

# Effect of methotrexate (mtx) administration on spermatogenesis: an experimental on animal model

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## ABSTRACT

An experiment was conducted to observe histomorphometric and cellular toxicity on rat testes after sixty days of methotrexate administration intraperitoneally (ip). Total 30 adult male rats were divided into one control and two experimental groups containing 10 rats in each group. Experimental groups received methotrexate in two different doses i.e 25 µg and 50 µg, whereas control one received normal saline intraperitoneally. At the end of the experiment, animals were sacrificed and testes were processed for paraffin sectioning and stained in haematoxylin and eosin. Further microscopic study of seminiferous tubules, interstitial spaces, primary spermatocytes and spermatids were carried out. Results revealed decreased diameter of seminiferous tubules, increased interstitial spaces in experimental groups in dose dependent manner and found to be statistically significant ( $p < 0.05$ ) as well as distortion of morphology of leydig cells in experimental group. Therefore, it can be concluded that these qualitative and quantitative changes in male gonads may alter the reproductive performance of animals.

**Keywords:** Methotrexate, Spermatogenesis, Wistar Albino rats.

## INTRODUCTION

Methotrexate (MTX) is an anti-neoplastic agent used for the treatment of malignancy. Initially it was developed for the treatment of cancers but recently it is being used for the treatment of nonmalignant conditions such as in Rheumatic disease (Rheumatoid arthritis) at much lower dose. Therefore it is also called as Rheumatrex.<sup>1,2</sup> It is also used for the treatments of psoriasis.<sup>3</sup>

Drugs used for cancer chemotherapy are well known to produce acute toxic side effects in multiple organ systems such as gastrointestinal tract, lung, kidney, liver, testes and skin. Ideally, this drug interferes with malignant or neoplastic cells. However, this currently available anticancer drug MTX specifically recognize neoplastic cells and also affect proliferating cells both of normal and abnormal origin. 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.<sup>3-12</sup> 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 continuous exposure of MTX on an animal model.

## MATERIALS AND METHODS

Total 30 healthy male Wistar albino rats obtained from the animal house of Anatomy department of B.P. Koirala Institute of Health Sciences, Dharan Nepal after obtaining ethical clearance from the concerned authority of the institute.

The weight of the animals ranged between 180 to 200 gm (age 4-5 months). These animals were acclimatized for 2 weeks and kept in separate cages (5 animals per cage). They were provided with adequate pellet diet and drinking water *ad libitum*.

Rats were divided into control and experimental (treated) groups. Experimental group further subdivided into different dose groups as 25 µg and 50 µg. Thus each group and subgroups contained 10 rats. The experimental groups were given MTX in the doses of 25 µg and 50 µg intraperitoneally daily for 60 days and control group were given normal saline for same duration. After the end of experiment, animals were sacrificed and testes were isolated and kept in the specimen bottle containing Bouins fluid solution and processed for paraffin sectioning. Sections (5µ thick) were obtained and stained with haematoxylin and eosin for microscopic study.

**Micrometry:** Micrometers (Ocular micrometer, Stage micrometer) were used for the measurement of the diameter of seminiferous tubules, primary spermatocytes, round spermatids, interstitial space and leydig cells. Five slides per animals were observed. The diameter of 25 seminiferous tubules was 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.

Statistical analysis: P-values were calculated using chi-square test, among control and experimental groups to find out the level of significance between two different doses of methotrexate comparing with controls.

## RESULTS

The morphometric discrepancies were observed such as discontinuity of basement membrane of seminiferous epithelium. It has been also observed the reduction in the number of the spermatocytes in experimental groups and marked differences were observed in high dose, apparently the number of leydig cells seemed to be affected when compared to control groups. (Fig. -1b, 2b, and 3b)

The diameter of seminiferous tubules was found to be significantly decreased ( $p < 0.05$ ) in both experimental groups I and II as compared to control. Significant differences ( $p < 0.05$ ) were also observed when Interstitial spaces were measured between control and experimental groups I and II (Table -1).

The changes in the cellular proliferation of gonads (testes) were also affected in experimental groups I and II. The diameter of primary spermatocytes and spermatids were observed both in control and experimental groups and were found significantly decreased in experimental groups (Table-2).

## 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 following drug toxicity by anti-cancer drugs both on cellular and molecular aspects during spermatogenesis. Gonad is an important component of reproductive system, associated with series of cellular interaction, differentiation to form mature germ cells through process of spermatogenesis. Any insult at this stage on gonads may impair fertility.<sup>3-13,15,16</sup> Previous study also indicated that low dose of MTX affects cellular contents, diameter of seminiferous tubules and interstitial space of testis during spermatogenesis.<sup>6,7,14</sup> However, similar findings were also noticed in the present study that the diameter of seminiferous tubules, primary spermatocytes and spermatids decreased with increase in dose of MTX due to antimetabolic activity of the drug, resulting significant decrease ( $p < 0.05$ ) in the diameter of seminiferous tubules followed by significant increase of interstitial space (Fig. 1b). The diameter of leydig cells in the present study did not reveal any alteration after MTX administration with respect to controls. This could be due to either increasing age for heterogeneous population of leydig cells in the testes<sup>14</sup> which made non-significant results through changes in morphological form from round to oval in shape was observed (Fig. 2b).

In the present study, the size of cellular contents of seminiferous tubules altered significantly that may be because primary spermatocytes and spermatids failed to replicate DNA due to inhibition of an essential enzyme dihydrofolate reductase required for normal DNA synthesis.<sup>15</sup> 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.

## REFERENCES

1. Chabner BA, Ryan DP, Ares LP, Carbonero RG, Calabresi P. Antineoplastic agent. Goodman and Gilman's the pharmacological basis of therapeutics. 10<sup>th</sup> edition. New york 2002: 1399-404.
2. Salmon SE, Sartorelli SE. Cancer chemotherapy. Bertman G. Katzung. Basic and clinical pharmacology. 8<sup>th</sup> edition. New York 2001: 932-3.
3. EI-Beheiry A, EI-Mansy E, Kamel N, Salman N. Methotrexate and fertility in men. *Arch Androl* 1979; 3: 177-9.
4. Sussman A, Leonard JM. Psoriasis, Methotrexate and oligospermia. *Arch dermatol* 1980; 116: 215-7.
5. Freeman-Narrod SA. Chronic toxicity of methotrexate in mice. *J Natl Cancer Inst* 1977; 58: 735-741.
6. Morris LF, Harrod MJ, Menter MA *et al.* Methotrexate and reproduction in men: case report and recommendations. *J Amer Acad Dermatol* 1993; 29: 913-6.
7. Koehler M, Waldherr R, Ludwig R, Heinrich U, Brandeis WE. Effects of methotrexate on rabbit testes. Part 1: Morphological changes. *Pediatr Hematol Oncol* 1986; 3: 325-34.
8. Lu CC, Meistrich ML. Cytotoxic effects of chemotherapeutic drugs on mouse testis cell. *Cancer Res* 1979; 39: 3575-82.
9. Johnson FE, Farr SA, Mawad M, Woo YC. Testicular cytotoxicity of intravenous Methotrexate in rats. *J Sug Oncol.* 1994; 55: 175-8.
10. Badri SN, Vanithakumari G, Malini T. Studies on methotrexate effects on testicular steroidogenesis in rats. *Endocr Res* 2000; 26:247-62.
11. Meistrich ML, Finch M, Dacuriha MF, Hacker U, Au WW. Damaging effects of fourteen chemotherapeutic drugs on mouse testis cells. *Cancer Res* 1982; 42: 122-31.

12. Russell L D, Russell JA. Short term morphological response of the rat testis to administration of five chemotherapeutic agents. *Amer J Anat* 1991; 192: 142-68.
13. Saxena AK, Dhungel S, Bhattacharya S, Jha CB, Srivastava AK. Effect of chronic low dose of methotrexate on cellular proliferation during spermatogenesis in rat. *Arch Androl* 2004; 50: 33-5.
14. Takano H, Abe K. Age related histologic changes in the adult mouse testis. *Arch Histol Japan* 1987; 50: 533-44.
15. Philippi MK, Banergee D, Malhotra S *et al.* Cells exposed to antifolate show increase cellular levels of proteins fused to dihydrofolate reductase: A method of modulate gene expression. *Proc Natl Acad Sci USA* 2002; 99: 3400-5.

**Table-1:** Diameter of seminiferous tubules and interstitial space in both control and experimental groups.

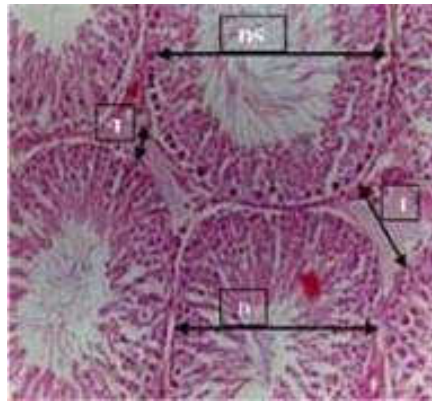
Groups	Diameter of seminiferous tubules ( $\mu\text{m}$ ) (mean $\pm$ SD)	Interstitial space ( $\mu\text{m}$ )(mean $\pm$ SD)
Control	319.5 $\pm$ 9.19	31.39 $\pm$ 3.29
Expt (Group I)	309.28 $\pm$ 10.71*	40.09 $\pm$ 4.91*
Expt (Group II)	295.7 $\pm$ 9.67*	51.93 $\pm$ 0.31*

\*P<0.05, Group I: 25  $\mu\text{g}/\text{day}$ , Group II: 50  $\mu\text{g}/\text{day}$ , Expt: Experimental

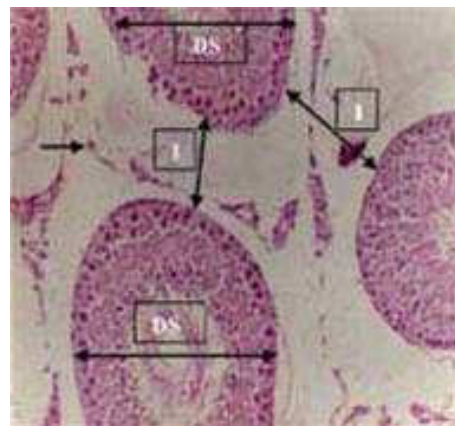
**Table-2:** Diameter of primary spermatocytes, spermatids and leydig cells in control and experimental groups.

Groups	Primary spermatocytes( $\mu\text{m}$ ) (mean $\pm$ SD)	Spermatids( $\mu\text{m}$ ) (mean $\pm$ SD)	Leydig cells( $\mu\text{m}$ ) (mean $\pm$ SD)
Control	8.24 $\pm$ 0.30	5.17 $\pm$ 0.25	4.81 $\pm$ 0.75
Expt (Group I)	7.08 $\pm$ 0.90*	4.67 $\pm$ 0.37*	5.85 $\pm$ 0.33
Expt (Group II)	6.34 $\pm$ 0.34*	3.32 $\pm$ 0.02*	4.85 $\pm$ 0.33

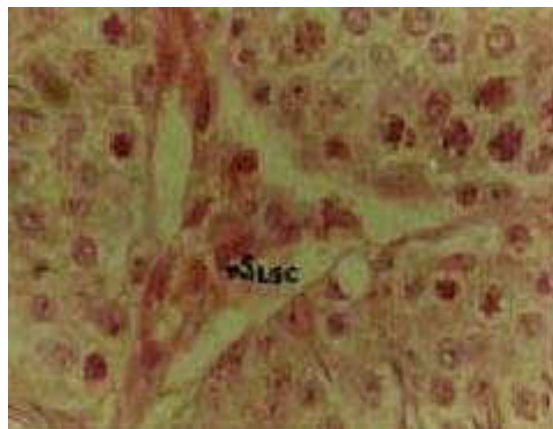
\*P<0.05, Group I: 25  $\mu\text{g}/\text{day}$ , Group II: 50  $\mu\text{g}/\text{day}$ , Expt: Experimental



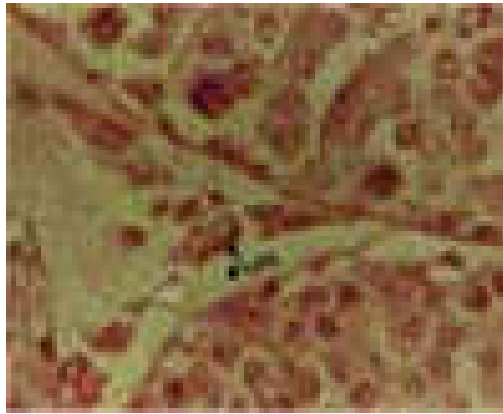
**Fig.1a.** Photomicrograph of testes of albino rat (control group) showing normal structure of seminiferous tubules. (DST- Diameter of Seminiferous tubules, IS- Interstitial space) (H and E X 100).



**Fig. 1b.** Photomicrograph of testes of albino rat (experimental group) showing decrease in diameter of seminiferous tubules (DST) and increase of interstitial space (IS) and distortion of basement membrane ( )→



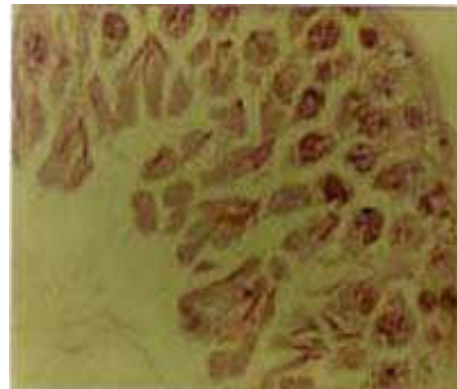
**Fig. 2a.** Photomicrograph of testes of albino rat (control group) showing normal structure of Leydig cells (LSC). (H & E X 400)



**Fig. 2b.** Photomicrograph of testes of albino rat (experimental group) showing the heterogeneous population of Leydig cells with alterations (ALSC) in morphological features. (H & E X 400)



**Fig. 3a.** Photomicrograph of testes of albino rat (control group) showing normal stage of spermatogenesis with clear visibility of spermatogonia (S) primary spermatocytes (S1), and spermatids (S3) with spermatozoa (S4). (H & E X 400)



**Fig. 3b.** Photomicrograph of testes of albino rat (experimental group) showing heterogeneous population of spermatogenesis and clear visibility of **loss of spermatids in the lumen** of seminiferous tubules. (H & E X 400)