University of Benghazi
Faculty of Education Al marj
Global Libyan Journal

جامعة بنغازي
كلية التربية المرج
المجلة الليبية العالمية

العدد الثاني والثلاثون – 30/ نوفمبر (2017)

Sperm abnormality toxicity due to Methotrex in male rat rats

DR . Zeinab. M . Gazi*

(Zoology Department, Faculty of Science, Omar El-Mokhtar University,El- Beda-Libya)

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Abstract

Methotrexate commonly used to treat cancer and caused reproductive damage in mice , . In this study, the effects of methotrexate on some parameters of sperms on mice testis after 35 days of MTX administration intraperitoneally (ip). twelve adult male mice were divided into control and treated groups composed of 6 mice in each group. Treated group received methotrexate in dose i.e 20mglkg, whereas control one received normal saline intraperitoneally, the results showed significant ($P<0.05$) increased percentage of abnormalities of sperms in mice treated with MTX compared to control, these data suggested that MTX administration induced sperm shape abnormalities.

Keywords: Methotrexate , Sperm abnormalities,mice.

Introduction

The use of chemotherapeutics is known to cause acute toxic effects in multiorgan systems (Kim and Chung ,1999) methotrexate (MTX) was introduced in 1950 as a chemotherapeutic agent.3 Methotrexate is used for a wide range of malignancies including acute and chronic leukemia, lymphoma, bladder cancer, breast cancer, and testicular tumor. (Vardi, etal.,2009 and Armagan,et al.,2008) Other applications of this medication include immunosuppression, treating the autoimmune disorders like arthritis rheumatoid and psoriasis. It has also immunosuppressive characteristic. (Gulgun, et al., 2010)(Shin, et al., 2008)There are lots of known and unknown causes for infertility. One of the most common sources of infertility is chemotherapy. (Richard L , et al.1980) due to decrease in sperm count and its altered quality. Reports available on effects of methotrexate on spermatogenesis (Shrestha, et al., 2007 and Padmanabhan, et al., 2008a) and steroidogenesis .(Padmanabhan, et al., 2008b) are scanty. Some histomorphometric effects e.g., reduction in diameter of seminiferous tubules, spermatocytes and spermatids were also noted down by some earlie.

Testicular toxicity of MTX has an important side effect which may cause subsequent infertility. Previous studies have shown disorganization in the seminiferous tubules of the testis, a decrease in sperm number, and sperm DNA damage following administration of MTX .(Padmanabhan, et al., 2008b and Nouri, et al., 2009) MTX prevents the synthesis of nucleotides through reaction with DHFR and subsequently prevents synthesis of DNA and RNA. (Novaković, et al., 2003 and Tian and Cronstein, 2007) Most chemotherapy agents act through reaction with DNA or its precursors preventing synthesis of new genetic materials. Damage to the genetic materials causes disorder in functions of somatic and reproductive cells. (Singh, et al., 2003 and Sukhotnik, et al., 2013) Toxic effects on reproductive cells may result in fetal imparities, changes in endocrine function, reproductive problems, and premature abortion.(Haines, et al., 2001 and Wyrobek,et al., 2005) Hence, an attempt has been made in the present study to investigate the effect of methotrexate on sperm morphology in male albino mice

Materials and methods

Materials

Animals

In this experimental study, 24 male healthy mice aged 10-12 weeks were used. These mice were kept in standard conditions including temperature 22±2oC and light/dark cycle of 14 and 10 hours respectively. The mice had free access to food and water

Chemicals

Methotrexate was obtained from (1200 hospital bengazi ,Libya)

Company: Ebewe , Austrla

methods

Experimental design

Study was performed on 12 mature male albino mice, divided into two groups; each group was consisted of 6 mice as following:

control group: Animals were injected intraperitoneally with physiological saline weekly for 5 weeks.

MTX treated group: Animals were injected intraperitoneally with MTX 20 mg\ kg per week for 5 weeks, (Alam ,etal., 2011).

Collection of epididymal sperm smears

The sperm smears were obtained from the caudae epididymis of the testes of adult control and treated males. The caudae epididymis were cut into small pieces in 1 cc. saline solution. Sperm smears were obtained from the resulting suspension. They were stained by eiosen. Approximately 1000 sperm cells were microscopically examined for each mice. A binocular microscope with _40 eyepieces and _100 oil immersion objective lenses were used for this study. Abnormally shaped sperm cells were recorded randomly

Statistical analysis

Data were collected, arranged and reported as mean±standard error of mean (S.E.M)

, summarized and then analyzed using the computer program SPSS/version (15.0). The statistical method was one way analysis of variance ANOVA test (F-test) and if significant differences between means were found, Duncan's multiple range test

(P <0.05) .

Results

Table 1 and Fig. 1 shows that the frequency of abnormally shaped sperms in the testes of male albino mice significantly increased (p< 0.05) after the injection of MTX at a dose of (20 mg/kg) weeklyfor 5 week. The percentage of total deformed sperms reached (6.05 %) compared to (1.43 %) in the control group. showed normal sperms (Figure.)and various forms of deformed sperms in (figure..)

Discussion

The use of chemotherapeutics is known to cause acute toxic effects in multiorgan systems.(Kim,et al., 1999), Permanent azoospermia and infertility have been reported as side effects of chemotherapeuticdrugs in males (Schilsky, et al .,1980) .Methotrexate is an anti-metabolic agent for a variety of neoplastic disorders and its toxic effects have been reported. (Chiang, et al., 2005) By acting on cell cycle, this agent prevents synthesis of DNA. (Novaković, et al., 2003) Increasing the number of abnormal sperms is an indirect result of genotoxicity. Aziz *et al*. showed that there was a significant relationship between Reactive oxygen species (ROS) and increasing of malformed sperms(p < 0.05). Disorders in morphology of sperms relates to spot mutations in reproductive cells34 or chromosomal defects. (Topham, 1980) In this study the number of abnormal sperms in MTX group showed significant increase compared to other groups (p < 0.05) that completely complies with previous studies. Therefore, it can be said that sperm disorders induced by MTX due to physiological, cytotoxic and genetic changes in DNA cause an increase in the number of abnormal sperms. (Jha and Kumar,2006) Padmanabhan et al. exposed mice with intraperitoneal injection of methotrexate weekly. They reported reduced sperm count and increased occurrence of sperm head abnormalities Administration of MTX is known to cause reproductive damage, including decreased epididymal and testicular weights, and reduc epididymal sperm counts and fertility (Saxena,et al.2004) . MTX treatment caused abnormal sperm morphology. The male reproductive function is under hormonal control

,spermatogenic process is under control of follicular stimulating hormone (FSH) and testosterone (Ganong, 1991) ,while the formation of type A spermatogonia and conversion of primary spermatocyte into secondary spermatocyte (Meiosis I) are dependent on testosterone and the final step of maturation of spermatids aredependent on FSH (Acosta,et al., 1988), so the abnormal sperm morphology mayreflect an abnormal intratesticular maturation as a result of drug treatment (Tesarik, et al.,199) , also the drug induce an alteration in androgen secretion that usually produce changes in the reproductive system ,such changes might include the production of abnormal sperms (Arab, et al., 1989),also any effect on spermatogenesis lead to production of abnormal sperms (AL-Rubaie, 1999) . the previous study had proved that MTX could bring out the reproductivetoxicity (Padmanabhan, et al., 2008).

Previous studies have demonstrated that various doses of MTX accelerate the apoptotic process via oxidative stress, and may cause harmful effects on spermatogenesis leading to infer-tility (Padmanabhan ,et al.,2009; Nouri ,et al .,2009 and Eid ,et al.,2002) . Therefore, for patients under MTX therapy, protection of germinal cells is important. Oxidative stress develops as a result of an imbalance between reactive oxygen radicals and antioxidant system. Excess amounts of reactive oxygen radicals induce production of abnormal sperms and infertility (Yuluğ ,et al. ,2013) . Therefore, it can be concluded that Our study shows that MTX inducedtesticular damage, By increasing the percentage of sperm abnormalities and thus decreasing fertility. However, this study needs to be supported with other experimental and clinical research To know the mechanism of action of methotrexate induced testicular damage And finding a protectors to reduce its toxic effects on the testicular.

Groups	No.of mice	No.of examind sperms 1000lmice	deformed sperms											
			Deformed head			deformed tail			Deformed head +tail			total deformed sperms		
			No.	%	mean+SE	No.	%	mean+SE	No.	%	mean+SE	No.	%	mean+SE
Control	6	6000	4	0.07	0.70+0.71	82	1.37	13.70+0.50	0	0	0.00+0.00	86	1.43	14.3+0.54
MTX	6	6000	36	0.6	6.00+0.44*	352	5.87	58.70+1.89*	2	0.03	0.30+0.32	400	6.5	65.00+0.25*

Values are mean ± S.E of six animals. significantly different from control at *p <0.05

(Table 1): Percentage of deformed sperms **in treated and control groups.**

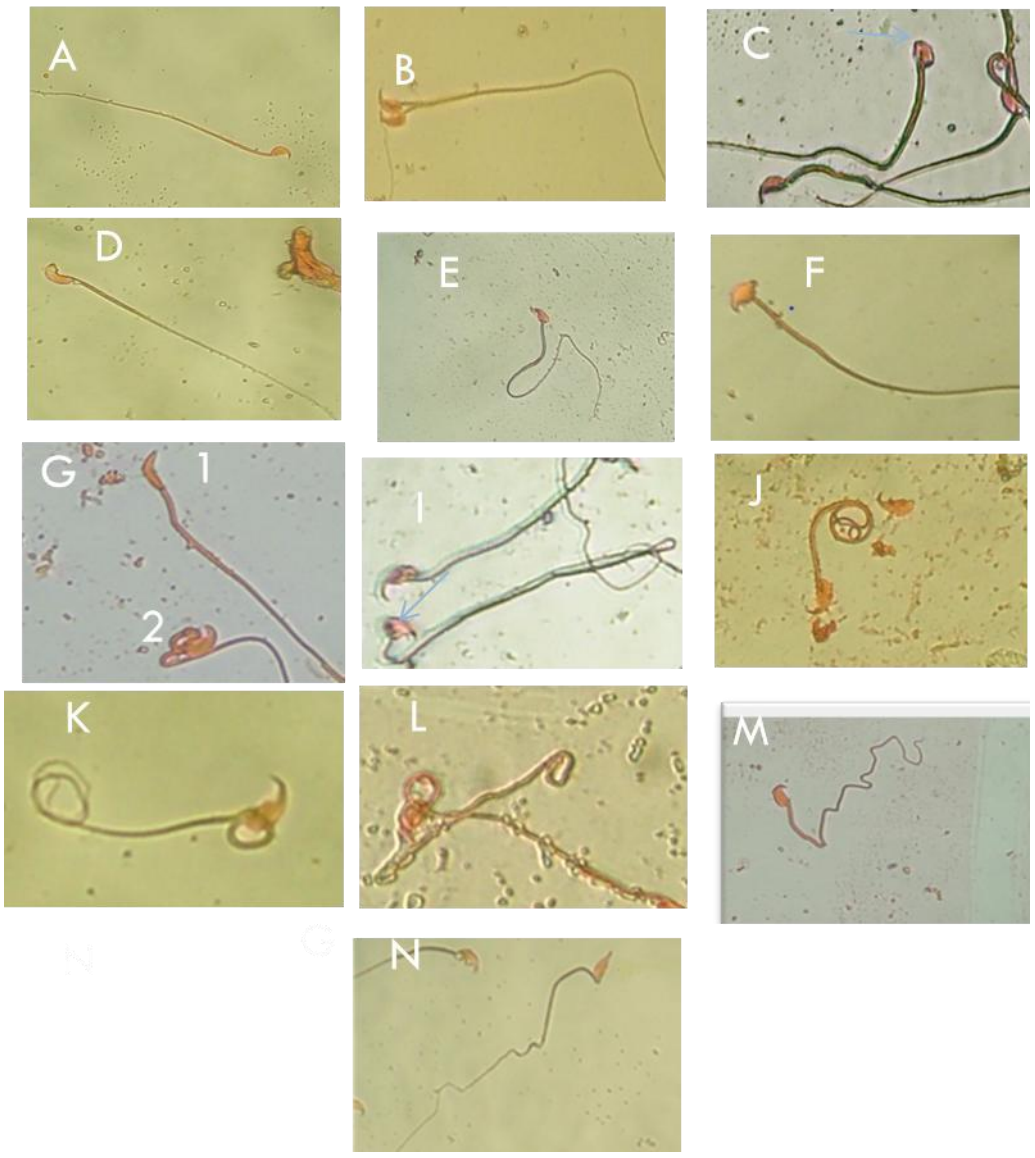

Figure (1): Different types of mice sperms abnormalities induced by Methotrexate

A) Normal sperm (B) double head sperm(C)ballon head(D) banana head (E) sperm withlong hook (F,I) amorphous head(G) defective head(2) bent neck, (1)(J) bent tail (k)bent neck and bent tail(L) bent tail and Self-adhesion(M) defective and looped tail (N) sperm without hook

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