17. Effect of Follic Acid Antagonist Methotrexate (MTX) on Changes in Body Weight and Reproductive Organ Weight of Funambulus Pennanti (Wroughton)

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Abstract

Methotrexate (MTX), an effective agent in treatment of cancer, is one of the most versatile antineoplastic agents in spite of severe toxicity problems due to cross linking of DNA–inhibition, protein synthesis and arrest of cell–cycle in S–phase are the important factors that interfere with the viability of cell to cell interaction, cell–proliferation and differentiation. The toxic effect of Methotrexate on the reproductive organs (testis and epididymis), accessory glands (seminal vesicle and prostate) and evaluation of testosterone levels have seen studied by intramuscularly injecting low dose of 3 mg/kgBW/day and 6 mg/kgBW/day for 15 days to adult male squirrel (*Funambulus pennanti*) during the breeding period January 2007. For comparing the effects the saline treated vehicle was injected same amount of saline and were maintained for the same duration. The low dose treatment resulted into an insignificant and high dose treatment into significant body weight loss. Similarly the reproductive organs and glands showed an insignificant change in weight with low dose whereas significant change with high dose.

Key Words: Methotrexate, Body weight, Organ weight

Introduction

Methotrexate is structurally related to dihydrofolate (the natural substrate for dihydrofolate reductase) that catalyzes the reduction of dihydrofolate to tetrahydrofolate and is a potent inhibitor of dihydrofolate reductase (DHFR). The inhibition of DHFR leads to an accumulation of dihydrofolate which is unable to act as substrate for any of the reaction converting tetrahydrofolate to its cofactor derivatives and, therefore, its accumulation is associated with depletion of the pool of the reduced folate cofactors. 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.

Observation and Results

General Conditions

All the treated animals as well the vehicle treated controls did not display sluggish behaviors. The general activities like alertness, locomotion, feeding, and excretion were partially hampered when compared to the treated groups. Administration of daily injection in the thigh resulted into loose texture of the musculature and inflammation. Squirrel treated with low dose and high dose Methotrexate (MTX) showed loss of body weight and hence the animal appeared skinny when compared to the control (figs 4, 5 and 6).

Vehicle Treated Control

Body Weight

The body weight of a mature adult varied from 90 gms to 125 gms during active breeding season i.e. from January to end of July (Table-1 and fig. 1 bar diagram).

Organ Weight

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 2 and fig. 4 bar diagram).

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

Body Weight

The total body weight of all animals treated with 3mg/kgBW/day showed insignificant decrease in the body weight as compared to control animals. (Table–1 and fig. 1 bar diagram).

Organ weight

All animals treated with 3mg/kgBW/day showed decrease in organ weight as compared to control animals (Table- 2 and fig.2 bar diagram). Similarly there was reduction in the size of testis.

High Dose Treatment (6mg/kgBW/day MTX for 15 days)

Body weight

The total body weight of all animals treated with 6 mg/kg BW/day showed significant decrease in their body weights as compared to control and low dose animals (Table-1 and fig. 1 bar diagram).

Organ weight

6 mg/kgBW/day for 15 days showed pronounced decrease in organ weight as compared to control and low dose animals (Table-2 and fig. 2 bar diagram).

Discussion

In the present work an insignificant reduction in the body weight of low dose treated group(3mg/kgBW/day for MTX 15 days) was observed but significant reduction in the high dose treated group (6mg/kgBW/day for 15 days)was registered . Similar reduction in the body weight from moderate to severe has been recorded by Labat et al., 1987and Iqbal et al., 1993, on the contrary no effect on the body weight was registered (Takeda et al., 1985).

A reduction in the total body weight with MTX treatment may be due to decline in the circulating blood serum androgen, since **androgen are potent stimulant of nitrogen retention and their administration readily leads to an increase in body weight in both men and women** (Kochakia, 1950; Forbes, 1985; Bhasin et al., 1997). **Reduction in the testosterone level** in the present work (Table-6) as well as the perusal of the earlier literature confirms our observations, (Sussman et al., 1980; Blatt et al., 1981; Shamberger et al., 1981b; Kohler et al., 1986 a, b and Badri et al., 2000).This is further supported by the **reduction of LH** in the blood as LH is precursor for the synthesis of testosterone in the Leydig cells (Shamberger et al., 1981a, b and Koehler et al., 1986). The alteration or occasional alteration in the **functionality of Leydig cells** which synthesizes testosterone Narrod et al., 1977; Lendon et al., 1978; Shamberger et al., 1981 a,b; Hensle et al., 1984; Saxena et al., 2004) or as suggested by Koehler et al., 1986b and Badri et al., 2000,the reduced plasma level of testosterone may be **an enzymatic defect** since

the specific activities of 3-β and 17-β hydroxyl-steroid dehydrogenase were markedly diminished.

Organ Weight

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

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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 results are in accordance with their results. Similarly no significant reduction in the prostate weight was recognized in MTX treated rats (Takeda 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. The light microscopic appearance of the testis revealed a slight change in the pattern of cytoarchitecture but the androgen deficiency was highlighted by the depletion in the germinal epithelium due to sloughing off of testicular tissue into the epididymis, a marked reduction in the diameter of seminiferous tubules with subsequent increase in intertubular spaces in both the treated testis, reduction in the diameter of seminiferous tubules (234.61 ± 3.38 x 157.21 ± 0.39) ($P< 0.05$). Size of primary and secondary spermatocytes was altered significantly. Vacuolization and decondensation of chromatin mass in primary/secondary spermatocytes led to change in the architectural features of the cells, significant reduction in the size of spermatid, and Leydig cells, partial arrest of spermatogenesis as evident by the change in the count of sperm from oligospermia to oligozoospermia, regression in the height and secretory activity of the epididymal epithelium, loss of secretion as well as atrophy and glandular degeneration as observed by the partially filled or empty lumen. The reason is attributed to the fact that **MTX being potent inhibitor of testicular 3α-hydroxy steroid oxido-reductase activity, itself binds to the catalytic binding sites of the substrates like DHT (5α-dehydroxytestosterone) thus reducing the ABP production which would have helped in the maintenance of the epididymis and accessories.**

From the foregoing it is inferred that Methotrexate (MTX) has the ability to cross blood into interstitial space and seminiferous tubule to induce such changes significantly and therefore indirectly causing reduction in the weight of the organs as the **nuclei in all the reproductive organs fail to replicate their DNA due to inhibition of an essential enzyme (dihydrofolate reductase) required for the normal synthesis of DNA** (Wheelar, 1962; Russell and Russell, 1991 and Saxena et al., 2004).

Table-1 Effect of 3mg and 6mg/Kg BW Methothrexate daily for 15 days on initial and final body weight of male Indian palm squirrel (values are mean ± SE).

Treatment	Mean Value	
	Initial	Final
Control	128.33 ± 1.67	131.67 ± 2.40
3 mg/Kg BW	115.00 ± 2.89	109.33 ± 3.48
6mg/KG BW	120.67 ± 3.54	111.33 ± 3.40
P value	P < 0.01	P < 0.01

Figure 1. Bar Diagram

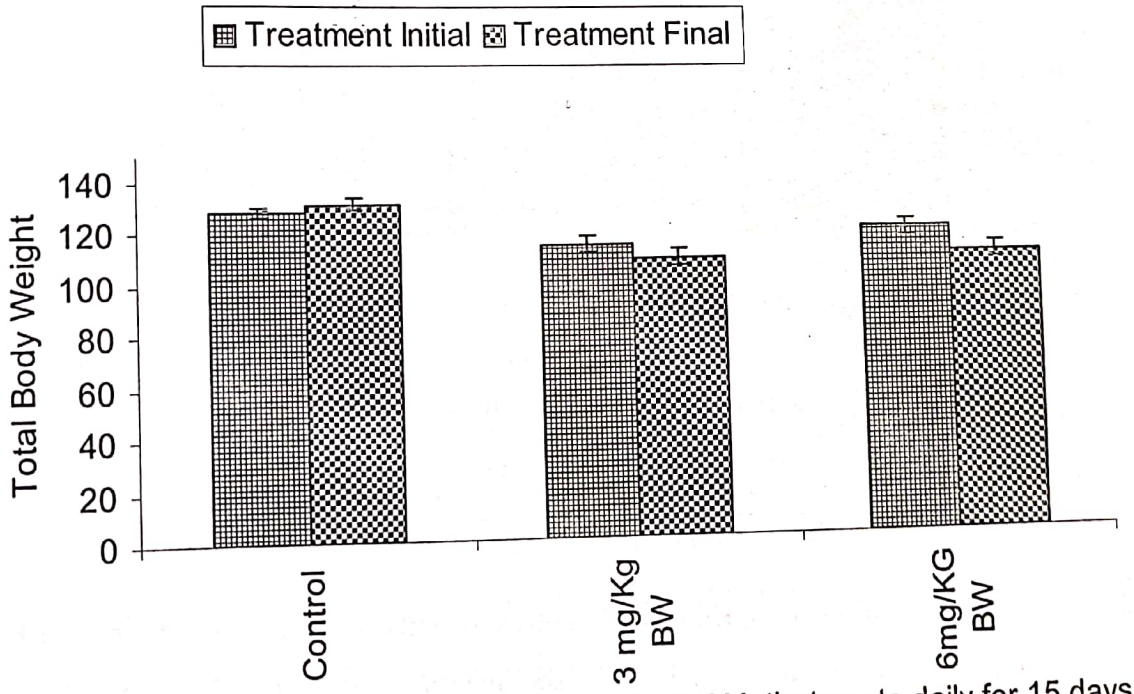

Fig. 37 Effect of 3mg and 6mg/Kg BW Methotrexate daily for 15 days on initial and final body weight of male Indian palm squirrel (values are mean ± SE)

Table -2 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

Figure 2. Bar Diagram

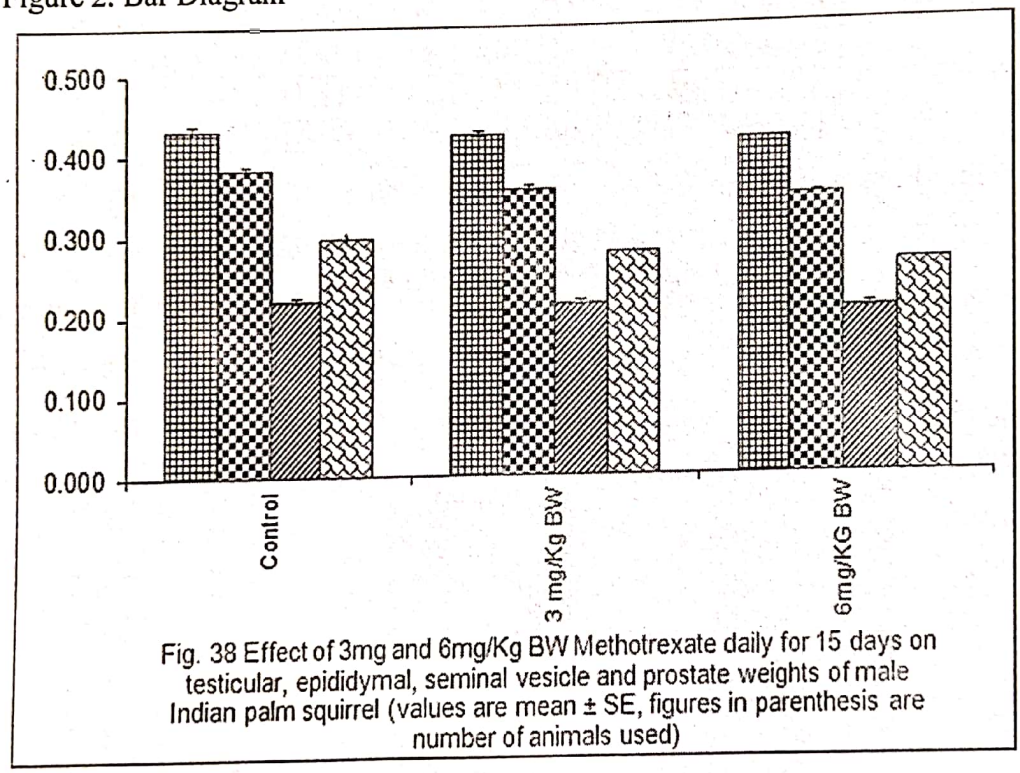

Fig. 38 Effect of 3mg and 6mg/Kg BW Methotrexate daily for 15 days on testicular, epididymal, seminal vesicle and prostate weights of male Indian palm squirrel (values are mean ± SE, figures in parenthesis are number of animals used)

Table 3 Effect of 3mg and 6mg/Kg BW Methotrexate daily for 15 days on diameter of Semineferous tubule, epididymal tubule, seminal tubule and prostatic acini (values are mean ± SE)

Treatment	Mean Value				
	Seminiferous tubule	Caput	Cauda	Seminal Vesicle	Prostate
Control	259.00 ± 0.96	154.34 ± 1.60	333.45 ± 3.90	348.54 ± 2.09	96.20 ± 2.39

3 mg/Kg BW	234.61 ± 3.38	150.80 ± 0.92	292.15 ± 3.93	328.60 ± 1.51	68.27 ± 1.45
6mg/KG BW	157.21 ± 0.39	143.87 ± 1.62	273.23 ± 0.64	274.94 ± 0.67	57.32 ± 1.63
P value	P < 0.05	P < 0.05	P 0.05	P < 0.05	P < 0.05

Figure 3. Bar Diagram

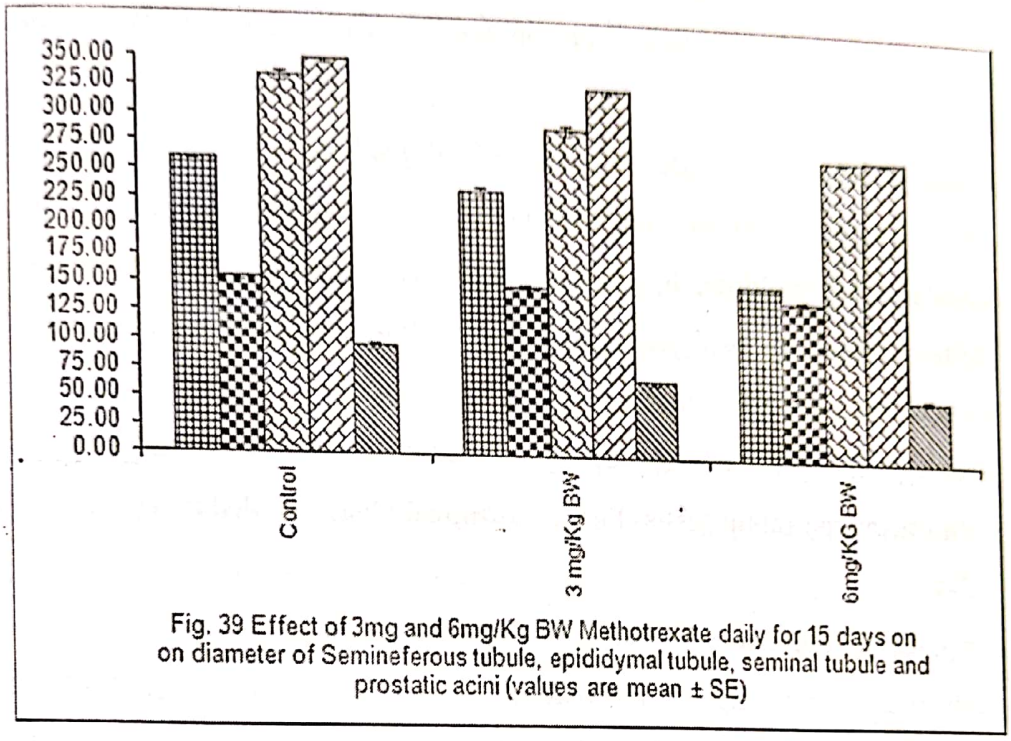

Fig. 39 Effect of 3mg and 6mg/Kg BW Methotrexate daily for 15 days on on diameter of Semineferous tubule, epididymal tubule, seminal tubule and prostatic acini (values are mean ± SE)

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