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Effect of folic acid antagonist methotrexate on prostate gland of Indian Palm Squirrel *Funambulus Pennanti* (Wroughton)

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Abstract

The toxic effect of Methotrexate on the prostate have been 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. For comparing the effects the saline treated vehicle was injected same amount of saline and was maintained for the same duration. The low dose treatment resulted into remarkable reduction in the size of prostatic acini, with moderate or considerable increase in the intertubular connective tissue, complete vacuolation in some cells but supra and infra nuclear in most of the cells, general disturbance in the secretory epithelium lining and exfoliation of nuclei into the lumen. These sloughed off nuclei were either lying in the partially dried prostatic secretion or they were extruded along with cytoplasm in any corner of the acinal lumen. Above mentioned effects were further enhanced with high dose as evident by total loss of secretory activity due to severe disruption of secretory epithelium, their degeneration, severe vacuolation, pyknosis of nuclei or total disintergration, general disruption in the basis architecture of the prostatic acini, enucleating of many of the cells.

Keywords: Methotrexate | toxic effects | prostate gland

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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, Felig and Frohman 2001, Prasad *et al.,* 1996). Methotrexate was formerly known as amethopterin, is an antimetabolite drug used in treatment of cancer and autoimmune diseases.

Objective

Thus the present study embodies:

1) Histopathological changes undergone by prostate gland.

Experimental protocol

In all three sets of experiments using low and high-doses of Methotrexate (MTX) were performed for the present study for the duration of 15 days (Tables 1 & 2).

Animals were sacrificed using chloroform 24 hours after the last day of each experiment. Immediately the organs were excised prostate, gland used for histological studies.

Number of animals and sex	Treatment	Dose mg/kg BW	Route	Duration
3 males (Experimental)	Methotrexate	3 mg daily	I.M.	15 days
3 males (Control)	Saline	E.V.	I.M.	15 days

E. V. = Equal volume,
I. M. = Intra muscular,
B W = Body weight

Table 1: Experimental Design for Low Dose Methotrexate treatment

Number of animals and sex	Treatment	Dose mg/kg BW	Route	Duration
3 males (Experimental)	Methotrexate	6 mg daily	I.M.	15 days
3 males (Control)	Saline	E.V.	I.M.	15 days

E. V. = Equal volume,
I. M. = Intra muscular,
B W = Body weight

Table 2: Experimental Design for High Dose Methotrexate treatment

Observation and Results

Histological Studies:

Prostate were fixed in Bouin's fluid for 24hrs and preserved in 70% alcohol. The tissues were dehydrated by passing through graded series of alcohol, cleared in xylol and after embedding in paraffin, blocks were prepared and serial sections were cut at various thicknesses between 5µ to 8µ. For routine histological study the sections were stained with Ehrlich's haematoxylin and counter-stained with eosin. Measurements when necessary were taken with the help of an occular micrometer calibrated to a stage micrometer. The photomicrographs were taken with the help of a Carl Zeiss camera attached to the microscope and enlarged to the required size.

The prostate was a compact compound tubular gland lying in the close approximation to the bladder. The glands have a thin membranous capsule. The glandular substance was spongy. The prostate showed two clearly marked regions, a cranial peripheral unit of secreting

tubules and a caudal ventral unit of collecting tubules. From the capsule thin trabeculae of fibromuscular tissue extended inward and formed the boundaries of the lobules. The lobules were formed of a closely packed network of glandular lobules or acini. Flat squamous epithelium lined the large distended acini with dense secretory material. The epithelial lining was infolded and intertubular connective tissue was thin (figs. 1 and 2).

Low dose treatment

(3mg/kgBW MTX for 15 days)

Histopathological study

All acini appeared partially shrunk in size due to a moderate increase in inter-tubular connective tissue.

The secretory epithelium also appeared straight probably due to loss in secretory activity, at many places in most of the acini the epithelium was lost and their nuclei were found to be accumulated in the centre. The cytoplasm also appeared extremely vacuolated and therefore feather-like. Some tubules showed partial secretory activity but most were empty. The lamina propria encasing each tubule was not distinctly demarcated. The fibro-muscular tissue even appeared free of smooth muscle cells, may be due to their loss (fig. 3 and 4).

High dose treatment

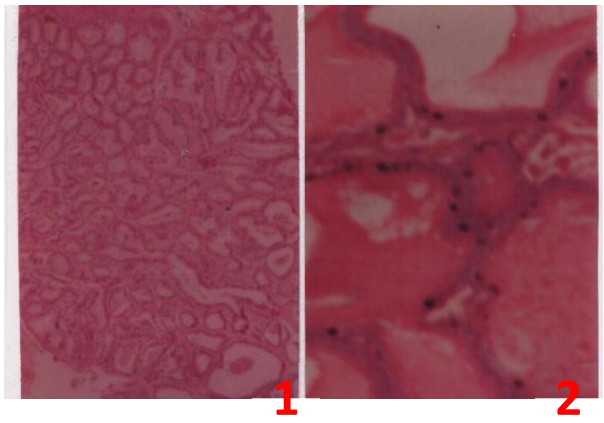
(6mg/kgBW/day MTX for 15 days)

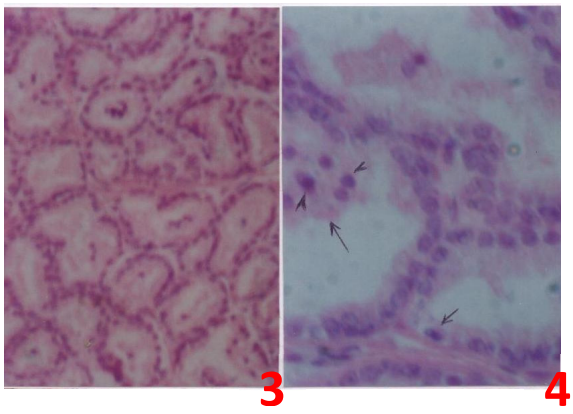
The high dose treatment has resulted into severe tubular contour distortion, smallness in size and distortion in linear arrangement of nuclei. The tunica propria lining each tubule was thickened at some places as well as broken at other places. Similarly, there was loss of nuclei as well as cells. Sometimes total loss of cytoplasm leaving only remnants of pyknotik nuclei. The intertubular connective tissue separating the tubules appeared fibrous and vacuolated. The tubules were completely empty due to loss of secretory activity. The cytoplasm showed extensive vacuolation (figs. 5 and 6).

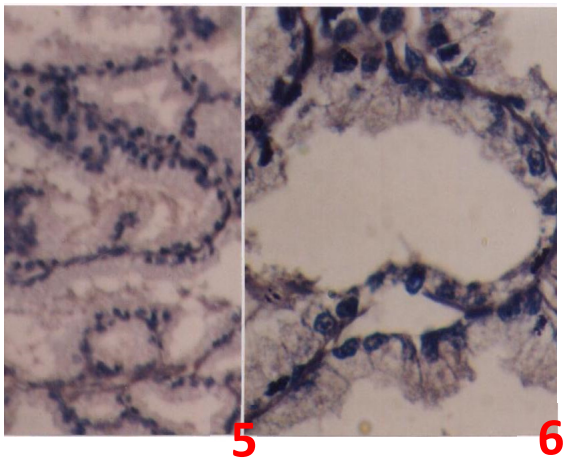
Discussion

In control squirrels the prostate revealed normal columnar epithelium which was reduced to cuboidal and squamous because of great distention of the tubules and copious amount of secretion in the lumen. The low dose treatment (3mg/kgBW/day MTX for 15 days) have resulted into remarkable reduction in the size of prostatic acini, with moderate or considerable increase in the intertubular connective tissue complete vacuolation in some cells but supra and infra-nuclear disturbance in most of the cells, general disturbance in the secretory epithelium lining, and exfoliation of nuclei into the lumen. These sloughed off nuclei were either lying in the partially dried prostatic secretion or they were extruded along with cytoplasm in any corner of the acinal lumen. The above mentioned effects have been further enhanced with high dose treatment (6 mg/kgBW/day MTX for 15 days) as evident by total loss of secretory activity due to severe disruption of secretory epithelium, their degeneration, severe vacuolation, pyknosis of nuclei or total disintegration, general disruption in the basic architecture of the prostatic acini, enucleation of many of the cells. This is the pioneer study on Methotrexate taken up in our laboratory since till now no previous worker has taken interest in the accessory sex glands excepting

a small note by Takeda *et al.,* 1985 who did a brief mention only on atrophy and degeneration of the glandular epithelium in the rat prostate.







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