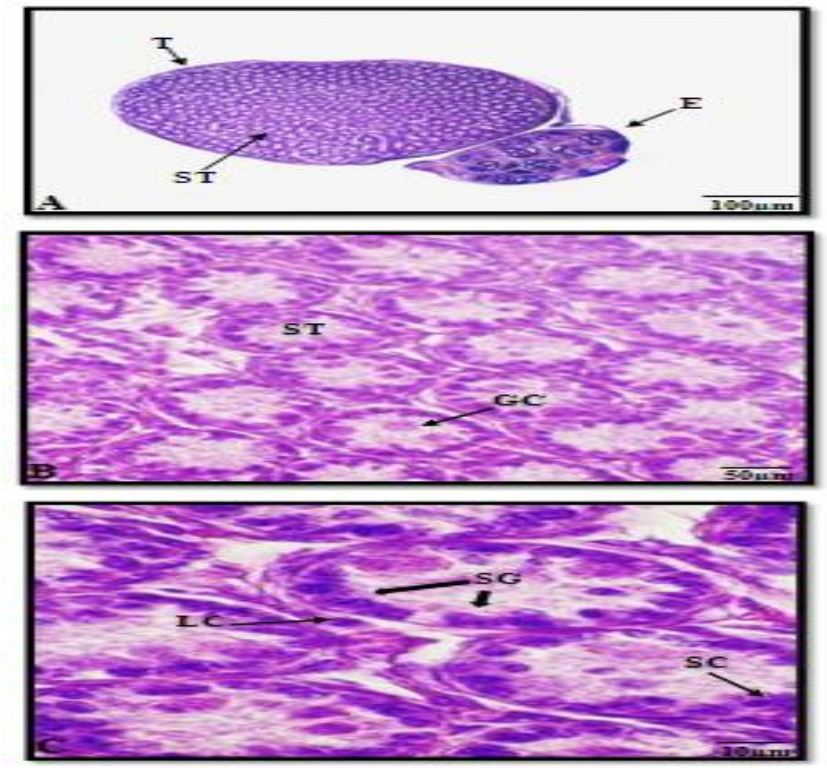

Figure (1): Cross sections of newborn's testis day seven of control group showing: A: normal structure of testis (T) and epididymis (E) 10X. B: seminiferous tubule (ST) and giant cell (GC) 40X. C: spermatogenic cell (SG), sertoli cell (SC) and leydig cell (LC) 100X. (H&E).

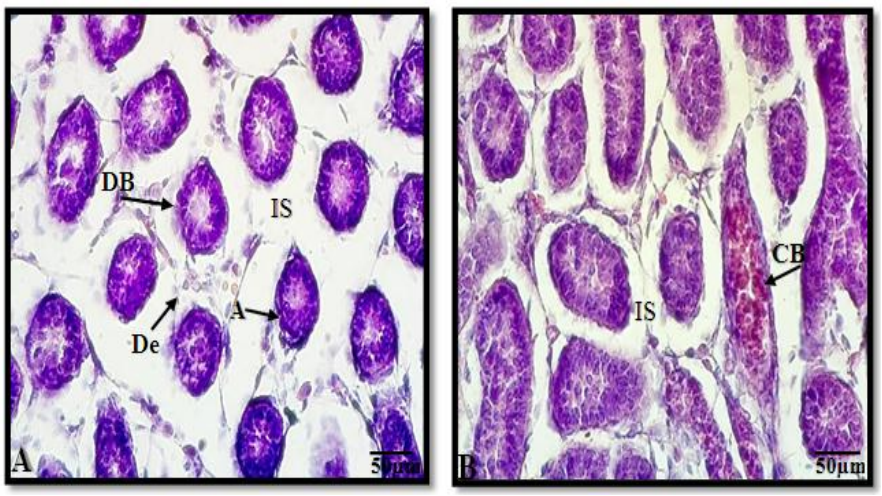

Figure (2): Cross sections of newborn's testis day seven of treated group with MTX (7.5 mg/wk.) showing: A: atrophy of seminiferous tubules (A), increase interstitial space with reduce interstitial connective tissue and leydig cell (IS), destruction of basement membrane (DB) and degeneration leydig cell (De). 40X. B: congested blood vessels (CB) 40X. (H&E).

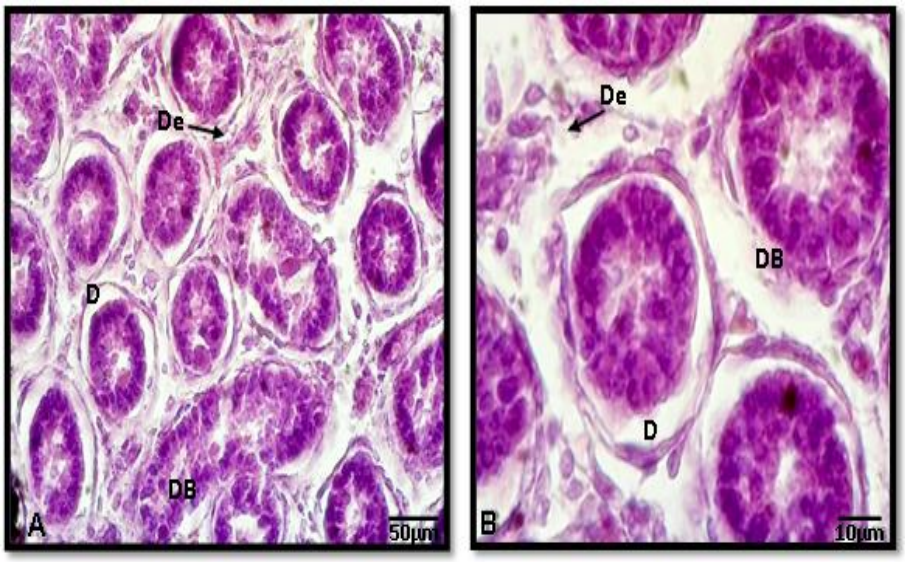

Figure (3): Cross sections of newborn's testis day seven of treated group with MTX (7.5 mg/wk.) showing complete detachment of spermatogenic cell from basement membrane (D), destruction of basement membrane (DB) and degeneration leydig cell (De). A: 40X. B: 100X. (H&E).

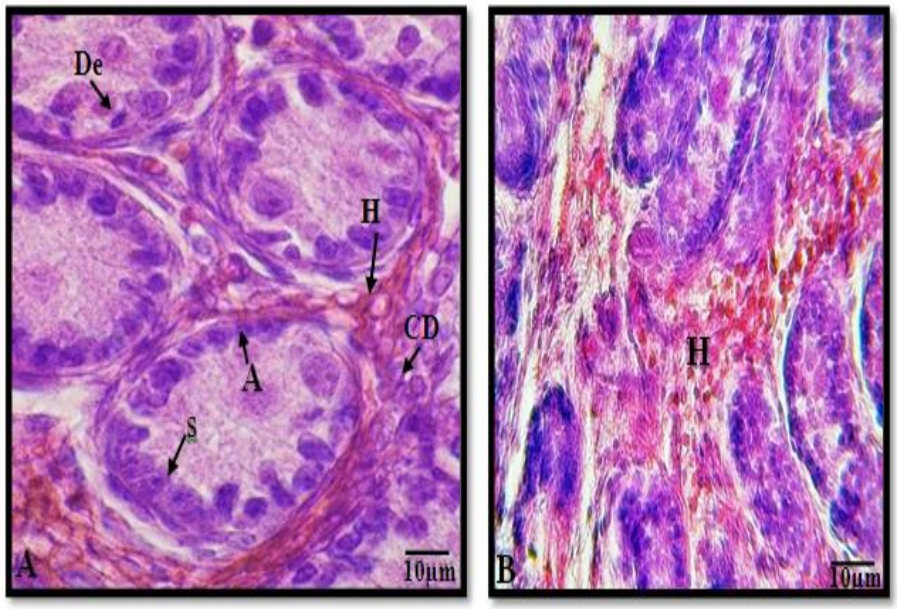

Figure (4): Cross sections of newborn's testis day seven of treated group with MTX (7.5 mg/wk.) showing: A: atrophy of spermatogenic cell (A), swelling spermatogenic cell (S), degeneration sertoli cell (De), cell death (CD) and hemorrhage (H) 100X. B: hemorrhage (H) 100X. (H&E).

Table (1): Effect of MTX on the parameter of newborn's testes day seven.

parameters	Day 7	
	Control group	Treated group
Weight of newborn (gm) (Mean±S.E.)	12.914±0.228	*6.648±0.14
Diameter of testis (µm) (Mean±S.E.)	1537.986±8.235	**1660.226±5.17
Diameter of seminiferous tubules (µm) (Mean±S.E.)	62.875±1.503	48.304±1.119***
Number of spermatogenic cells (Mean±S.E.)	15.80±0.416	14.40±0.4*

* Significant differences (P<0.05)

** Significant differences (P<0.001)

*** Significant differences (P<0.01)

DISCUSSION

Chemotherapeutic agents are extensively used for the treatment of various types of cancers. These drugs bring drastic improvement in the illness of patient as well as increase the life expectancy of cancer patients. But most of chemotherapeutic agents are mutagenic and carcinogenic.[8] Koehler et al. were the first to find out effects of methotrexate on rabbit testis. They evaluated fertility rate and spermatogenetic activity using tubular fertility index.[9] Badri et al. reported decrease in steroidogenesis due to decrease in testosterone level by effect of methotrexate after intramuscular injection.[10]

The present study show detachment of spermatogenic cell from basement membrane of seminiferous tubule in newborn's testis day seven, this result agree with other study.[11] Van dewater *et al.* shown detachment of cell from basement membrane may cause cell death due to loss of cytoskeleton and destruction of plasma membrane.[12]

The present study show destruction basement membrane of seminiferous tubule in newborn's testis day seven, this result agree with other study.[13]

In newborn's testis day seven show atrophy of seminiferous tubules this result agree with other studies.[14] [15] and increase interstitial space with reduce interstitial connective tissue and leydig cell in newborn's testis day seven this result agree with other research.[16] Shrestha *et al.*[17] revealed increased interstitial space.

The other effect is atrophy of spermatogenic cell inside the seminiferous tubules shown in newborn's testis day seven, this due to decreases the cell substance and results in cell

shrinkage.[18]

In newborn's testis day seven show congested blood vessels, Balasubramanian *et al.*[19] explained the congestion of blood vessels as being due to the inhibition of prostaglandins synthesis, since these compounds are known to be involved in the regulation of testicular blood flow. Robbins and Kumar[20] also showed the congestion of blood vessels caused by acute inflammation which causes changes in blood flow inside the vessels and gets relaxation and dilation in the blood vessels that lead to accumulation of blood inside the vessels.

Also degeneration of sertoli and leydig cell in newborn's testes day one and seven and degeneration of stromal cell in newborn's ovary day one and seven, this result agree with other study.[21] They suggest the cause of degeneration either due to drug accumulation in lysosomes then rupture the cell and death, or due to inhibition of protein synthesis.[22] Shapiro and Harper[23] refers that the drug may cause cell death due to damage DNA or Spindle apparatus. Also degeneration of cell may be due to destruction of cytoskeleton and disappears plasma membrane.[24]

The statistical analysis of the current study shown significant decrease ($P<0.05$) in newborn weight compared with control group. Embryonic growth delay induced by folic acid antagonists was first described in chickens by Karnofsky.[25] Highly significant increase ($P<0.001$) in diameter of testes compared with control group in day seven, this increase due to increase interstitial space. Heidari *et al.* seems that probably opiates could affect testicular volume and induce arrested spermatogenesis, sloughed germinal epithelium, destroyed Sertoli cells and thickened and irregular basement membrane as well as signs of apoptosis.[26] Significant decrease ($P<0.01$) in diameter of seminiferous tubules in newborn's testes compared with control group in day seven. Shreshta *et al.*[17] studied effect of intraperitoneal injection of methotrexate on rat testis. They revealed decreased diameter of seminiferous tubules, increased interstitial space as well as distortion of morphology of Leydig cells. Significant decrease ($P<0.05$) in numbers of spermatogenic cell in seminiferous tubules in newborn's testes compared with control group in day seven. This result agree with other study.[27] Other study[28], they showed that the Metformin may be interfere with normal physiology of testicular processes, leading to spermatogenic failure and subsequently decrease in the number of spermatogonia cell/ tubule.

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