HISTPATHOLOGICAL EFFECTS OF METHOTREXATE ON MALE AND FEMALE REPRODUCTIVE ORGANS IN WHITE MICE

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(Received 22 September 2008, Accepted 19 March 2009,)

Keywords; methotrexate**,** spermatogenesis**,** ovaries

ABSTRACT

The present study was conducted on (40) white mice of approximately the same age (4-6 weeks) and body weight (23-25 gm) for the aim of observing the histopathological changes for male and female reproductive organs due to prolonged treatment (6 months) with anticancer chemotherapeutic agent namely methotrexate.

Forty mice were divided into 4 groups (10 mice of each group 5 mice per sex). The first group (low or therapeutic dose group) was received 0.15 mg/kg B.W. The second group (intermediate dose group) received 0.3 mg/kg B.W. The third group (toxic dose group) received 0.45 mg/kg B.W. the fourth group was a control group; it received 0.2 ml buffered physiological saline.

All these groups injected intramuscularly, once weekly for 6 months. The results showed that methotrexate can cause suppression of spermatogenesis. In female, methotrexate can cause obvious pathological changes in uteri and ovaries such as reduced endometrial glands and ovarian follicles respectively.

INTRODUCTION

Chemotherapy involves one or several antineoplastic (anticancer) drugs to inhibit cancer cells. Methotrexate is an example of an anticancer drug that interferes with cellular reproduction and is also used in the treatment of psoriasis and certain inflammatory disease (1).

Among the antimetabolic drugs, one of the most important and widely used is methotrexate. It's a chemical agent that acts as inhibiting the enzyme dihydrofolic acid reductase, which catalyses the conversion of folic acid into active form called as folinic acid by binding to it (***2***).The workers provided that methotrexate can cause major effects with different body organs, especially reproductive organs and several cases reports have documented reversible sterility in men using methotrexate (3).

There are important differences between male and female gametogenesis relevant to the effects of cancer therapy on fertility. The high rate of cell division makes the germ cells particularly sensitive to cytotoxic agents in contrast to the Ledig or Sertoli cells. Spermatogonia are the most sensitive to cytotoxic compounds with the other germ cells (4)

Oligospermia has been reported in association with methotrexate treatment (5)

Also female infertility may occur as a result of either oocyte or granulosa cell impairment because the granulosa cells are susceptible to cytotoxic drug (6).

The main problem in cancer chemotherapy is the lack of highly selectivity toxic agents. That means methotrexate used antiproliferative anticancer drugs, many rapidly dividing normal cells (bone marrow, gut epithelium, spermatogenic cells, lymphoid tissue, ovarian and uterine tissues and fetus) are also killed (7).

This study designed to have the knowledge of side effects of methotrexate on male and female reproductive organs.

Aims of study:

The aims of present study are to aid clinicians in the counseling and education of patients taking or about to start taking low dose methotrexate.

MATERIAL AND METHODS

Forty mice (20 males and 20 females) of 1-1.5 months old were divided into 4 equal groups (each groups consisted of 5 males and 5 females).

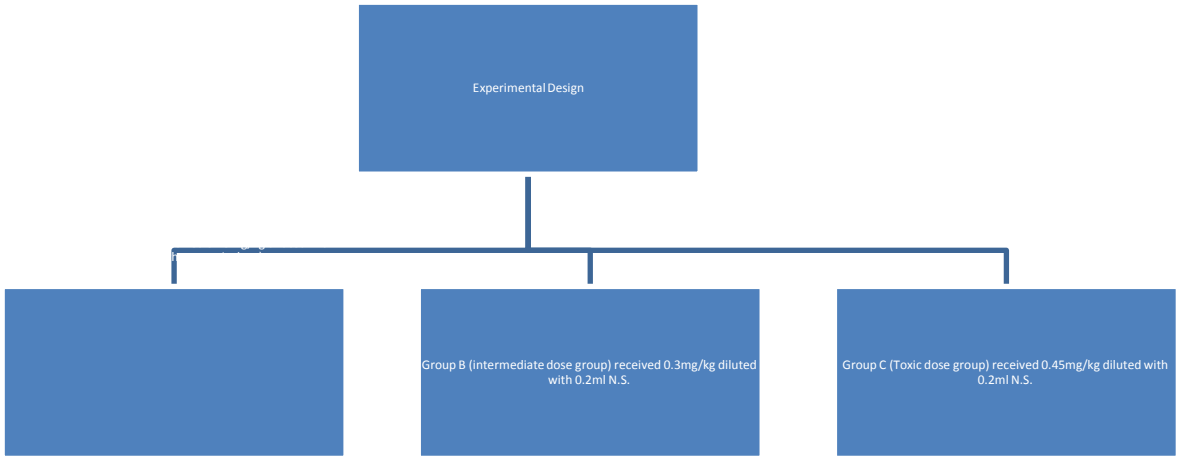
The animals housed in a 6*4*3 m3 room in animal house of veterinary medicine college of Al-Qadisia University under 10 hours light / 14 hours dark at 21+ _4C and put as 5 mice in each standard plastic cage.

Methotrexate (Trixilem) is a clear yellowish solution vial of 5 mg/2ml for injection (lemery Uppsala Sweden).

Each 5 mg/2ml was diluted with 333 ml physiological normal saline and the mixture was injected intramuscularly to animals once weekly for six months. Untreated control received equivalent amount of physiological normal saline.

Experimental Design:

Forty mice divided into four equal groups as in diagram:



- Control group (received only normal saline at a dose 0.2 ml).
- Each groups injected I/M once weekly for six month

For histopathology, pieces of 1-2cm from male and female reproductive organs (testes, epididymus, uterus and ovaries) respectively were fixed with 10% neutral buffered formalin for fixation, processed routinely in histokinette, cut at 5 *M*m thickness by rotary microtome and stained with Haematoxylin and Eosin stain then examined under light microscope (8).

RESULTS

The reproductive organs of both sexes of mice in all groups were tested.

Effects of methotrexate on male reproductive organs:

Group A (Low dose group):

The testes showed complete suppression of spermatogenesis, vaculation of spermatogonia. The seminefrous tubules contained multinucleated spermatid giant cells (Figure 1).

Group B (intermediate dose group):

The testes showed similar effects in the previous group, there are suppression of spermatogenesis, vaculation of spermatogonia and reduced numbers of spermatozoa (Figure 2).

Group C (Toxic dose group):

The histological changes in toxic dose of methotrexate showed that effects on testes and epididymus of mice in this group.

The testes showed complete suppression of spermatogenesis, vacuolation of spermatogonia. Only occasional seminiferous tubules with vacuolation and spermatid giant cells appeared clearly (Figure 3). Also epididymal tubules were empty with no spermatogonia (Figure 4).

Control group:

There are no significant microscopic signs in control untreated animals (male and female).

Effects of methotrexate on female reproductive organs:

Group A (low dose group):

The uterus in this group seemed inactive with few uterine glands and there was thickening of squamous epithelia.

Group B (intermediate dose group):

The histopathological changes of uterus in this group are more than low dose group. The uterus showed thickening of uterine wall associated with hyperplasia of endometrial glands and thickened squamous epithelium of cervix of uterus, also there was sub mucosal fibrosis and infiltration of inflammatory cells.

Group C (Toxic dose group):

The histological changes of female reproductive organs of mice (uterus and ovaries) are severing more than both low and intermediate doses.

There is thickened uterine wall characterized by hyperplasia of endometrial glands. Also there are few dilated and cystic endometrial glands. Thickening of squamous epithelium of cervix and papillary projections of mucosal epithelium (Figure 5).

The ovaries showed absence of corpora leutea, few follicles and graaffian follicles(Figure 6).

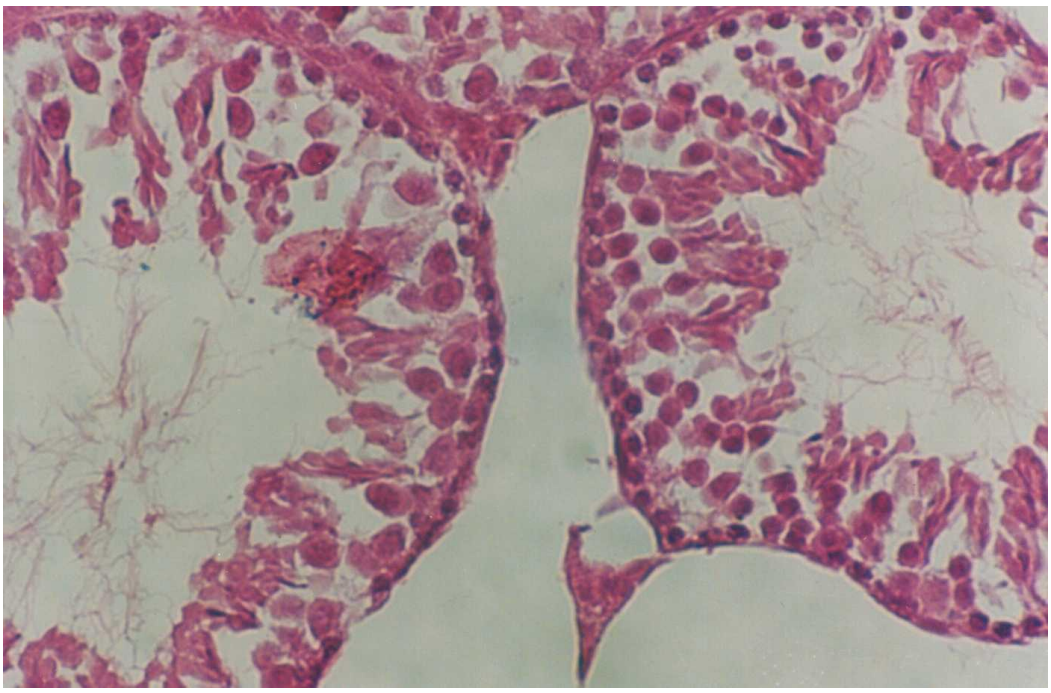


Figure (1): Testes (Low dose group), note suppression of spermatogenesis,

Reduced numbers of spermatozoa in the lumen and presence of spermatid multinucleated giant cell in one of the tubules (X100

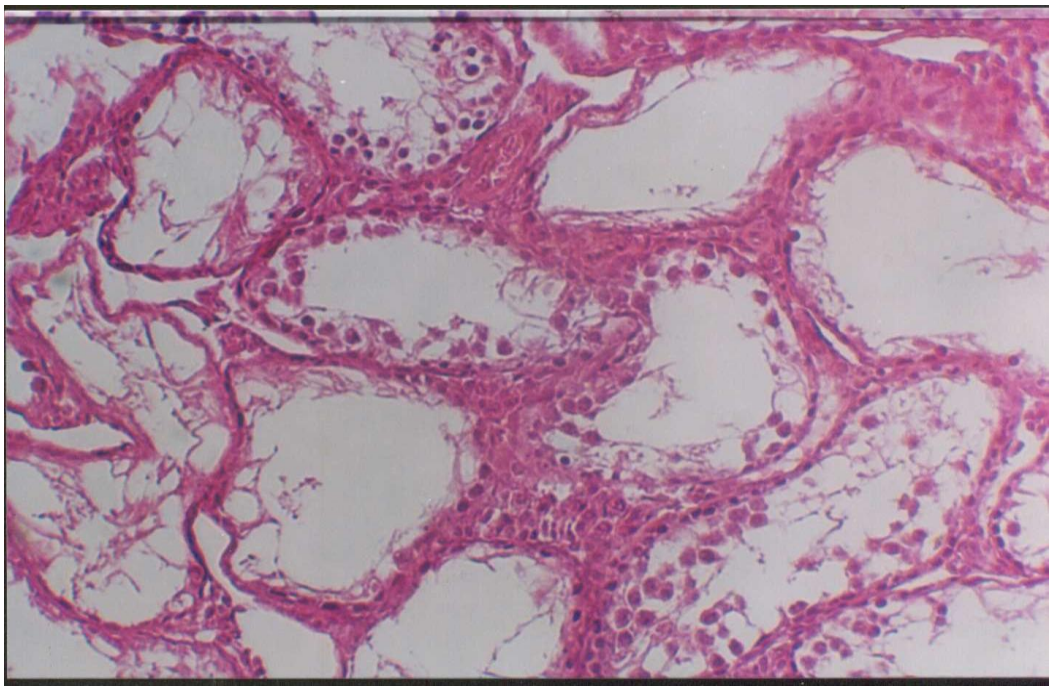


Figure (2): Testes (intermediate dose group) note sever suppression of Spermatogenesis, reduced numbers of spermatogonia (X 50 H&E).

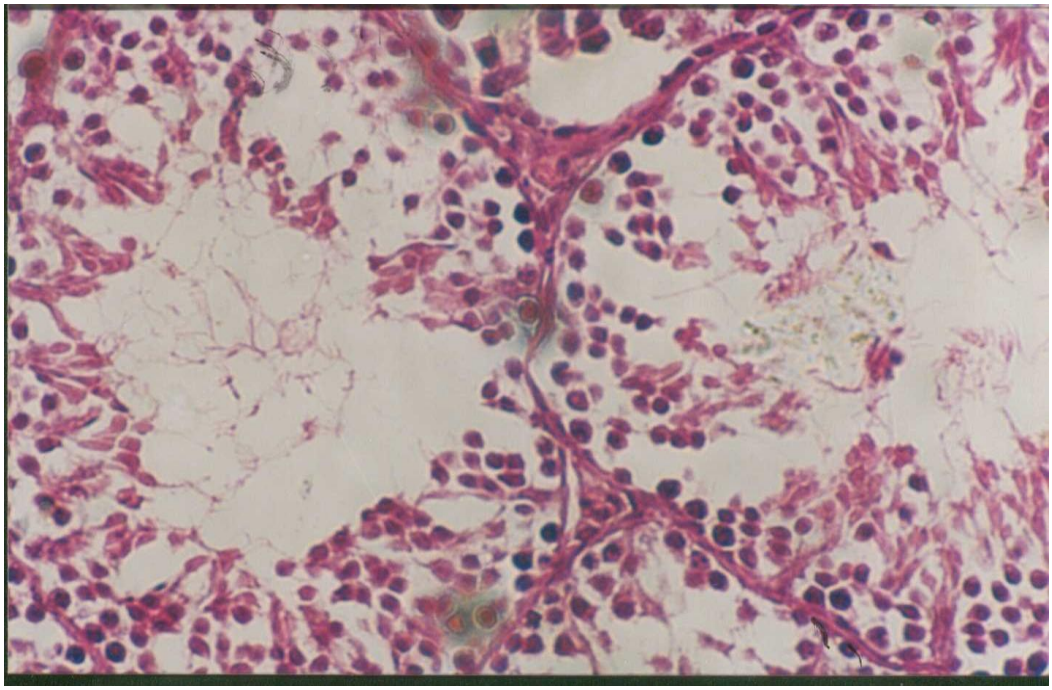


Figure (3): Testes (toxic dose group) there is sever suppression of spermatogenesis, reduced numbers of spermatozoa in seminephrous tubules lumen and vaculation of spermatogonia (X 100 H&E).

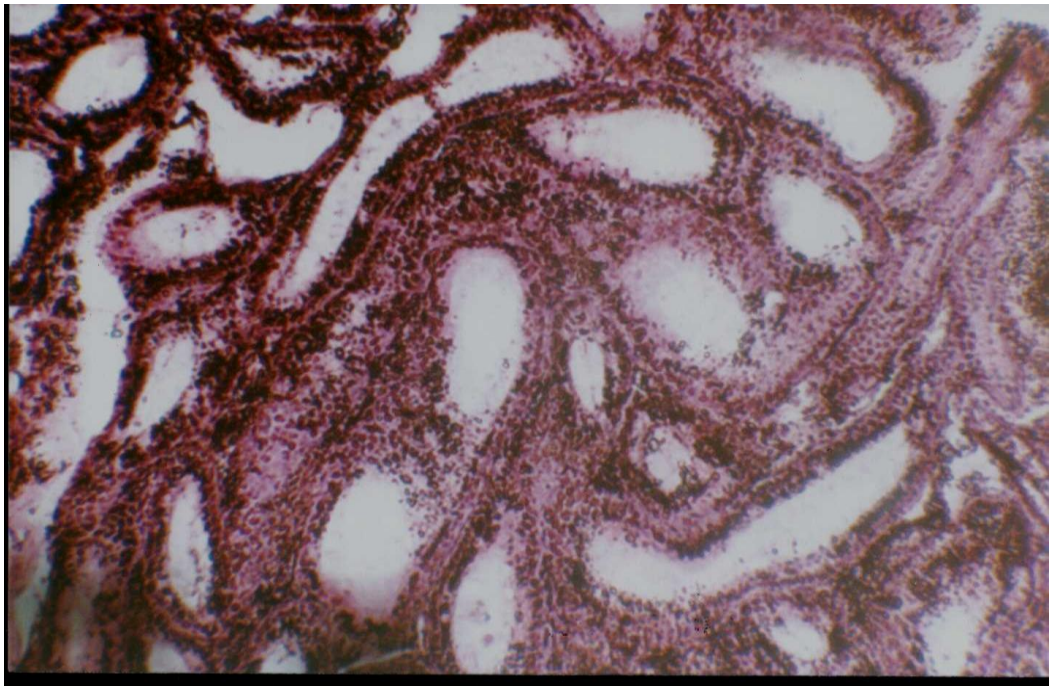


Figure (4): Epididymus (toxic dose group) note majority of tubules are empty (X 50 H&E).

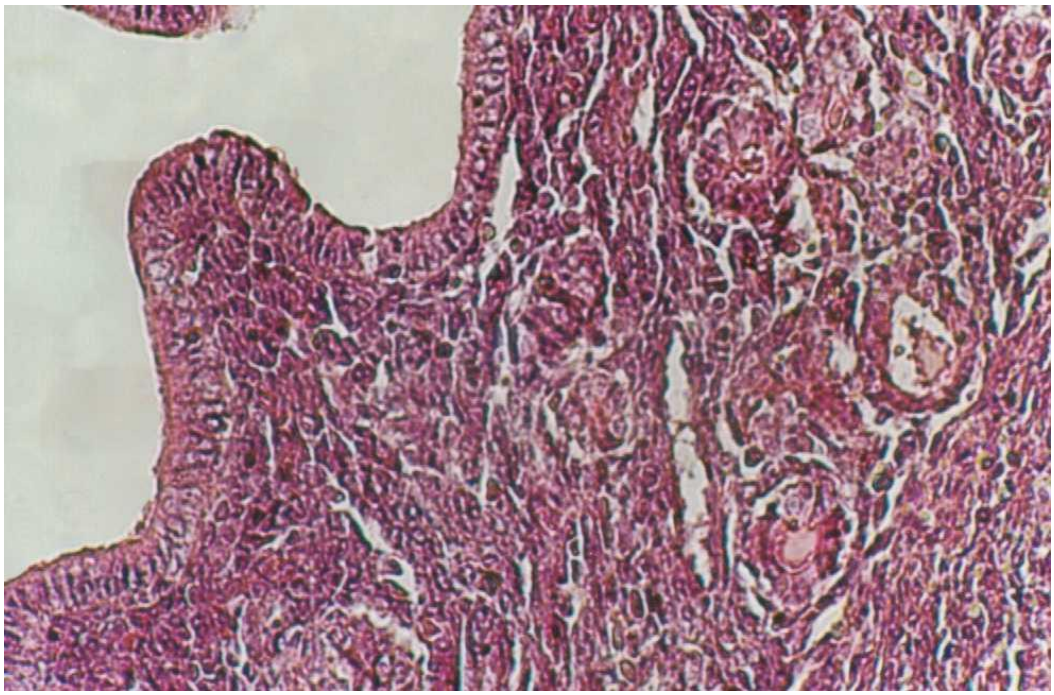


Figure (5): Uterus (toxic dose group) note proliferation of sub-mucosal uterine glands with inflammatory cells (X 100 H&E).

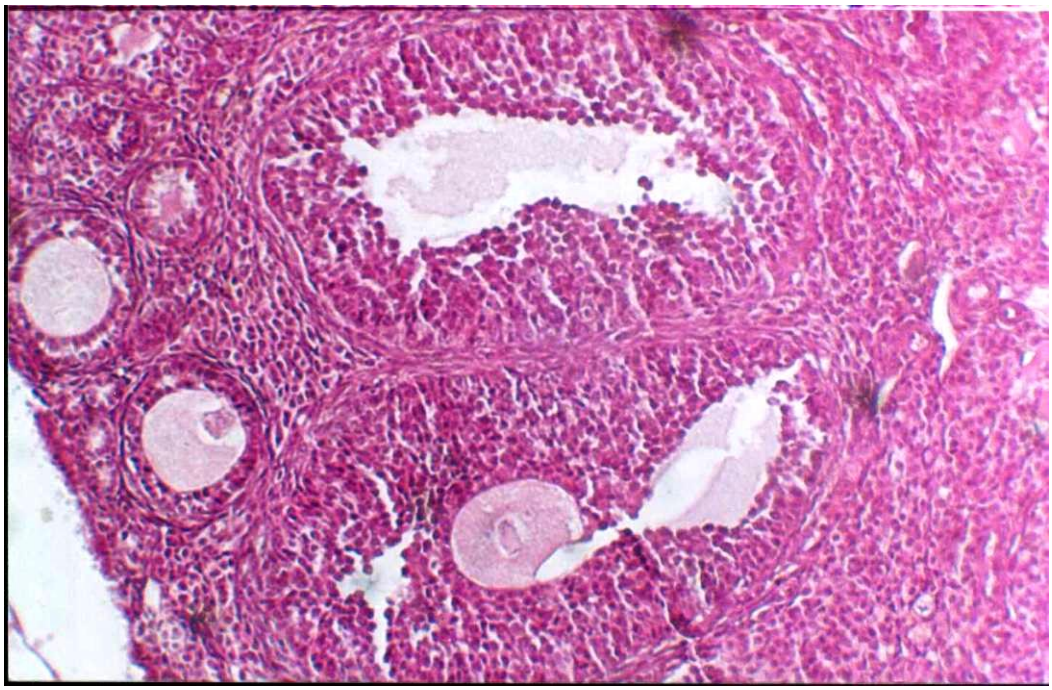


Figure (6): Ovary (toxic dose group) note absence of corpora leutea, few follicles and Graaffian follicles (X 50 H&E).

Discussion

Methotrexate is used to treat certain cancers. With methotrexate, cancer cells can not make DNA; consequently kills cancer cells (9). However, methotrexate can also be harmful to other normal cells in the body, this harmful effect is called methotrexate toxicity, using methotrexate for long period and it distinguished by longer methotrexate stays in the body, can increase the risk of toxicity (10).

Methotrexate can cause infertility because of effect toxicity of male and female reproductive organs for these reasons and others; this study has been focused on the genital organs for both sexes (male and female) in mice.

The most important pathological lesions in the testes were represented by suppression of spermatogenesis leading to evidence of inhibition of spermatogenesis result in a case called (Oligospermia), it was reported by (5), and they reported oligospermia occurred after administration of methotrexate in human being. In the present study, the results showed presence of multinucleated spermatid giant cells and empty epididymus.

The multinucleated spermatid giant cells not langhan's giant cells or foreign body giant cells but they are special cells indicating that there was degeneration process occurring in the testes. While empty epididymus indicating that there was no production of sperms in the testes because epididymus is the pathway of the sperms to outside from the testes.

The uterus, showed a clear changes in our results, epithelium proliferation, in group A and B animals (low and intermediate dosage levels), also there was hyperplasia of uterine epithelia at high dose. From those results, we noted that the lesion in the low dose group and intermediate dose group was restricted in the superficial surface of the endometrium. That was considered the classical response of those organs to the stimulation caused by methotrexate therapy. As well as, uteri under the influence of high dose of methotrexate demonstrated hyperplasia of uterine endometrium. This change could be considered as preneoplastic lesion as some authors reported that (11), they considered the proliferate lesion of the uteri like hyperplasia are preneoplastic lesion. Also Takashi et al. (12) reached the same conclusion.

The ovaries under the effect of high doses of methotrexate and for a long period of time are reflecting a particular type of lesions such as absence of corpora leutea, few immature follicles and few graffian follicles and no ovulation will occur as a squally, these changes in ovaries occurred because methotrexate causes hypothalamic or pituitary dysfunction, then resulting in decrease levels of FSH & LH, causing inhibition of maturation of follicles

and no ovulation. Those results were reported by Chapman (13), he was reported that methotrexate causes reproductive dysfunction due to hypothalamic – pituitary damage.

Oktay et al. (14) also indicated that chemotherapy could inhibit follicle stimulating hormone (FSH) and or/ leutinizing hormone (LH).

التغيرات المرضية النسجية للميتوتركسات على الأعضاء التناسلية الذكرية و الأنثوية في الفئران البيضاء

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الخلاصة

أستخدم في التجربة الحالية (40) من الفئران البيضاء، والتي كانت متقاربة في العمر (4-6) أسابيع والأوزان (23-25 غرام)، ومن أجل معرفة التغيرات المرضية النسجية للأعضاء التناسلية الذكرية والأنثوية في الفئران خلال ستة أشهر من المعالجة الكيميائية المضادة للسرطان والمسمى الميتوتركسات Methotrexate. قسمت الفئران إلى أربعة مجاميع متساوية (10فئران لكل مجموعة – 5 فئران لكل جنس المجموعة الأولى (مجموعة الجرعة العلاجية) أو الجرعة الواطئة استلمت 0.15 ملغم\كغم، المجموعة الثانية (مجموعة الجرعة المتوسطة) استلمت 0.3 ملغم\كغم، المجموعة الثالثة (مجموعة الجرعة السمية) استلمت 0.45 ملغم\كغم، وجميع الجرعات أعطيت مرة واحدة أسبوعيا بالعضل.

أظهرت النتائج أن الميتوتركسات ممكن أن يسبب العقم في الذكور و الإناث، ففي الذكور يسبب كبح عملية تكوين النطف، وفي الإناث يمكن أن يسبب الميتوتركسات تغيرات مرضية واضحة في المبايض والأرحام مثل الغدد الرحمية المختزلة و جريبات المبيض المختزلة على التوالي

REFERENCES

1- Connaughton K. (2003). Effects of dietary supplementation with RNA on recovery of intestinal function after administration of methotrexate. J. undergraduate Research; PP: 1-7.

2- Duran N.; Allahverdiyev A.M.; Cetiner A. (2001). Vepesid on the HEp-2 cell cycle. Turk J Med. Sci.; 31: 187-192.

3- Shamberger R.C., Sherins R.J., Rosenberg S.A. (1981). The effects of postoperative adjuvant chemotherapy and radiotherapy on testicular function in men undergoing treatment for soft tissue sarcoma. Cancer; 47:2368_74.

4- Sussman, A. and Leonard, J.M. (1980). Psoriasis,methotrexate and oligospermia. Arch Dermatol; 116: 215-217

5- Diehl V.,Sex tro M. , Franklin J. (1999). Clinical presentation, course and prognostic factors in lymphocyte predominant Hodgkin`s disease and lymphocyte rich classical Hodgkin`s disease: report from the European task force on lymphoma project on lymphocyte_ predominant Hodgkin`s disease. J. Clin Oncol.; 17:776.

6-Vaug han Hudson B. (1998). The BNLI: past and present. Clin. Oncol.; 10:212.

7- Boulton A.J.M., Carpenter J. R., Clark B., Dascombe M.J., Deakin J.F.M., Dive C., Duxbury A.J. and Foster R.W. (1996). Antiparasitic Chemotherapy. In Basic pharmacology. 4th Ed., Oxford Auckland Boston and Johannesburg Melbourne, New Delhi; PP: 269-274.

8- Luna, L.G. (1968). Manual of Histological staining methods of the armed forces. Institute of Pathology. 3rd Ed., McGraw Hill Book Company, N. Y., Toronto. London, Sydney; 12-31.

9- Cronstein B.N.; Naime D.; Ostad E. (1993). The anti-inflammatory mechanism of methotrexate: increased adenosine release at inflamed sites diminishes leukocytes accumulation in an in vivo model of inflammation. J. Clin. Invest,; 92: 2675-2682.

10- Rosenthal, G.J.; Weigand, G.W.; Germolec, D.R. (1988). Suppression of B cell functions by methotrexate and trimethotrexate. Evidence for inhibition of purine biosynthesis as a major mechanism of action. J. Immunol.; 141: 410-416.

11- Mackawa, Akihiko.; Masakazu, Takashi.; Jin, Ando. And Midori, Yoshida. (1999). Uterine carcinogenesis by chemicals/ hormones in rodents. J. Toxicol. Pathol.; 12: 1-11.

12-Takahashi, M.; Takasumi, Shimomoto.; Katsuhiro, Miyajima.; Seiichi, Iizuka; Takoa, Watanabe; Midori, Yoshida; Yuji, Kurokawa and Akihiko, Mackawa. (2002). Promotion but not progression effects of tamoxifen on uterine

carcinogenesis in mice initiated with N-ethyl-N-nitro-N-Nitrosoguanidine. Carcinogenesis; 23 (9): 1549-1555.

13- Chapman R.M. (1992). Gonadal toxicity and teratogenicity. In perry MC (ed) the Chemotherapy Source Book. Williams & Wilkins, Baltimore, PP: 71-753.

14- Oktay, K.; Newton, H.; Mullan, J.; Gosden, R.G. (1998). Development of human primordial follicles to antral stages in SCID/ hpg mice stimulated with follicle stimulating hormone. Hum Report.; 13: 1133-1138.