# Study The Effects Of Methotrexate (MTX) On Some Parameters Of Sperms And Histopathological Changes Of Testis Mice

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## Abstract

Methotrexate commonly used to treat cancer and caused reproductive damage in mice , an early sign of which is sloughing of germ cells with associated sertoli cell fragments. In this study, the effects of methotrexate on some parameters of sperms and histopathological on mice testis after 35 days of MTX administration intraperitoneally (ip). Twenty-four adult male mice were divided into one control and two treated groups composed of 8 mice in each group. Treated groups received methotrexate in two different doses i.e 25 µg and 50 µg, whereas control one received normal saline intraperitoneally, the results showed significant ($P<0.05$) decreased in sperms motility , increased percentage of dead sperms and abnormalities of sperms in mice treated with MTX, while the results showed significant decreased in diameters of seminiferous tubules, primary spermatocytes and spermatids, increased interstitial spaces in treated groups as well as distortion of morphology of leydig cells in treated mice.

## الخلاصة

يستخدم عقار الميثوتركسيت بصورة شائعة لعلاج الامراض السرطانية ، وعادة مايسبب ضررا تناسليا لدى الفئران من خلال احداث اضرار في الخلايا الجرثومية وتكسر خلايا سرتولي . وفي هذا البحث، تمت دراسة تاثير عقار الميثوتركسيت في بعض قياسات الحيامن والتغيرات النسيجية المرضية لخصى الفئران بعد ٣٥ يوما من المعاملة بالميثوتركسيت داخل الخلب البريتوني. تم تقسيم ٢٤ ذكرا ناضجا من الفئران الى مجموعة اولى لاغراض السيطرة ، ومجموعتين تمت معاملتهما بالعقار المذكور، تحتوي كل منها على ثمانية ذكور. تم اعطاء المجاميع المعاملة جرعا مختلفة من الميثوتركسيت بواقع ٢٥ و ٥٠ مايكروغرام على التوالي واعطاء المحلول الملحي الفسيولوجي الى مجموعة السيطرة. وقد اظهرت النتائج انخفاضا معنويا في حيوية الحيامن ، وزيادة معنوية في نسب الحيامن الميتة والمشوهه في الفئران المعاملة بالميثوتركسيت ، و اظهرت النتائج انخفاضا معنويا في اقطار النبيبات المنوية ، وخلايا النطاف الاولية والنطاف ، وزيادة في المسافات البينية للنبيبات المنوية لخصى الذكور المعاملة بالميثوتركسيت بالاضافة الى وجود تغيرات شكلية في خلايا لايدك في مجموعة الفئران المعاملة.

## Introduction

Methotrexate is a mild immunosuppressant that also exhibits anti-inflammatory activity. Methotrexate is commonly used for the treatment of certain cancers including but not limited to leukemia, Hodgkin's disease and head and neck cancers. In these illnesses, methotrexate is used in very large doses so that it interferes with the reproduction of the cancer cells. Methotrexate is used in much smaller doses for the treatment of rheumatoid arthritis, Crohn’s disease and psoriasis [1].

Methotrexate is known to be teratogenic in women, but few data are available on the effects of methotrexate on male reproductive capability. Studies in animals have shown altered spermatogenesis, cytotoxicity and degeneration of spermatocytes, Sertoli cells, and Leydig cells [2,3]. In patients with psoriasis who were treated with methotrexate, there are case reports of oligospermia that resolved when the methotrexate was stopped [4].The effects of MTX also documented in reproductive system. It induces cellular alteration in male gonads (testes) of human being as well as in animals [5,6].But there is little documentation in literature regarding mode of action and mechanism of cell death on testes during proliferative stage of reproductive system after long term continues exposure of MTX on an animal model. The aims of this study is to evaluate the effects of chemotherapy drug (methotrexate) on some parameters of sperms and histopathological on testes.

## Materials and Methods:

### Animals, Treatment of males

Adult male mice (30-36 gm) were purchased from Biotechnology Research Center and maintained on a 14:10-hour light dark cycle in the animal house control and treated mice were provided with food and water ad libitum; there were no differences in food intake. One week after arrival males were randomly divided into 3 treatment groups, each composed of 8 mice. Mice were treated with the 25 µg and 50 µg of methotrexate given intraperitoneally were administered for 35 days [7] and the third group was administration normal saline as a control group. The animals in each group were sacrificed by dislocation of cervical vertebrate. testes fixed with Bouin fluid (BDH Inc, Toronto, Canada). Spermatozoa were obtained from the two tails of epididymies by mincing in 500 µl TCM-199, and maintained at 37°C in 5% CO2 incubator and the percentages of motility , dead and abnormalities of spermatozoa were measured**.**

### Histological Examinations:-

The perfuse-fixed testes placed in Bouin fluid overnight, and processed for routine paraffin embedding. The testes were cut into 5-µm sections. Three serial sections per testes were mounted on slides, deparaffinized, rehydrated, and stained with hematoxylin - eosin stain. Sections of the testes were examined by light microscope. Seminiferous tubules, interstitial spaces, primary spermatocytes and spermatids diameters were assessed in each testes using a previously calibrated Micrometers (Ocular micrometer, Stage micrometer).

The diameter of 25 seminiferous tubules were measured in 5 fields (5 seminiferous tubules per field). In similar manner diameter of primary spermatocytes, spermatids, leydig cells were measured in 5 fields and the mean value of each was calculated. The interstitial space observed between to consecutive seminiferous tubules by using the ocular micrometer.

### Microscopical examination

Spermatozoa were assessed according to WHO laboratory manual [8] for viability , percentage dead/live spermatozoas, motility and abnormalities.

### Statistical analysis

Statistical analysis was performed to compare two different groups by using ANOVA-test. Statistical significance was determined at $P<0.05$. [9].

## Results and Discussion

Methotrexate is a well-known anti-cancer agent used for the treatment of malignant and non-malignant conditions. In recent years, large number of reports has been published on potential gonadal damage. In this study the results showed significant decreased of motility , increase abnormalities of sperms (Figure.1) , while percentage of dead sperms increased significantly in the groups treated with MTX compared with control group which showed normal sperms (Figure.2 and Table 1).

**Table 1: percentage of sperms motility, dead sperms, sperms abnormalities in treated and control groups.**

| Groups | Motility of sperms (%) (mean+SD) | Dead sperms (%) (mean+SD) | Abnormalities of sperms (%) (mean+SD) |
|---|---|---|---|
| **Treated with 25 µg of MTX** | B<br>55.00+8.21 | B<br>23.85+4.69 | B<br>20.83+3.61 |
| **Treated with 50 µg of MTX** | B<br>50.00+7.53 | B<br>27.07+3.82 | B<br>26.41+4.89 |
| **Control** | A<br>80.00+6.32 | A<br>15.32+4.61 | A<br>12.94+2.63 |

Differences A, B are significant (P<0.05) to compression rows

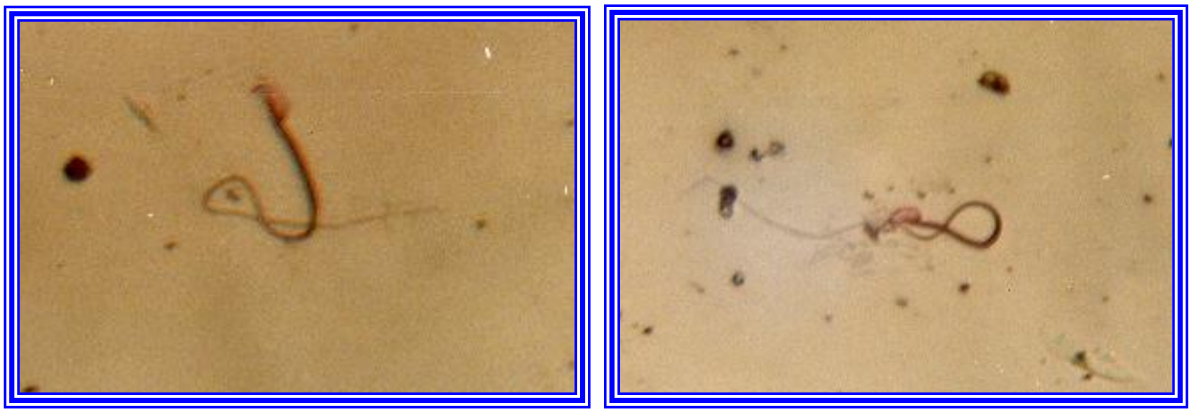

**Figure 1: Showed abnormal sperms treated with MTX from treated groups (E X40)**

**Figure 2: Showed normal sperms treated with normal saline from control group(E X40)**

Methotrexate, an immunosuppressive drug used to treat cancer, psoriasis, and rheumatic diseases, is a folic acid antagonist that binds to the enzyme dihydrofolate reductase. This inhibits synthesis of thymidylate, serine, and methionine, which disrupts sythesis of DNA, RNA, and protein and leads to cell death [10].

Several case reports and series have documented reversible sterility in men using methotrexate.[11,12] They reported a decrease in sperm count or quality with use of the agent. When the medication was discontinued, the sperm returned to normal levels and quality. Van Scott and Reinertson[13] reported a decrease in sperm count 12 to 14 days after a single intravenous injection of methotrexate, but patients were not followed up to determine whether this was temporary. Shamberger et al[12] also observed an age-dependent effect in terms of reversibility of the altered spermatozoa; men younger than 40 were more likely to experience recovery. Azoospermia was confirmed in men how treated with MTX.

A case series published in 1970[14] reported no change in sperm concentration, motility, or morphology in 11 men treated with methotrexate. Semen was analyzed both before and during long-term treatment with the medication. Grunnet et al[15] compared the ejaculates of 10 men using topical corticosteroids for severe psoriasis with 10 men using methotrexate therapy for the same indication. They found no adverse effects of methotrexate on semen quality. In fact, men treated with methotrexate were more likely than men treated with corticosteroids to have normal semen. De Luca et al[16] also reported minimal to no suppression of spermatogenesis with methotrexate therapy.

Another study of 26 patients with psoriasis who had received methotrexate found no abnormalities in semen, testicular histology, and spermatogenic function, although there was no long-term follow-up to exclude teratogenocity in the off spring [17].

Administration of MTX is known to cause reproductive damage, including decreased epididymal and testicular weights, and reduced epididymal sperm counts and fertility[18]. Histopathology of the testis is characterized by vacuolization of Sertoli cells, and sloughing of elongating spermatids and spermatocytes when damage is severe [19]. A corresponding decrease in the number of microtubules in Sertoli cells after MTX treatment has been observed using electron microscopy and immunohistological techniques [20].

The results showed significant decreased in diameters of seminiferous tubules (table 2)figure.4 , primary spermatocytes, spermatids (table 3) and increased in interstitial space (table 2) figure.4 when treated with MTX compared with control group (figure.3) this due to antimitoic activity of the drug, The diameter of leydig cells (table 3) in the present study did not reveal any alteration after MTX administration with respect to controls.(figure.5) This could be due to either increasing age for heterogeneous population of leydig cells in the testes[21] which made non-significant results hrough changes in morphological form from round to oval in shape was observed (figure.6).

**Table 2: Diameter of seminiferous tubules and interstitial space in both treated and control groups.**

| Groups | Diameter of seminiferous tubules (µm) (mean±SD) | Interstitial space (µm) (mean±SD) |
|---|---|---|
| **Treated with 25 µg of MTX** | B<br>186.60+12.43 | B<br>32.61+1.05 |
| **Treated with 50 µg of MTX** | B<br>173.71+13.62 | B<br>41.80+3.52 |
| **Control** | A<br>209.32± 3.63 | A<br>20.11+1.68 |

Differences A, B are significant (P<0.05) to compression rows

**Table 3: Diameter of primary spermatocytes, spermatids and leydig cells in treated and control groups.**

| Groups | Primary spermatocytes(µm) (mean±SD) | Spermatids(µm) (mean±SD) | Leydig cells(µm) (mean±SD) |
|---|---|---|---|
| **Treated with 25 µg of MTX** | B<br>5.10+0.72 | B<br>3.4+0.83 | A<br>4.63+0.89 |
| **Treated with 50 µg of MTX** | B<br>4.38+0.80 | B<br>2.67+0.54 | A<br>3.91+0.52 |
| **Control** | A<br>6.20+0.72 | A<br>4.18+0.34 | A<br>4.21± 0.24 |

Differences A, B are significant (P<0.05) to compression rows

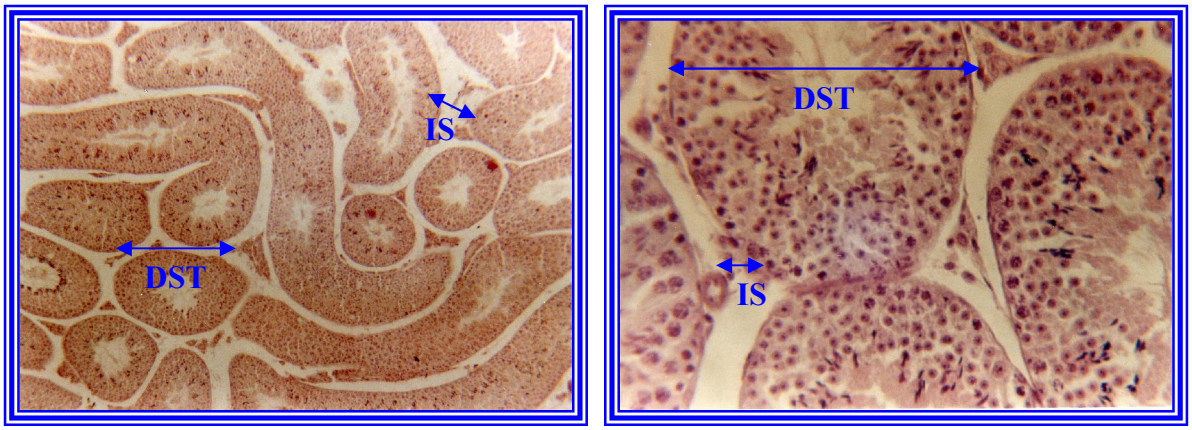


Figure 3:Photomcirograph of testes of mice (control group) showing normal structure of seminiferous tubules. (DST- Diameter of Seminiferous tubules, IS- Interstitial space) (H and E X 10).

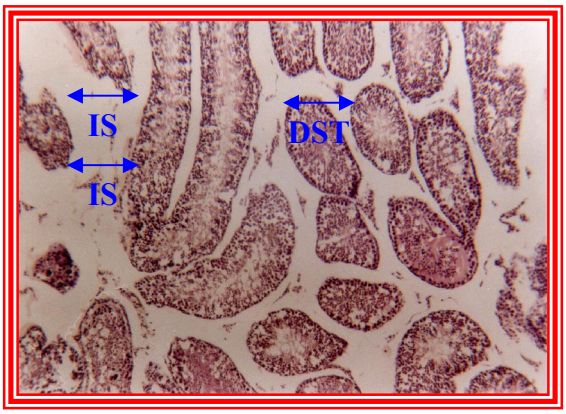


Figure 4:Photomicrograph of testes of mice (treated group) showing decrease in diameter of seminiferous tubules (DST) and increase of interstitial space (IS) (H and E X 10).

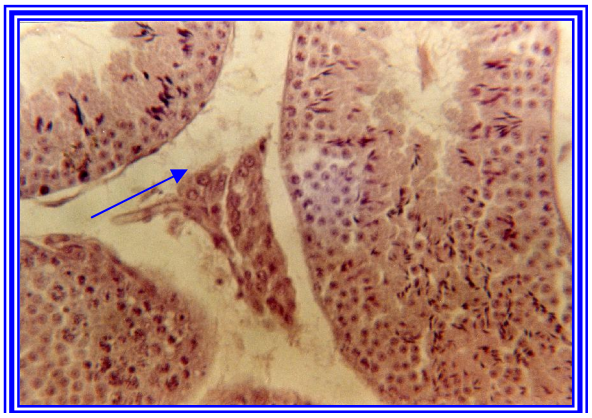

Figure 5:Photomcirograph of testes of mice (control group) showing normal structure of Leydig cells (LSC). (H & E X 40)

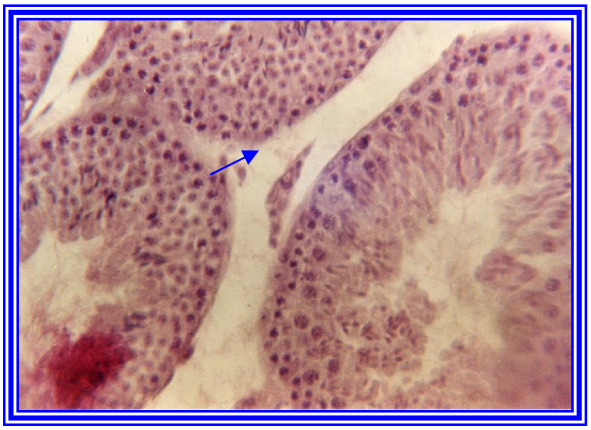

Figure 6:Photomcirograph of testes of mice (treated group) showing the heterogeneous population of Leydig cells with alterations (ALSC) in morphological features. (H & E X 40)

replicate DNA due to inhibition of an essential enzyme dihydrofolate reductase required for normal DNA synthesis.15 Therefore, it can be concluded that these qualitative and quantitative changes in male gonads may alter the reproductive performance of animals, if not reversible in nature. However, further study is required at ultra-structural and molecular level to explore the mechanism of action of methotrexate.

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