EFFECT OF FOLLIC ACID ANTAGONIST METHOTREXATE (MTX) ON TESTIS AND ACCESSORY GLANDS OF *FUNAMBULUS PENNANTI* (WROUGHTON)

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ABSTRACT

Effect of Methotrexate on reproductive organ and glands of Funambulus pennanti has 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. For comparing the effects saline treated vehicle was injected same amount of saline and were maintained for the same duration. After treating weight of testis and reproductive accessory glands (Seminal vesicle, prostate gland and epididymis) measured. It is concluded that Methotrexate has adverse effect on weight of reproductive and accessory organs which are dose and duration dependent besides being toxic, therefore certainly causing reduction in the fertility rate.

Keywords: *Methotrexate, Organ weight, Antifertility.*

Intoduction

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

Testis, epididymis, seminal vesicle and prostate of all the experimental and control animals were weighed separately on an electric balance and a data was maintained.

Observation and Results

Vehicle Treated Control

During active breeding, the weight of the testis varied from 0.430 to 0.450 gms. The epididymis weight varied from 0.060 to 0.080 gms. The seminal vesicle weight varied from 0.210 to 0.240 gms and prostate weight varied from 0.300 to 0.310gms (Table 4 and fig. 38 bar diagram).

Low Dose Treatment (3mg/kgBW MTX for 15 days)

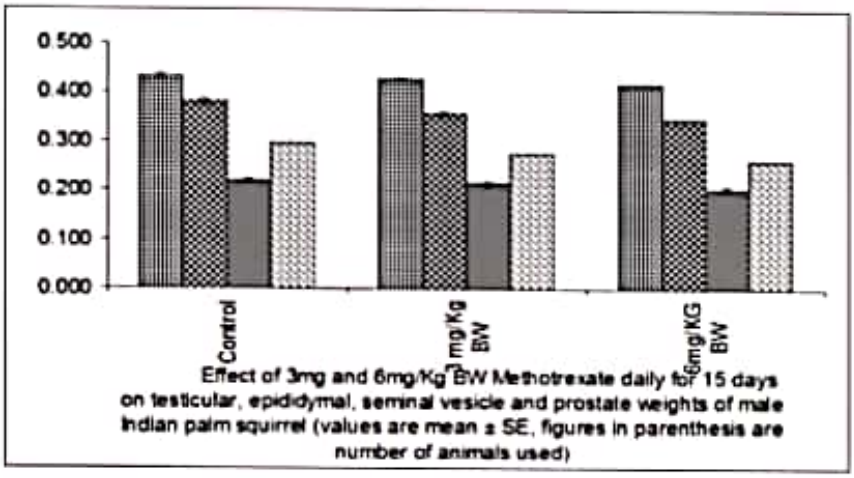
All animals treated with 3mg/kgBW/day showed decrease in organ weight as compared to control animals (Table-1 and fig.1 bar diagram). Similarly there was reduction in the size of testis.

High Dose Treatment (6mg/Kgbw/Day MTX for 15 Days)

6 mg/kgBW/day for 15 days showed pronounced decrease in organ weight as compared to control and low dose animals (Table-1 and fig.1 bar diagram).

Table - 1 Effect of 3mg and 6mg/Kg BW Methotrexate daily for 15 days on testicular, epididymal, seminal vesicle and prostate weights of male Indian palm squirrel (values are mean ± SE, figures in parenthesis are number of animals used)

Treatment	Mean Value			
	Testicular Wt.	Epididymal Wt.	Seminal Vesicle Wt.	Prostate Wt.
Control	0.433 ± 0.0053	0.382 ±0.0050	0.220 ±0.0036	0.297 ±0.0039
3 mg/Kg BW	0.426 ±0.0032	0.357 ±0.0069	0.213 ±0.0035	0.278 ±0.0015
6mg/KG BW	0.417 ±0.0004	0.347 ±0.0034	0.204 ±0.0035	0.262 ±0.0003
P value	P < 0.001	P < 0.5	P < 0.5	P < 0.005



Effect of 3mg and 6mg/Kg BW Methotrexate daily for 15 days on testicular, epididymal, seminal vesicle and prostate weights of male Indian palm squirrel (values are mean ± SE, figures in parenthesis are number of animals used)

Discussion

The reduction in the weight of testis and accessory organs or glands of the MTX treated squirrel, (both the low dose and high treated groups) points to **reduced level of androgen level of androgen binding protein (ABP)** in the testis and a reduction in the circulating androgen as described by (Sussman et al., 1980; Blatt *et al.*, 1981; Shamberger *et al.*, 1981 a, b; Koehler et al., 1986b and Badri *et al.*, 2000) and as result of diminished gonadotrophin activity (Sussman and Leonard, 1980; Blatt et al., 1981; Shamberger *et al.*, 1981a, b; Kohler *et al.*, 1986). This is because the biosynthesis and secretion of ABP appear to be regulated by both FSH and androgens. (Tindall and Means 1997; Buchanan and Riches, 1986). The Sertoli cells of the rat secrete tubular fluid rich in proteins of which androgen binding protein (ABP) is of atmost importance (Hansson *et al.*, 1976). It is the FSH which activates the Sertoli cells thus helping in the formation of ABP. The bound androgen in the form of ABP leaves the testis through the efferent duct fluid conveying the bound androgen to the caput epididymis where it may be used for sperm metabolism and maturation. The accessories like the seminal vesicle and the prostate are morphologically and physiologically dependent on the production of the androgens and circulating androgen levels. Due to the dose and duration specificity of MTX various conclusion were drawn by previous workers regarding the maintenance and weights of accessories as described in the previous paragraph.

Though the initiation of spermatogenesis takes place in the testis, the transport, storage, capacitation and nutrition of the spermatozoa are totally dependent on androgenic hormones to maintain their normal structure and function (Mainwaring, 1977; Tuohima, 1980; Buchanan and Riches, 1986; Sugimura, 1986) and are also very sensitive to the level of androgens (Parrot, 1974), as the secretory activity depends upon the circulating androgens. Previous studies by (Sussman *et al.*, 1980; Blatt *et al.*, 1981; Shamberger *et al.*, 1981 a, b; Koehler *et al.*, 1986b and Badri *et al.*, 2000) also support the hypothesis that fall in the testosterone level after MTX treatment lowers the organ weight as in our studies. MTX which is antiandrogenic, therefore causes involution in weight and the size of testis, epididymis, seminal vesicle and prostate. The reduction in the germinal epithelium due to necrosis and apoptosis and therefore their depopulation by extensive vacuolation and slough off into testicular lumen, in the production rate of sperm and hence decrease in weight of testis, decrease in the volume of secretory fluids, all causes reduction in the weights of reproductive organs for example testicular weight (Johnson *et al.*, 1994) who registered reduction in the organ weight. Our res[illegible]ecordan with their results. S[illegible]nific reduction in the [illegible]t w recognized in MTX trea[illegible]eda *et al.*, 1985) which disagree with our results since the growth of the prostate depends upon the level of androgens in the body (Buchanan and Riches, 1986). Decrease in the sperm production correlate well with decrease in

testicular weight (Robaire *et al.*, 1979). Above statement is also supported by an insignificant reduction in the values of testosterone with low dose and significant reduction with high dose MTX treatment.

In our study we found that the decrease in the weight of testis and accessories had a direct relation to the nature and duration of the treatment. In case of low dose (3mg/kgBW/day for 15 days) treatment of MTX the testis and accessories registered an insignificant decrease in their weights but significant in the high dose (6mg/kgBW/day for 15 days). In both the treatment the gross morphological appearance of the testis and accessories appeared the same, but they all looked like miniature replicas of their original structure.

Summary and Conclusion

Reproductive organs and glands showed an insignificant change in weight with low dose whereas significant change with high dose.

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