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Alterations in Spermatological Parameters due to Endosulfan Toxicity in Swiss Albino Mice

Priyanka Sinha[1,*] and A. Nath[2]

[1]Department of Zoology, Patna University, Patna, Bihar, India.

[2]Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India.

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ABSTRACT

Pesticides are one of the most significant environmental factors that cause various metabolic and physiological dysfunctions in the male reproductive system. In the present investigation, alterations in sperm morphology, sperm count and sperm motility, as well as in the testosterone levels and histopathology of testicular cells due to endosulfan toxicity was studied. Sexually matured male Swiss albino mice (*Mus musculus*), weighing 30±2g, were segregated into 4 different groups with ten mice in each group. 3 groups were administered with the dose of 3mg/kg body wt per day of Endosulfan by oral gavage method for 6, 12 and 18 weeks respectively, and one group served as control. After the last treatment, the animals were sacrificed on 6^{th}, 12^{th}, 18^{th} weeks and the sperm parameters were estimated. The mice testes were fixed for light microscopy study and serum testosterone levels were estimated. Endosulfan significantly decreased the sperm motility and sperm count, and there were distinct histopathological abnormalities in testicular tissue. Significant decline in the testosterone levels was also observed. Endosulfan causes deleterious effects on the spermatological parameters of Swiss albino mice, thus negatively affecting the fertility.

Introduction

The increasing population poses one of the greatest challenges to the mankind in the twenty first century. This rapid growth in the global population continues to challenge the world's ability to provide enough food, thus increasing the pressure on the agricultural land. Therefore, to augment the yield of cereals, coffee, tea, cotton, oilseeds, fruits and vegetables, various pesticides are being indiscriminately used by the farmers worldwide. Tremendous benefits have been derived from the use of pesticides in agriculture, forestry, public health and domestic sphere.. A number of animal studies as well as human epidemiological studies have demonstrated that exposure of males to pesticides could result in reproductive toxicity. Endosulfan is an organochlorine pesticide, which, through biomagnifications keeps on accumulating as toxin and getting concentrated in the tissues of higher organisms through the food chain. Endosulfan is classified in India as an "Extremely Hazardous" pesticide (ITRC, 1989), Moderately hazardous chemical by (WHO-class II), highly toxic substance (ATSDR, 1993; EXTOXNET, 1998) and moderately hazardous pesticide on the basis LD_{50} value. There is substantial evidence which suggests that it acts as endocrine disruptor interfering with hormonal function of estrogen, testosterone and other steroidal hormones (Scelto, 2006). It is reported that Endosulfan exposure may result in reproductive deformities (Cheek et al, 1998; Saiyed et al, 2003, 2004). Though it is moderately toxic to humans, the genetic and carcinogenic risk of Endosulfan on human beings or animals is much higher than other chemicals with greater toxicity (Schettler et al, 2003). A number of studies have been done to see the toxic effect of pesticides on the reproductive organs of mice (Kumar & Nath, 1997; Russel, 1995, Sinha et al, 2005). Endosulfan may cause decrease in semen quality, increase in testicular and prostate cancer and an increase in defect in male sex organ (Hileman, 1994; Solo, 1983). Endosulfan is known to be toxic to gonads, but available literature fails to provide the in depth analysis of time dependent damage to sperms in male Swiss albino mice. Keeping these aspects in mind, the effect of Endosulfan on sperm parameters that is sperm morphology, sperm count and sperm motility was investigated and correlated with hormonal and histological parameters.

Materials and Methods

In the present investigation, experiments were performed on 12 weeks old male Swiss albino mice, *Mus musculus*.For optimal growth and reproduction, the maintenance of animals was in the ideal conditions at the laboratory as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the ,Animal Welfare Division, Govt. of India. All the animals were housed in polypropylene cages using paddy husk bedding at 28±1°C temperature and 50±5% humidity.

The oral LD_{50} value of Endosulfan for mice was estimated by standard interpolation method, which was 7mg/kg body weight. The standard data reference for LD_{50} of Endosulfan for mice is 7.36 mg/kg body weight (EXTOXNET, 1998). Endosulfan manufactured by Excel industries Mumbai (E.C-35%) was dissolved in distilled water to prepare sub-lethal dose of 3 mg/kg body weight and administered by gavage method for 6 weeks, 12 weeks and 18 weeks respectively. A vehicle of control group of mice was established and given equal volume of distilled water by gavage method. The animals were sacrificed according to the above mentioned treatment plan.

Sperm Morphology Assay: After sacrificing the mice, testes were removed and cauda epididymis was separated. Sperm

Tele:

E-mail addresses: psinhazoology@gmail.com

suspensions were prepared by mincing the cauda epididymis in 2 ml in phosphate buffered physiological saline (PBS, pH=7.2). Suspension was pipetted and filtered through 80µm nylon mesh to remove tissue fragments. A fraction of suspension was then mixed (10:1) with eosin Y and 30 minutes later the smears were made, air dried and mounted.

Epididymal sperm count and sperm motility: After separating the cauda epididymis, sperm number per epididymus was determined by haemocytometer. Dilute sperm suspension was prepared with phosphate buffered saline and introduced into a counting chamber. The total sperm count in 8 squares of 1 mm ^{2} each was determined and multiplied by $5x10^4$ to calculate the number of sperms per epididymis. Sperm motility was also counted in same eight squares and percentage of motile sperm was recorded (Vega et al, 1988).

Estimation of testosteroneby ELISA method: Blood samples were collected after each sacrifice and serum was isolated. Testosterone kit of LILAC Medicare (P) Ltd. Mumbai was utilized for the experiment.

Light Microscopic Study: The Testis was dissected and fixed in 10% formalin and embedded in paraffin. 4-5 µm thick sections were cut and stained with Haemotoxylin and Eosin. The sections were examined under light microscope.

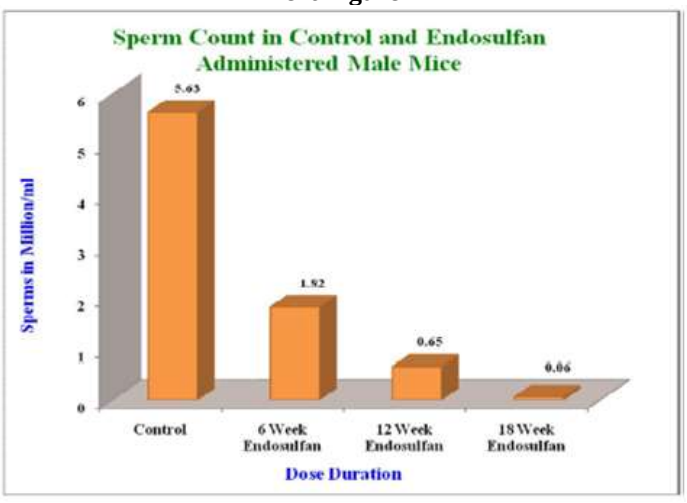
Statistical Analysis: Data obtained from the experiments were correlated and analyzed by one way ANOVA and values of $P<0.05$ were considered as statistically significant.

Results:

Effect on sperm morphology: On 6 weeks Endosulfan administered mice, the abnormal sperm increased in number but increase was not as significant as compared to the control. On 12 weeks Endosulfan treatment, number of abnormal sperms increased. The abnormal sperms are classified as (a) head abnormality - that included hook less, banana shaped, double headed and amorphous (b) tail abnormality – which includes the coiled and double tailed sperm. On 12 weeks of Endosulfan administration the number of hookless sperms increased. On 18 weeks Endosulfan treatment the head were amorphous and the tail were mostly coiled and double tailed. The percentage of abnormal sperms was highest at 18 weeks. (Plate-I)

Effect on epididymal sperm count: 6 weeks Endosulfan administration caused decrease in sperm count. The decrease happened in a time dependent manner. On 12th week, the effects were significant and the highest effect was found on 18th week. There is a significant reduction in sperm count in experimental group as compared to the control group. (Table-1, Text Figure-I)

Text Figure-I

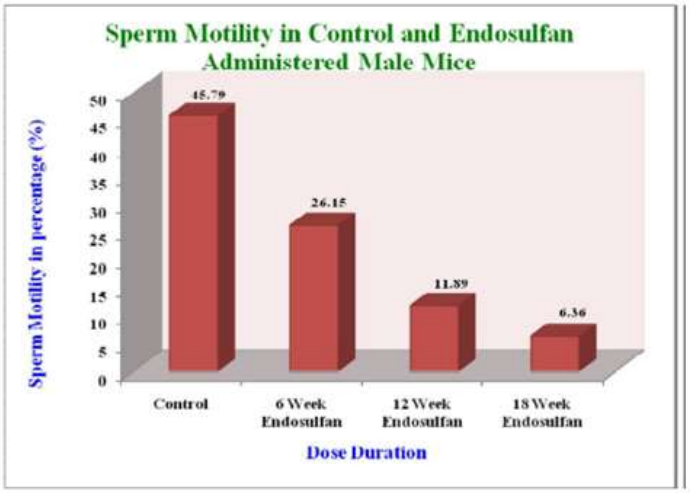


Effect on sperm motility: After the treatment of Endosulfan for 6 weeks, the sperm motility decreased to some extent. At 12 weeks the numbers of sperms were much more and there were few straight moving sperms, while sperms showing zigzag movement were a little higher. On 18 weeks Endosulfan administration the motility of sperm reduced significantly with number of non- motile sperm showing an increase in number. The percentage of motile sperms was least at 18 weeks. (Table-1, Text Figure-II)

Table-1. Table of Spermatological Parameters in Control and Endosulfan Administered Mice

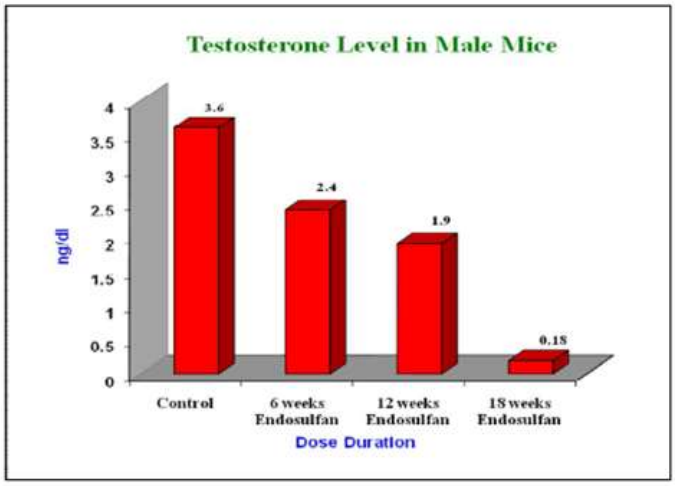
S. No	Parameters	Control	6 weeks Endosulfan	12 weeks Endosulfan	18 weeks Endosulfan	P-Values
1	Sperm No. (Million/ml)	5.63 ± 0.969	1.82 ± 0.539	0.65 ± 0.307	0.06 ± 0.030	< 0.0001
2	Sperm Motility (%)	45.79	26.15	11.89	6.36	-
3	Testosterone (ng/dl)	3.6 ± 0.888	2.4 ± 0.767	1.9 ± 0.818	0.18 ± 0.101	< 0.0001

Text Figure-II



Estimation of Testosterone: The testosterone level shows a significant decline with increase in number of days. (Table-1, Text Figure-III)

Text Figure-III



Light Microscopic Study: The control testis of *Mus musculus* show normal feature. The seminiferous tubules are closely packed together and masses of interstitial cells are found in between the tubules. All the spermatogenic cells namely spermatogonia, primary spermatocyte, secondary spermatocyte,

spermatids as well as mature spermatozoa are visible in the seminiferous tubules (Plate-II, Fig-A)

At 6 weeks Endosulfan treatment, the seminiferous tubule boundary membrane starts degenerating. The interstitial space show enlargement of leydig cells (Plate-II, Fig-B). Further exposure of Endosulfan for 12 weeks causes high degree of degeneration in interstitial cells. The membrane of seminiferous tubule is ruptured at many places (Plate-II, Fig-C). The 12 weeks Endosulfan exposed testicular cells showed highly degenerative changes. Number of testicular cells decreased in seminiferous tubules. The lumen of tubules was completely vacuolated. There is complete degeneration of spermatocytes and spermatogonia (Plate-II, Fig-D).

Plate-I

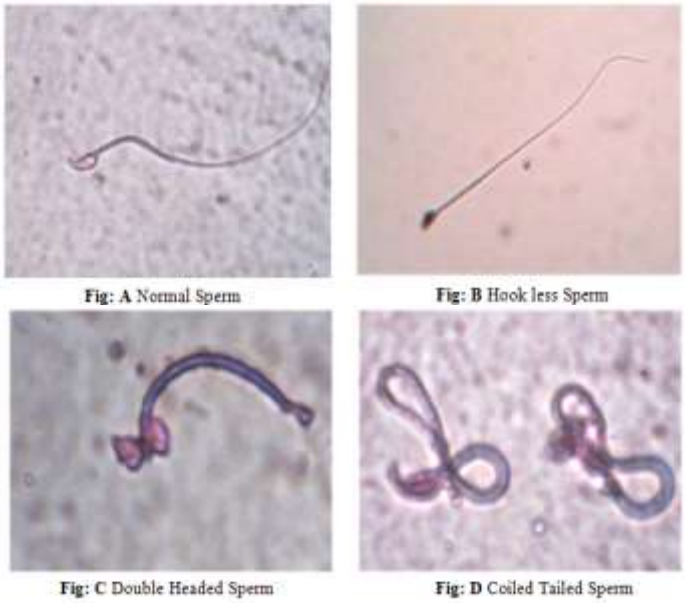


Fig: A Normal Sperm **Fig: B** Hook less Sperm

Fig: C Double Headed Sperm **Fig: D** Coiled Tailed Sperm

Plate-II

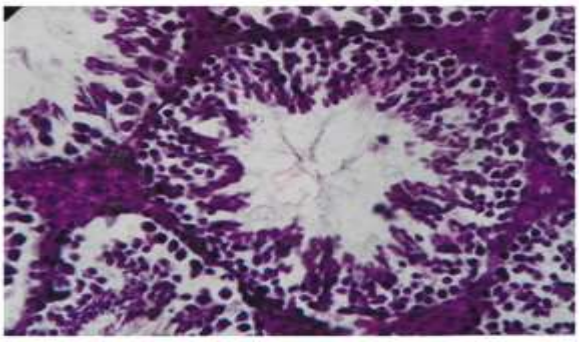


Fig: A Photomicrograph of testis of control male mice – Showing normal section of seminiferous tubules. x 800

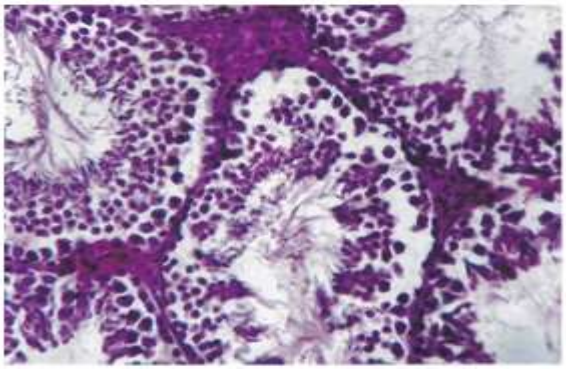


Fig: B Photomicrograph of testis of 6 weeks Endosulfan administered male mice – Showing thin epithelial germ layer and decreased number of spermatogonia. x 800

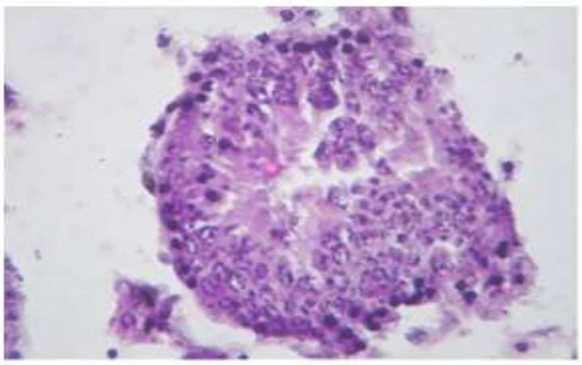


Fig: C Photomicrograph of testis of 12 weeks Endosulfan administered male mice – Showing damaged epithelial layer and degeneration of spermatogonia and spermatocytes; increased interstitial spaces. x 800

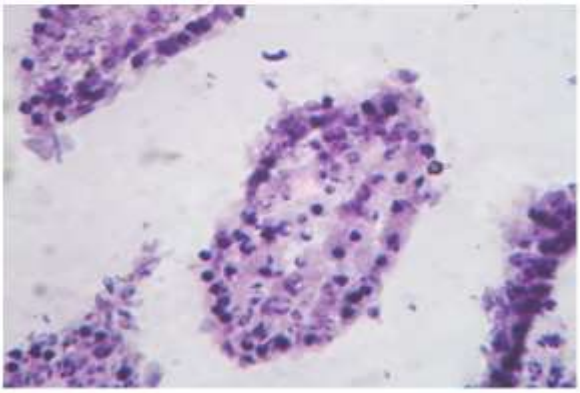


Fig: D Photomicrograph of testis of 18 Weeks Endosulfan administered male mice – Showing complete degeneration of spermatogonia, spermatocytes, spermatids and sperms. x 800

Discussion

Our study shows that Endosulfan exposure caused a significant increase in the percentage of spermatozoa with abnormal morphology. Sperm shape abnormality test is one of the most reliable, rapid methods used as an in vivo assay for genotoxicity (Wyrobek, 1982; Wyrobek et al, 1975, 1983). Endosulfan is known to damage sperm architecture and acrosome formation which in turn affect sperm function (Nath, 2007). It is also reported that Endosulfan induces possible occurrence of apoptosis in testis of mice (Singh et al, 2011). All these studies support our finding that Endosulfan is genotoxic to germs cell. Sperm shape abnormality caused by Endosulfan may be due to interference with DNA synthesis during mitotic stage of spermatogenesis or interference with chromosome structure.

Sperm count is an important indicator of male fertility (Meistrich et al, 1983). Any agent that interferes with meiotic division is also known to reduce the sperm count (Aarnoud et al, 2002). Endosulfan caused large reduction in sperm count and at 18 weeks the number of sperms was significantly low. The Endosulfan may affect the leydig cells which lead to decreased testosterone levels. Decrease in testosterone level can cause sloughing of germinal epithelium hence declining sperm count (Thust et al, 2002). It is reported that Endosulfan causes reproductive toxicity showing degenerative changes in the seminiferous epithelium induction of rate limiting enzyme in testosterone production and histological changes in testicular atrophy in male rat (Naqvi et al, 1993). Defective sperm motility is one of the causes of untreatable infertility or subfertility in men (Acacia et al, 2000). Reduction in sperm count resulting from adverse effect on spermatogenesis and less motile defective spermatozoa after Endosulfan treatment have also

been observed by Pandey and Ratna (2003).Chronic effect of Endosulfan on testis of rat has been studied (Chitra et al,1999). Endosulfan decreases sperm motility starting from 6 week exposure and highest reduction in motility is observed at 18 week. It has been reported that the decrease in sperm count and motility are valid indices of male infertility in laboratory animals (Working et al, 1993; Lemasters et al, 1993). However sperm motility is often used as a marker of chemical induced testicular toxicity (Bitman and Cecil, 1970). They have also stated that the disruption of seminiferous epithelium is indicative of male reproductive hazard, therefore our study suggest a gonadotoxic potential of endosulfan. The testosterone level decreased as the period of Endosulfan treatment increased showing hormonal imbalance. Ichihara et al (1993) have observed the correlation of ultrastructural and testosterone levels in aging rats. Fusion of lobular boundary membrane and obliteration of interstitium causes damage to seminiferous tubules.

In conclusion this study clearly demonstrates that Endosulfan causes severe histopathological anomalies and depleted testosterone levels. Endosulfan exposure negatively affectsall the spermatological parameters in male mice. Thus, endosulfan induces severe reproductive toxicity ultimately leading to infertility.

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