Effect of phosphamidon on the testes of albino rats: a histological study

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ABSTRACT

The present study shows the qualitative and quantitative histological changes in testes of albino rats treated with two doses of phosphamidon 35 and 50 parts per million(ppm) for 1 month time period. Rats were treated by drinking water containing 35 ppm (low dose) and 50ppm (high dose) concentration of phosphamidon for30 days. After 30 days, they were sacrificed, the testes were fixed in vivo and were taken out. The histological slides of these testes were prepared and were studied under light microscope. The decrease in the weight of testes and 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 the findings of present study. phosphamidon seems to be toxic on male reproductive system if exposed for prolong period. The awareness regarding the impact of phosphamidon 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: Phosphamidon, Testes, Albino rat, Spermatogenesis, Germ cells

INTRODUCTION

Phosphamidon is widely used pesticide like other organophosphorus. It exerts its toxic effects to the pest by phosphorulation of serine residues in the active centre of acetylcholineesterase, which leads to accumulation of acetylcholine.[1] Phosphamidon has been detected in the environment as the contaminants in the drinking water and pesticide residues in food stuffs because of their extensive uses.[2] Thus human exposure of this compound may occur through occupational setting as well as via a verity of environmental sources.

The spermatogenesis is a highly sensitive process toward the physical and chemical agents in which precursor cells form mature haploid spermatozoa within seminiferous tubules. It is very important to know the cytotoxic effect of phosphamidon on male reproductive system, as it is not well studied earlier according to available literature.

There has been a genuine decline of sperm count among healthy men in world, fallen to about 50.0% and the quality of semen over past 50 years. This indicates reduction of male fertility and there is significant correlation between the fertility and the exposure to pesticides.[3]

MATERIALS AND METHODS

Thirty healthy rats weighing 160-200 gm albino rats were randomly selected from the animal house of BPKIHS Dharan .They were given standard pallet diet and housed in well ventilated room at control ambient temperature (25±5ºc) and natural day light cycle.

These rats were equally divided into 3 group comprising 10 rats in each group. The first group was provided the plane tap water, acts as control while the second and thirds groups were provided the tap water containing 35 and 50ppm doses of phosphamidon for 30 days *ad libitum* and were designed as experimental groups. The phosphamidon used was commercially manufactures by Anu product Ltd (Agro chemical ,Tigon ,Fariabad) the formulated chemical was diluted in drinking water to make 35 and 50 ppm solution of phosphamidon .

All the experimental rats along with their control immediately after completing 30 days duration were anaesthetized and their testes were fixed in vivo perfusion in Bouin’s fluid. As the perfusion completed the testes were taken out by dissection. The weights and sizes of testes were measured by electronic balance and vernier callipers. After 18 hours of fixation in Bouin's fluid, testis tissues were processed.

The slides were prepared and stained in H.E. for microscopic study.

RESULTS

Quantitative measurement: This was accomplished by measuring different parameter like diameter of seminiferous tubules, interstitial space, number and sizes of germ cell, sertoli cells and leydig cells. Micrometer were used for the measurement.

Qualitative measurement: Different types of qualitative changes in the section were observed.

Statistical Analysis:

Statistical analysis was done by using SPSS program.Wilcoxon test and Mann Whitney Tests were used to see the level of significance of differences. $P<0.05$ was considered as significant.

Weight of the testes: The weight of the right testis in both experimental groups was decreased significantly than the control (control 1.20 ±0.01) Low dose (1.08 ±0 .08), High dose (1.11 ±0.05) $P<0.05$, $P<0.01$. The weight of the left testes treated with high dose group was decreased significantly in comparison to control group (control 1.2 ± 0.1, High dose 1.13 ± .04)

Diameter of seminiferous tubules: The diameter of the seminiferous tubules was found significantly decreased in 35 ppm dose experimental groups (control 424.8 ± 39 Low dose 375.3 ± 32.28 $p<0.05$) In 50 ppm treated group decrease in diameter was highly significant (control 424.8 ±39 High dose 328.17 ±9.35 $p<0.001$)

The interstitial space was signicantly increase in both experimental groups. The number of germ cell,leydig cell, and sertoli cells were significantly decreased .

Qualitative changes: In treated groups, pronounced cytoplasmic vacuolization of germ cells towards the periphery of tubules were seen. It was more pronounced in high dose. The number of spermatozoa is less in both treated group. The lumen of some of the tubules were clear and the spermatozoa were not seen .In high dose group some of tubules were degenerated, the shape of the tubules was also distorted in both groups .Few pycnotic nuclei were observed among the germ cells.

DISCUSSION

Many pesticides cause to decrease the weight of the testes, the decreased in weight of the testes by phosphorothionates (RPR-) and parathion has been documented.[4,5] but they did not compare the weight between right and left testes. In our study, the weight of right testes was more affected by the pesticides, which has not been reported earlier.

Organophosphorus compounds like phosphorothionates, quinolphos cause the reduction of the diameter of seminiferous tubules.[4,5] This is further supported by this study and decrease of the diameter of seminiferous tubules by phosphamidon has been found, which is followed by increased in the interstitial spaces. In the present study the number of primary spermatocytes, round spermatids, sertoli cell and leydig cells per tubules have been decreased with increasing dose .Similar result had been obtained in mice treated by high dose of malathion.[6]

Present study showed more pronounced cytoplasmic vacuolization of germ cells and sertoli cells in high dose treatment .Such type of finding was reported only in high dose treatment of dimethylmethylphosphate, another commonly used pesticides.[7]However in our study we couldn't observe the formation of giant cell derived from germ cells. In our study we found significant decreased in the size of leydig cells($P<0.001$) than other cells .This may be due to lack of testis blood barrier which is existed only inside the seminiferous tubules .This finding has not been mentioned in literature so far consulted .

In conclusion phosphamidon is found to be toxic on male reproductive system if exposed chronically .The reduction of weight of testes, decreased in diameter of seminiferous tubules, increased in interstitial space, reduction in number of germ cells,sertoli cells and leydig cells,cytoplasmic vacuolization of germ cells ,tubular deformities in testes shows the cytotoxic effects of phosphamidon .

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Table-1: Showing the weight of testes of control and Experimental group

Types	Right testes (mean ± SD	Left testis (mean ± SD)
Control	1.20 ± 0.10	!.20 ±0.02
Low Dose	1.08 ±0.08*	1.14 ±0 .10
High Dose	1.11 ±0.05**	1.13 ±0.04***

*P<0.05 ** P<0.01 ***P<0.001

Table-2: Showing the interstitial space and diameter of seminiferous tubules of control and experimental groups.

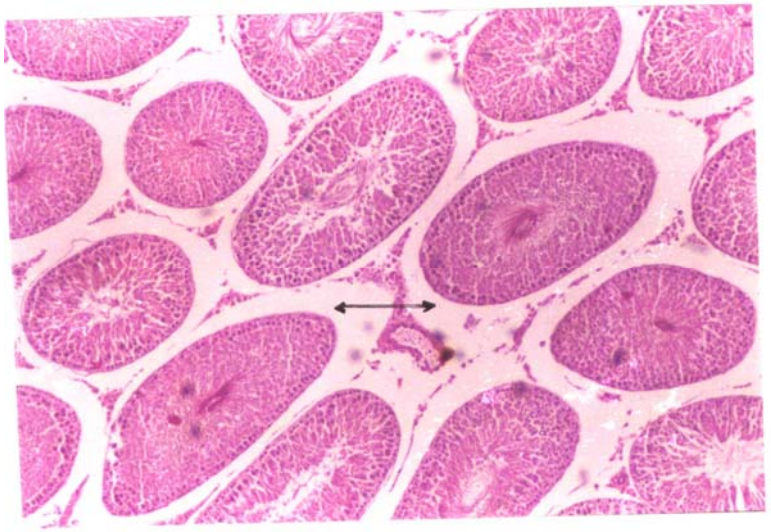
Types	Interstitial space (μm) (mean ± SD)	Mean diameter of seminiferous tubules(μm)) (mean ± SD
Control	29.81± 3.21	424.8± 39.00
Low dose	46.82± 4.89***	375.3 ±32.28**
High dose	50.41 ±3.93***	328.17±19.35***

*P<0.05 ** P<0.01 ***P<0.001

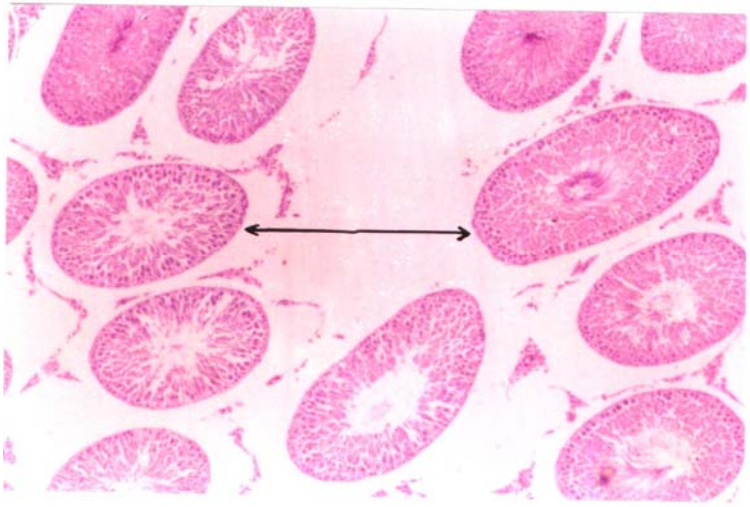
Table-3: Showing the number of different types of germ cells (primary spermatocytes and spermatids) and non-germ cells (sertoli cells and leydig cells) per seminiferous tubules

Types	No of primary spermatocytes (mean ± SD)	No of spermatids (mean ± SD)	No of sertoli cell (mean ± SD)	No of leydig cells (mean ± SD)
Control	72.57± 8.21	185.10 ±15.03	21.27± 2.01	72.43± 5.28
Low dose	53.09 ±6.62***	122.5 ±13.07**	14.71± 1.62***	38.87± 7.76***
High dose	44.13 ±4.4***	121.48±35.08**	13.29± 1.47***	39.96± 8.00***

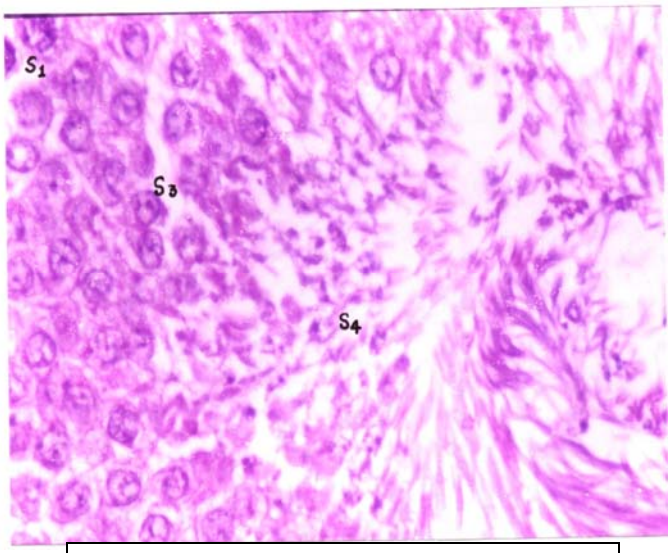
P<0.05 ** P<0.01 ***P<o.001



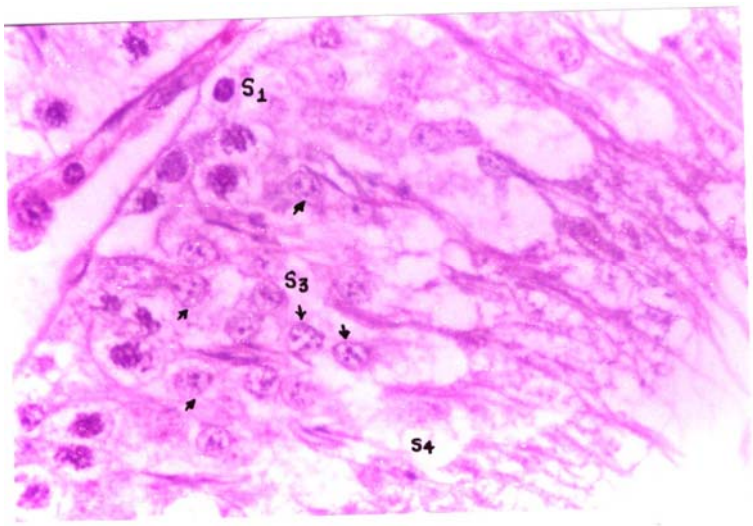
Photomicrograph of T.S. testis of control group showing seminiferous tubules and interstitial space. (H.E.100X)



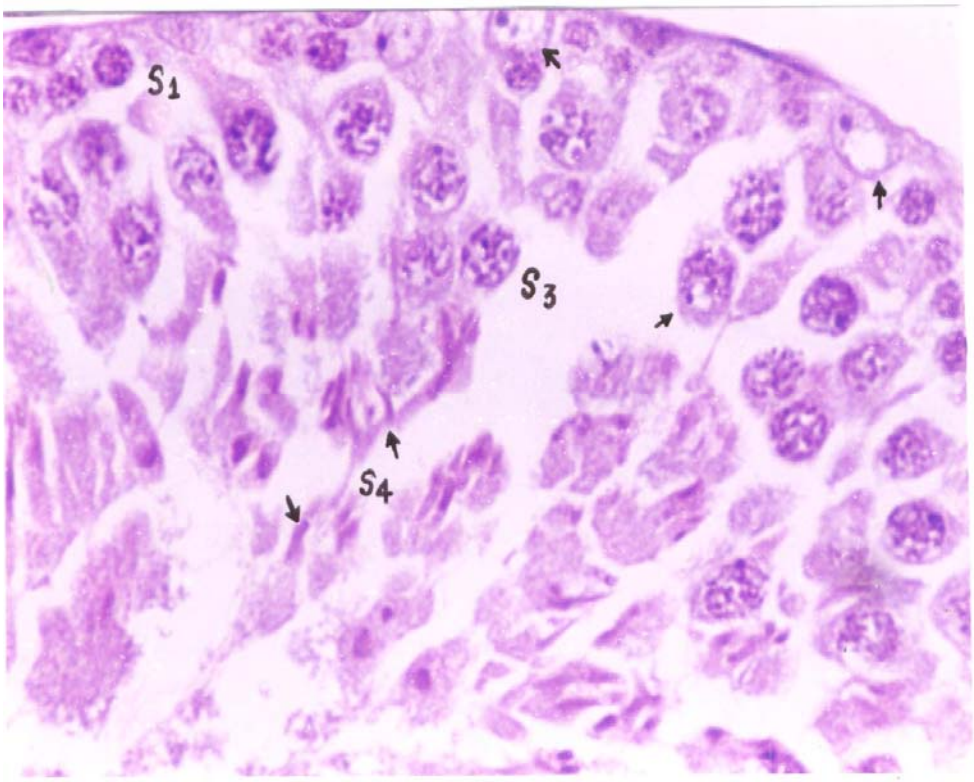
Photomicrograph of T.S.of testis of experimental group showing decreased in diameter of seminiferous tubules and increased in interstitial space.(H.E.100X)



Photomicrograph of T.S. of testis of control group showing large number of germ cells, sertoli cells and spermatozoa.The lumen is filled by spermatozoa (H.E.400X) S1 primary spermatocyte, S3 spermatid, S4 spermazoa



Photomicrograph of testis (Experimental group) showing few number of germ cells ,sertoli cells and spermatozoa. (H.E.400X) S1primary spermatocyte, S3 spermatid, S4 spermazoa



Photomicrograph T.S. of testis (Experimental group50 ppm) arrows showing pronounced cytoplasmic vacuolization of germ cells (H.E.400)