



EFFECT OF FOLLIC ACID ANTAGONIST METHOTREXATE (MTX) ON THE LEVEL OF TESTOSTERONE OF *FUNAMBULUS PENNANTI* (WROUGHTON)

Biological Science

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ABSTRACT

Effect of Methotrexate on the level of testosterone have been studied by intramuscularly injecting low dose of 3 mg/kg BW/ per day and 6 mg/kg BW/ day for 15 days to adult male squirrel (*Funambulus pennanti*) during the breeding period January. For comparing the effects saline treated vehicle was injected same amount of saline and were maintained for the same duration. Level of testosterone was determined by Enzyme Linked Fluorescent Essay (ELFA), significant decrease in the testosterone level after high dose treatment and insignificant decrease after low dose treatment observed. From the foregoing it is concluded that Methotrexate has adverse effect on spermatogenesis which are dose and duration dependent besides being toxic, therefore certainly causing reduction in the fertility rate.

KEYWORDS

Methotrexate, Testosteron, ELFA, Antifertility

INTRODUCTION

Methotrexate (Rheumatrex) is a medicine that is used to treat Rheumatoid arthritis (RA), psoriatic arthritis, Reiter's syndrome and other conditions. Aside from its antineoplastic activity, Methotrexate has also been used with benefit in the therapy of common skin disease psoriasis (McDonald, 1981). Additionally Methotrexate inhibits cell mediated immune reaction and is employed as an immunosuppressive agent, for example, in allogenic bone marrow and organ transplantation and for the treatment of dermatomyositis, rheumatoid arthritis, Wegener granulomatosis and Crohn's disease (Messmann and Allegra, 2001; Feagan *et al.*, 1995). Methotrexate was formerly known as amethopterin, is an antimetabolite drug used in treatment of cancer and autoimmune diseases.

MATERIAL AND METHODS

Enzyme Linked Fluorescent Assay (ELFA) For The Measurement Of Serum Testosterone

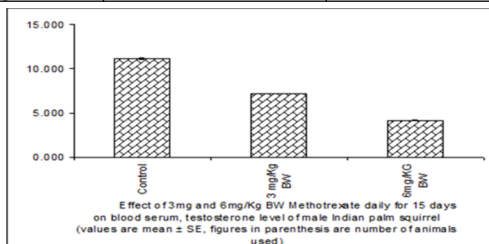
For the determination of testosterone level in blood, squirrels were anesthetized by ether and 2ml of blood was drawn by cardiac puncture with 2ml sterile syringe. The blood was allowed to clot at room temperature for half an hour. The clotted blood was sent to NRPL Pathology laboratory, Nagpur for further processing (Delahunt, 1993).

Observation and results

The low dose group showed insignificant decrease but the high dose treatment resulted into significant decrease in Testosterone concentration compared to control values (Table-1 and fig. 1 bar diagram).

Table and Graph : 1. Effect of 3mg and 6mg/Kg BW Methotrexate daily for 15 days on blood serum, testosterone level of male Indian palm squirrel (values are mean \pm SE, figures in parenthesis are number of animals used)

Treatment	Mean Value Testosterone	P vale
Control	11.147 \pm 0.0867	
3 mg/Kg BW	7.137 \pm 0.0088	P < 0.001
6mg/KG BW	4.153 \pm 0.0027	P < 0.01



DISCUSSION

Determination of testosterone values is very important during the study of male reproduction. Weight, sizes and cytologic structure of testis and accessory reproductive organs and the glands, sperm motility, its viability, morphology and the count, the enzymes the

substrates and the mitotic activities of accessory reproductive glands and their secretory activity etc. all depend upon the level of testosterone in the blood.

Even though for the present work the values of testosterone are measured by very sophisticated technique of Enzyme Linked Fluorescent Assay (ELFA) where an enzyme immunoassay competition method with a final fluorescent detection is used as a principal and is also a more rapid way of determination of hormonal values, a correlation of its biological activities with the different parameters like body weight, organ weight, histological observations of testis, epididymis, seminal vesicle and prostate has been studied.

An overall decrease in the circulating testosterone by ELFA was observed for both the doses of MTX as described by earlier workers.

A number of previous workers have observed a decrease in the values of Testosterone after the administration of various doses of MTX (Sussman *et al.*, 1980; Blatt *et al.*, 1981; Shamberger *et al.*, 1981a, b; Kohler *et al.*, 1986b and Badri 2001). These authors have stated that administration of MTX significantly increases the metabolic clearance rate of testosterone due to **an increase in the testosterone A ring reductase activity or probably due to the increase in hepatic Δ^4 steroid reductase activity or causes suppression of gonadotrophin (LH) secretion and therefore decrease in testosterone level** (Shamberger *et al.*, 1981a,b and Kohler *et al.*, 1986b) or according to Narrod and Narrod 1977; Lendon *et al.*, 1978; Shamberger *et al.*, 1981a, b; Hensle *et al.*, 1984 and Saxena *et al.*, 2004, the fall in the blood testosterone concentration could be a direct inhibitory effect of MTX on **Leydig cell steroidogenesis** or displacement of sex steroid binding protein or as suggested by Kohler *et al.*, 1986 b and Badri *et al.*, 2001, the reduced plasma level of testosterone **may be an enzymic defect since the specific activities of 3- β and 17- β hydroxy steroid dehydrogenase were markedly diminished.**

In the present work level hepatic steroid A ring reductase activity has not been studied, however, atrophy and reduction in the number of Leydig cells suggest that MTX has direct inhibitory effect on steroidogenesis or the production rate of testosterone from the Leydig cell and therefore on the gonadotrophin (LH) secretion rate which controls the biosynthesis of testosterone from the Leydig cells.

Significant decrease in the testosterone level after high dose treatment, and insignificant decrease after low dose treatment has also been supported by general appearance of the animal, their sluggish behavior, the skinny appearance, due to body and organ weight loss, testicular atrophy, damage to all spermatogenic elements, distortion of tunica propria, damage to Leydig cells, impairment as well as arrest of spermatogenesis resulting into severe reduction of sperms- a condition of oligozoospermia, damage to all cells type of epididymis, abundance of clear cells, limpness of steriocilia, similarly the accessory sex glands which are said to be dependent upon androgen showed severe atrophy in their architecture as well as their secretory activities.

From the foregoing, it is concluded that Methotrexate (MTX) has antispermatogenic, antigonadotrophic and antiandrogenic properties and therefore severe obstruction to all male reproductive physiological process which are androgen dependent.

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