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Study on the Effect of Endosulfan on Testosterone Level and Seminiferous Tubule of Testis of Mice

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Abstract: The increasing knowledge of the reproductive toxicity of environmental chemicals has raised public concern as to whether the current use of pesticides could adversely affect human reproduction. Among pesticides and their related chemicals, organochlorine insecticides have so far drawn the primary attention. Endosulfan is widely used pesticide due to its quick effect on insects. Endosulfan formulations are used in commercial agriculture and home gardening. They are also used for wood preservation. The present study aimed to illustrate the effect of endosulfan on testosterone level and seminiferous tubule and spermatid in testis of mice. Endosulfan was administrated 2 mg/kg b.w daily by gavage method for five weeks. Mice were sacrificed after completion of schedule, blood were collected for testosterone estimation and tissues for histology. Testosterone level was declined with degenerated seminiferous tubule and spermatid. Primary and secondry spermatocyte were also degenerated after endosulfan exposure. Thus it was concluded that endosulfan exposure causes decline level of testosterone in mice which causes improper spermatogenesis due to degeneration of seminiferous tubule. Spermatid and secondary spermatocyte were also degenerated which causes azoospermic condition leading infertility in mice.

Key words: Seminiferous tubule % Testosterone % Spermatid % Endosulfan

INTRODUCTION

The increasing knowledge of the reproductive toxicity of environmental chemicals has raised public concern as to whether the current use of pesticides could adversely affect human reproduction. Among pesticides and their related chemicals, organochlorine insecticides have so far drawn the primary attention. Dischlorodiphenyl Trichloroethane (DDT), dieldrin and toxaphene have instrinsic estrognic activity and are known as possibly endocrine-disrupting chemicals [1]. Endosulfan is a pesticide belonging to the organochlorine group of pesticides, under the Cyclodiene subgroup. Endosulfan is used world wide on food crops such as tea, vegetables and deciduous fruits (and nuts), as well as nonfood crops such as tobacco and cotton. This pesticide is also used on forage crops such as alfalfa [2]. Endosulfan formulations are used in commercial agriculture and home gardening [2]. They are also used for wood preservation [3]. Endosulfan causes spermatozoa degeneration [4] as well as declined testosterone level. Endosulfan exposure lead to ovarian nuclear degeneration [5]. Biochemical changes in endosulfan treated testes of rats was observed by *Sinha et al*. [6]. Endosulfan treatment in pubertal rate inhibits testicular functions [7]. Now a days endosulfan is popularly used by farmers of Bihar but they are not aware about its health impacts.

The present study aims to illustrate effect of endosulfan on testosterone level and seminiferous tubule and spermatid of testis of mice.

MATERIALS AND METHODS

Animals: The mice were reared in our laboratory. The age group of mice selected for the study was 12 weeks old with 30±2 g. b.w.

Chemicals: Pesticide Endosulfan, manufactured by Excel India Pvt. Ltd., Mumbai with EC 35% was utilized for the experiment.

Study Groups and Sampling: The control group of 10 mice received distilled water as drinking water.

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The 'treatment' groups (n=10) received Endosulfan 2 mg/kg b.w daily by gavage method for five weeks. Mice were sacrificed after completion of schedule, blood were collected for testosterone estimation. The testes from all the animals were removed and washed three times in isotonic saline (0.85 v/w%) and fixed in neutral formalin for light microscopy.

Estimation of Serum Testosterone Level in Mice: ELISA Method: Blood sample were collected after each sacrifice and there serum were isolated. Using the ELISA method Testosterone kit of LILAC Medicare (P) Ltd., Mumbai was utilized for the experiment.

Method: The normal range was calibrated and then 25 µl serum samples were taken in the well plates. 100 µl of enzyme conjugate was added in each well. After that, it was left for incubation at 37^0C in incubator for 1 hour. Then, the wells were washed with 300 µl distilled water for at least 3 times and blotted. Then, 100 µl TMB solution was added as substrate in each well plate and was again left for the incubation for 15 minutes for the colour. Finally, 100 µl stop solution was added in each well to stop the reaction. Reading was taken at 630nm through Merck ELISA reader in ng/ml value.

RESULTS

The control group of mice having testosterone level 7.117 ± 0.06 ng/ml while after administration of endosulfan it became gradually decreased by increasing duration of doses i.e: 3.733 ± 0.04 ng/ml after 3 weeks and 1.633 ± 0.04 ng/ml after 5 weeks administration of endosulfan (Figure 1). P Value are < 0.0001 which is highly significant (Table 1).

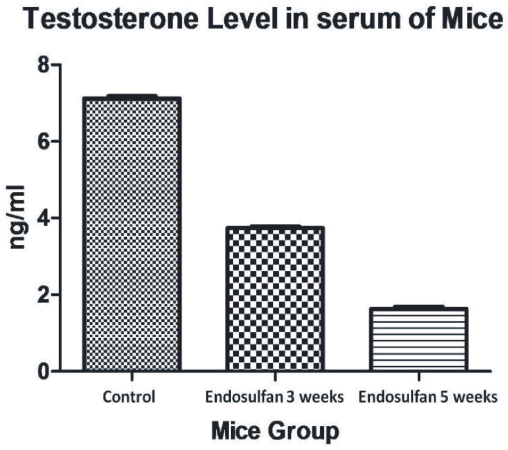

Fig. 1:

Table 1:

Mice Group	Number	Mean ± SEM	P value
Control	6	7.117 ± 0.06009	< 0.0001
Endosulfan 3 weeks	6	3.733 ± 0.04944	< 0.0001
Endosulfan 5 weeks	6	1.633 ± 0.04944	< 0.0001

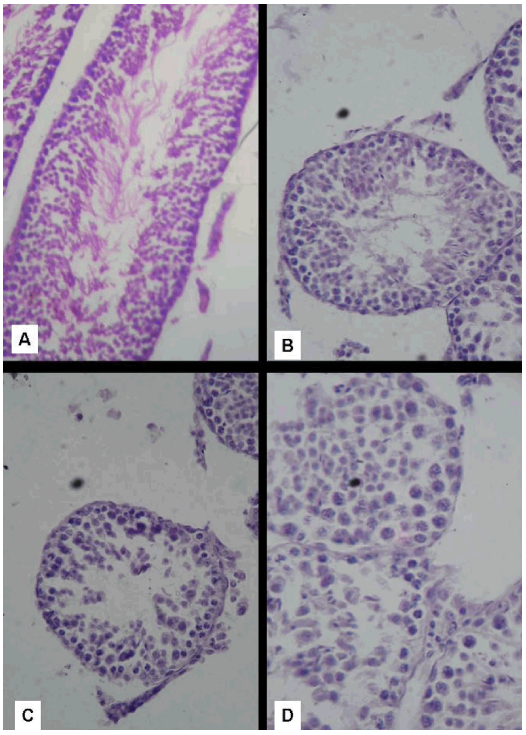

Plate 1: Microphotographs section of testis of control and endosulfan administered mice stained with haematoxylene and eosin

Fig. A: T.S of testis of control mice showing well organized seminiferous tubule with distinct primary and secondary spermatocyte. Spermatid was also normal. X400

Fig. B: T.S of testis of endosulfan administered mice for three weeks @ 2mg/kg b.w/ day showing thin walls of seminiferous tubule. Degeneration was observed in secondary spermatocyte. Malformed spermatids were also observed. X200

Fig. C: T.S of testis of endosulfan administered mice for five weeks @ 2mg/kg b.w/ day showing clustered cells of seminiferous tubules with fragmented walls. Scanty spermatid was also observed. X200

Fig. D: T.S of testis of endosulfan administered mice for five weeks @ 2mg/kg b.w/ day showing degenerated seminiferous tubule. Degeneration were also observed in primary as well as secondary spermatocyte. Rudimentary spermatids were observed in different shapes. X600

The control group of mice showed well organized seminiferous tubule with distinct primary and secondary spermatocyte. Spermatic were also normal (Plate: I, Fig-A). Testis of endosulfan administered mice for three weeks @ 2mg/kg b.w/ day showing thin walls of seminiferous tubule. Degeneration was observed in secondary spermatocyte. Malformed spermatid was also observed (Plate: I, Fig-B). Endosulfan administered mice for five weeks @ 2mg/kg b.w/ day showing clustered cells of seminiferous tubules with fragmented walls. Scanty spermatids were also observed (Plate: I, Fig-C). Degenerated seminiferous tubules were observed. Degeneration were also observed in primary as well as secondary spermatocyte. Rudimentary spermatids were observed in different shapes (Plate: I, Fig-D).

DISCUSSION

Setchell [8] and Cram[9] have observed the infertility in men and it causes due to hormonal imbalance. Ichihara[10] has observed the effect on testosterone due to chemically caused ageing. Anomalies in sperm and hormonal imbalance (Testosterone) of Mus musculus due to Endosulfan exposure were studied in detailed by Ali [11]. Testosterone level was also declined to greater extent which finally causes degeneration in seminiferous tubule and spermatids because normal level of testosterone maintains these functions.

It was evident from study that endosulfan exposure causes histological degeneration in testis of mice. Chitra [7] have also studied the effect of endosulfan on the testis of growing rat. Seminiferous tubules become gradually thinner and finally observed in rudimentary condition after prolong exposure of endosulfan. Scanty secondry spermatocyte were also observed, least degeneration were observed on primary spermatocyte, which indicates that endosulfan causes more degeneratin effect on latter phase of spermatogenesis. Deshaped spermatids were frequently observed which finally leads to azoospermic condition in mice. Decline level of testosterone with increasing period of dose were also evident due to which seminiferous tubule become rudimentary and causes infertility in mice.

Thus it is concluded that endosulfan exposure causes decline level of testosterone in mice which causes improper spermatogenesis due to degeneration of seminiferous tubule. Spermatid and secondary spermatocyte were also degenerated which causes azospermic condition leading infertility in mice.

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