THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Endosulfan Induced Male Infertility Study in Swiss Albino Mice: Evaluation of Effect on Testosterone and Sperm Count

Dr. Manisha Lall
Research Assistant, Mahavir Cancer Institute, Phulwarisarif, Patna, Bihar, India
Dr. Kumud Bala
Associate Professor, Amity Institute of Biotechnology, Amity University, Noida, U.P., India
Dr. Arun Kumar
Scientist, Mahavir Cancer Institute, Phulwarisarif, Patna, Bihar, India

Abstract:
A number of animal studies as well as human epidemiological studies have demonstrated that exposure of males to various agents could result in abnormal reproductive toxicity. Endosulfan is known to cause deleterious effects on the reproductive organs of human, but the extent of damage to sperm parameters of swiss albino mice is not known clearly. Hence this study on sperm morphology, sperm count and sperm motility.The dose of 3mg/kg body weight of Endosulfan was administered to 12 weeks old male swiss albino mice by oral gavage method for 6, 12 and 18 weeks respectively.100 Mice were segregated into 4 groups. 3 groups were administered with Endosulfan and the rest 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 serum was analyzed for Testosterone and testis was fixed for light microscopy study. It significantly decreased the sperm motility and sperm count. Endosulfan exposure significantly decreased the testosterone levels. There was distinct histopathological alterations in testicular tissue. Endosulfan has negative effects on the sperm parameters of Swiss albino mice, thus affecting fertility.

Keywords: *Endosulfan, mice, sperm count, sperm motility, sperm morphology. histopathology of testis*

1. Introduction

Pesticide poisoning is an important health issue because intensive use of pesticide puts the target as well as non target species at increased risk of pesticidal illness. The pesticide used in this investigation is Endosulfan. Endosulfan is an organochlorine compound which through biomagnifications keeps accumulating as toxins 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 after taking 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. It is reported that Endosulfan has an effect on male 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).Endosulphan induced neoplastic changes in the liver cells of mice has also been observerd (Kumar and Nath 2014). Many researchers has also reported the malformation of the ovaries and the ovules in endosulfan exposed mice(Kumar et.al 2012). 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). 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.

2. Materials and Methods

Male Swiss albino mice (12 weeks old) were bred in animal house of the institution. Breeding and maintenance of animals were done according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Animal Welfare Division, Govt. of India for the use of laboratory animals. 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 sublethal dose of 3 mg/kg body weight and administered by gavage method for 6 weeks, 12 weeks and 18 weeks respectively. A 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.

2.1. Sperm Morphology Assay

After sacrificing the mice, testes were removed and cauda epididymis was separated. Sperm suspensions were prepared by mincing cauda 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.

2.2. Epididymal Sperm Count and Sperm Motility

After separating the cauda epididymis, sperm numbers per epididymis were 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).

2.3. Estimation of Testosterone by 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.

2.4. 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.

2.5. 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.

3. Results

3.1. 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)

3.2. Effect on Epididymal Sperm Count

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

3.3. 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)

3.4. Estimation of Testosterone

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

3.5. Light Microscopic Study

The control testis of *Swiss albino* mice shows 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 there is a 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).

4. Discussion

Our study shows that Endosulfan caused an increase in the percentage of abnormal sperms. 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 damages sperm architecture. Hyposecretion of testosterone and low sperm count ultimately lead to infertility in male mice.

5. Acknowledgement

The authors are indebted to the Indian Council of Medical Research, New Delhi for financial support. The authors are also thankful to Mahavir Cancer Sansthan & Research Center for providing infrastructural facilities.

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

Table 1: Table of Spermatological Parameters in Control and Endosulfan Administered Mice

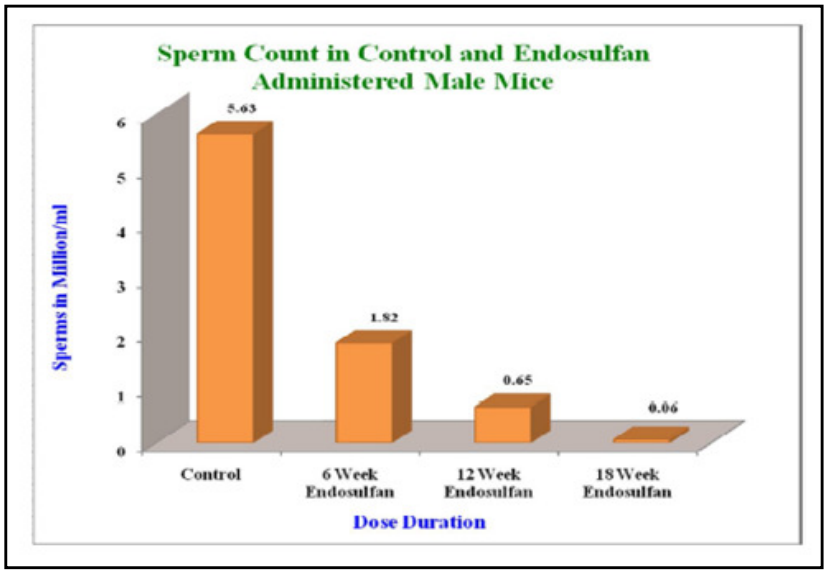

Figure 1

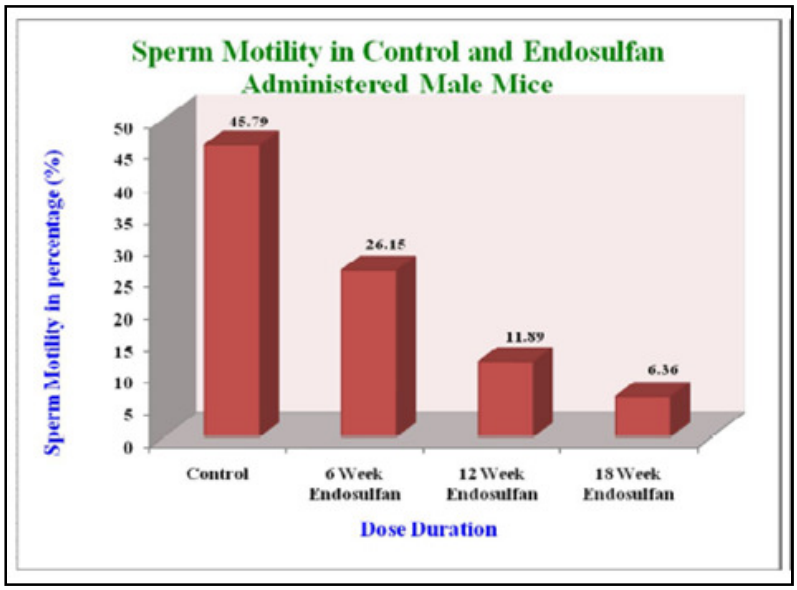

Figure 2

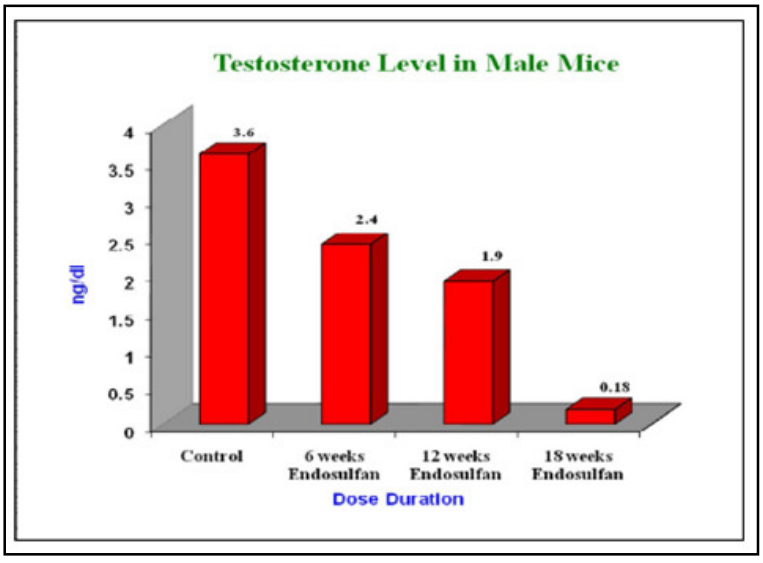

Figure 3

PLATE - I

Fig: A Normal Sperm

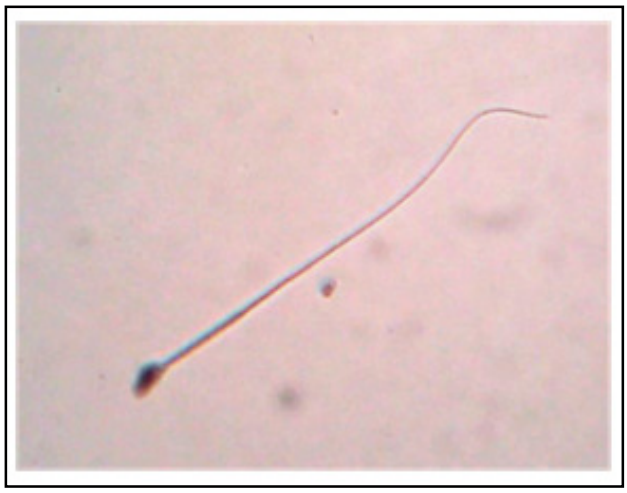

Fig: B Hook less Sperm

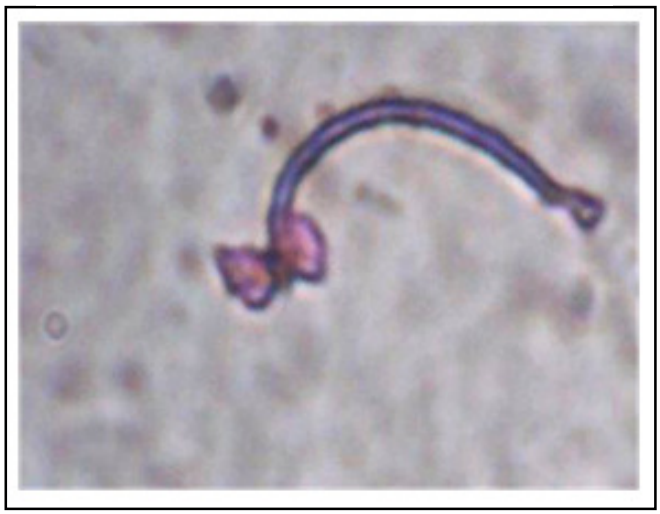

Fig: C Double Headed Sperm

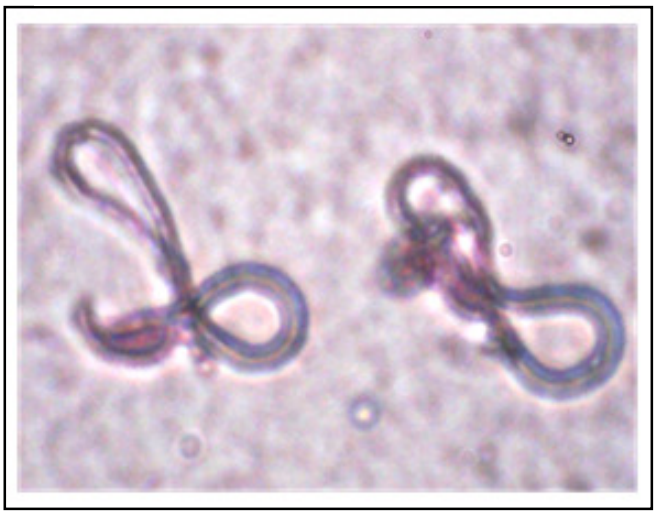

Fig: D Coiled Tailed Sperm

Figure 4

PLATE-II

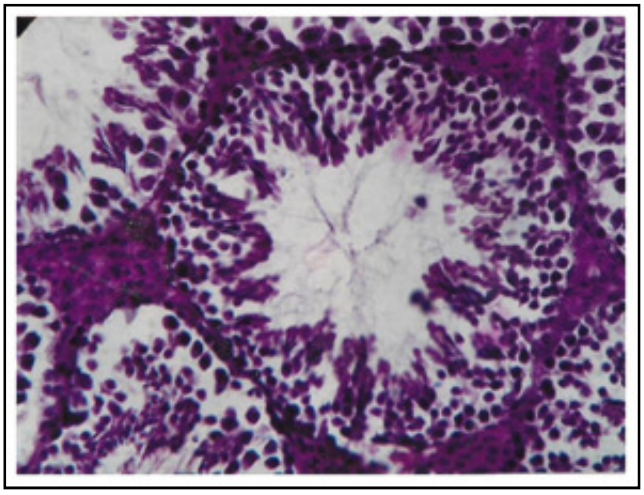

Fig: A Photomicrograph of testis of control male mice – Showing normal section of seminiferous tubules. x 800

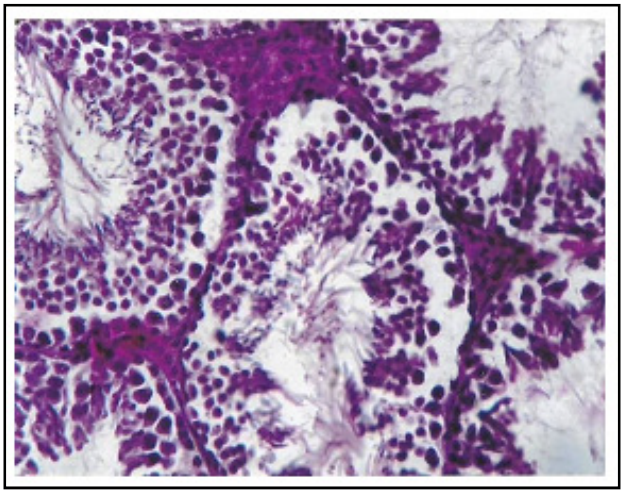

Fig: B Photomicrograph of testis of 6 weeks Endosulfan administered male mice – Showing thin epithelial germ layer and decreased number of spermatogonia. x 800

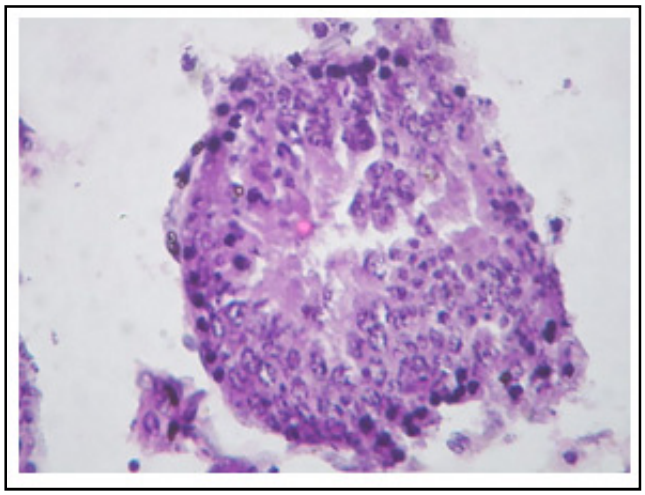

Fig: C Photomicrograph of testis of 12 weeks Endosulfan administered male mice – Showing damaged epithelial layer and degeneration of spermatogonia and spermatocytes; increased interstitial spaces. x 800

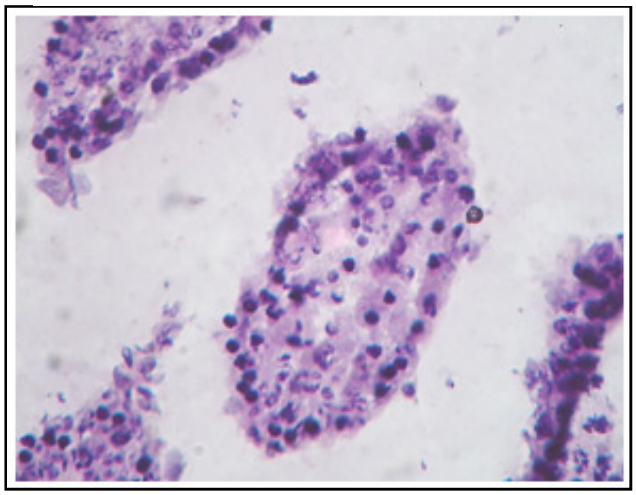

Fig: D Photomicrograph of testis of 18 weeks Endosulfan administered male mice – Showing complete degeneration of spermatogonia, spermatocytes, spermatids and sperms. x 800

Figure 5

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