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Research Article

EFFECT OF NANO MOLAR CONCENTRATION OF METHYL PARATHION ON GOAT TESTIS

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ABSTRACT

Objective: The present study shows the quantitative and qualitative histological changes in testes of goat treated with Methyl parathion (1 nanomole/ml) for three different time exposures (4 hrs, 8 hrs and12 hrs) *in vitro*.

Methods: After stipulated time, the tissues were harvested and fixed in Bouin's fixative. The histological slides of tissues were prepared and were studied under light microscope.

Results: The decrease in the diameter of seminiferous tubules, increase in the interstitial space, the decrease in the numbers of germ cells and supporting cells, Cytoplasmic vacuolization of the germ cells, distortion of seminiferous tubules were observed in present study.

Conclusion: Methyl Parathion suggested to be toxic on male reproductive system if exposed for prolong period. The awareness regarding the impact of Methyl parathion should be given to farmer and they should be encouraged to practice biological means to control pests and herbs instead of these harmful chemical compounds.

Keywords: Testis, Germ cell, Seminiferous tubule, Lumen.

INTRODUCTION

Methyl parathion (O,O-dimethyl O-4-nitrophenyl phosphorothioate) is an organophosphate pesticide widely used in agricultural activities as an acaracide and an insecticide. It is commonly used in order to kill the insects in different products such as cotton, corn, apple, bean, rice, wheat, peach, clover and sunflower [1].Thus human exposure of this compound may occur through occupational setting. The present investigation deals with the qualitative and quantitative evaluation of the Methyl parathion on testicular morphology of goat. Effect on diameter of seminiferous tubules, interstitial space, numbers of germ cells and supporting cells were studied.

MATERIALS AND METHODS

Reagents: The reagents used during the study were of analytical grade and procured from standard laboratory suppliers.

Experiment design: The testis of *Capra hircus* procured from the slaughter houses near Kurukshetra and brought to the laboratory in culture media. The testis was cut into small pieces and processed for *in vitro* experimental protocol. After washing with normal saline, the tissue was placed in culture medium (TCM-199) which was fortified with antibiotics (200 unit penicillin 10 IU/ml and streptomycin 1µg/ml). The 1 nanomole/ml Methyl parathion in culture medium is employed for toxicity assessment.The tissues were divided into four groups. The Group A was the zero hour control and kept in Bouins fixative. The Group B was exposed to drug for 4 hour, Group C was exposed to drug for 8 hour and Group D was exposed to drug for 12 hours, all with their respective control.

Histological slides: The tissue was harvested after stipulated time and processed for histological slide preparation. For histological slides the tissue was fixed in aqueous Bouins fixative for 24 hours. Then tissue was washed in running tap water for 6 hours. The specimens then were dehydrated in various grades of alcohol. After proper dehydration specimens were then embedded in paraffin wax at 58^0-60^0C. The tissue was sectioned serially at 5 µm thickness and the sections were stained with the haemotoxylene for 10 minutes and allowed to develop for 5 to 15 minutes in tap water. After dehydration in 70% ethanol, the sections were stained with eosin (2% eosin in 70 % alcohol) for 1 to 2 minutes. The slide were washed in 70% ethanol and dehydrated in 90% and absolute alcohol and cleared in xylene and were mounted in DPX. Each section was examined under light microscope to study the morphological characteristics of testis tissue.

RESULTS AND DISCUSSION

The group A showed the normal testicular morphology. The seminiferous tubules arranged in bundles and possessed germ cells and Sertoli cells. The Leydig cells were localized in interstitial space. The mean seminiferous tubules diameter was 54 µm and mean lumen diameter was 14.5 µm (Figure 1 and 2, Table 1 and 2).

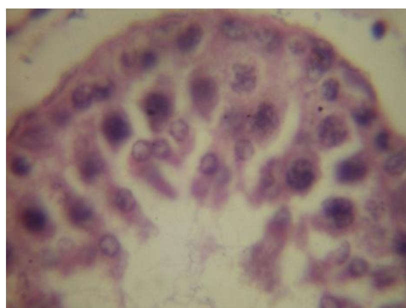

Fig. 1: Zero Hour Control (Group A) (1000X)

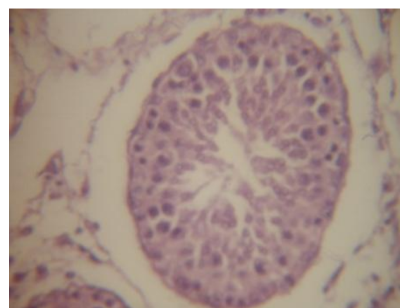

Fig. 2: Zero Hour Control (Group A) (400X)

The group B showed reduction in the mean seminiferous tubules diameter from 52.2 µm, in control group, to 46.5 µm, in treated group (Figure 2 and 3).The one-tailed P value is 0.0302, considered significant (Table 1). The mean lumen diameter was increased from 14.2 µm, in control group, to 19.3 µm, in treated group (Figure 2 and 3).The one-tailed P value is 0.0030, considered very significant (Table 2).

The group C showed disturbance in architecture of semiferous tubles with marked detechment. The treatment result in decreased mean diameter of seminiferous tubules from 49.6 µm, in control group, to 43.6 µm, in treated group (Figure 5 and 6). The one-tailed P value is 0.0189, considered significant (Table 1). The mean lumen diameter was increased from 14.1 µm, in control group, to 20 µm, in treated group (Figure 5 and 6).The one-tailed P value is 0.0004, considered extremely significant (Table 2).

The group D showed distortion of seminiferous tubules. The mean diameter of seminiferous tubules declinedfrom 46.4 µm, in control group, to 40.1 µm, in treated group (Figure 7 and 8,). The one-tailed P value is 0.0141, considered significant (Table 1). The

mean lumen diameter was increased from 13.6 µm, in control group, to 17.4 µm, in treated group (Figure 7 and 8). The one-tailed P value is 0.0116, considered extremely significant (Table2).

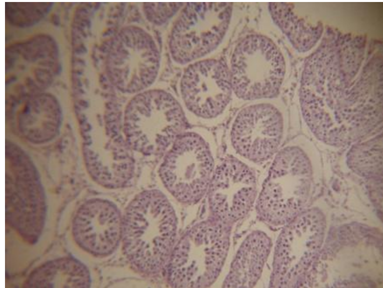

Fig. 3: Four Hour Control(Group B) (100X)

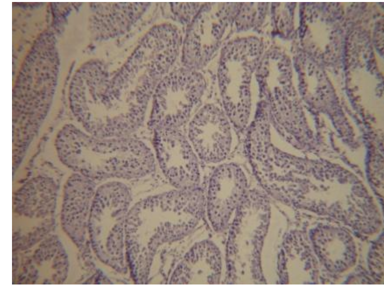

Fig. 4: Four Hour Treated (Group B) (100X)

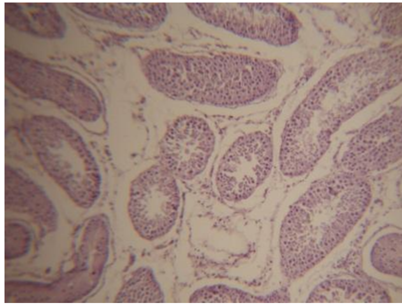

Fig. 5: Eight Hour Control (Group C) (100X)

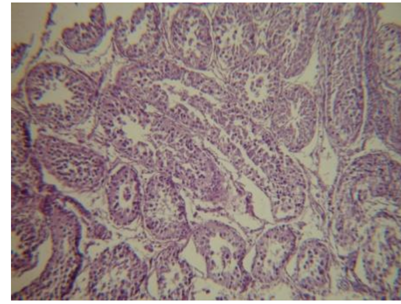

Fig. 6: Eight Hour Treated (Group C) (100X)

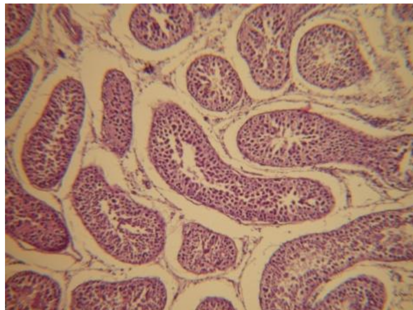

Fig. 7: Twelve Hour Control (Group D) (100X)

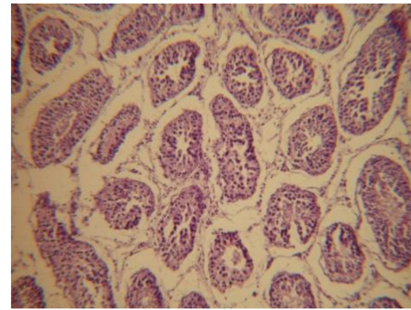

Fig. 8: Twelve Hour Treated (Group D) (100X)

Table 1: Effect of Methyl Parathion on Seminiferous tubules diameter

Group	Duration of Exposure (Hours)	Seminiferous tubules Diameter (µm) (Mean ± SD)		P Value
		Control	Treated	
A	Zero	54.0 ± 4.22		
B	4	52.2 ± 6.53	46.5 ± 3.95	0.0302 (Significant)
C	8	49.6 ± 6.18	43.6 ± 3.88	0.0189(Significant)
D	12	46.4 ± 4.50	40.1 ± 5.78	0.0141(Significant)

Table 2: Effect of Methyl Parathion on Lumen diameter

Group	Duration of Exposure (Hours)	Lumen Diameter (µm) (Mean ± SD)		P Value
		Control	Treated	
A	Zero	14.5 ± 3.15		
B	4	14.2 ± 4.29	19.3 ± 1.94	0.0030 (Very Significant)
C	8	14.1 ± 3.54	20.0 ± 2.49	0.0004(Extremely Significant)
D	12	13.6 ± 3.65	17.4 ± 2.22	0.0116(Significant)

Reduction in seminiferous tubule dimensions and declining in number of germinal cells and Leydig cells after exposure of methyl parathion is similar to those observed after exposure of Diazinon in rats [2].

The disorganization of seminiferous tubule structure and vacuolization observed during present investigation show similar set of changes as already recorded after exposure of Endosulphan [3] and Malathion [4].
The reduced number of Leydig cells observed after exposure of methyl parathion strongly indorses earlier findings after atrazine treatment in rats [5].
Degenerative changes including degranulation and sloughing off germ cells are similar to those observed after exposure of different pesticides [2-5].
From pattern of changes observed after exposure of pesticides by various workers strongly advocates that the molecular mechanism associated these degenerative changes involve similar pathway which might be due to enhance oxidative stress as reported in enormous studies.
The changes observed after exposure of Methyl parathion in testicular tissue are possibly due to agonistic or antagonistic activity of pesticides to hormone or hormone receptors.

CONCLUSION

Present studies opines that Methyl parathion disturb normal testicular function either via enhancing oxidative stress or interfering with underlying endocrine regulation. It is therefore recommended that use of such pesticide should be allowed at SOS situation. Frequent exposure as well as acute toxicity of Methyl parathion may lead to infertility problems. It is suggested that biological alternative should be explored as an alternative to these toxic agents.

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