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Research Article

Effect of Endosulfan Toxicity on 1st Stage of Spermiogenesis Leading to Infertility

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Abstract

Toxicities of organochlorine pesticides like Endosulfan show adverse effects on many organs and organ systems like liver, kidney, testes, muscles, immune system and hematological system. Most of the previous studies focused on effects of organochlorine on activity of spermatogenic cells. However, there is very little research reporting effects of endosulfan on spermiogenesis till date. Therefore, the current study was aimed to assess the effect of Endosulfan on spermiogenesis in the testes of mice model. A 5 mg/gm body wt. Endosulfan was administered to mice by oral gavage method. Normally in the beginning of spermiogenesis, the sperm head gets embedded in the sertoli cell and finally released from the seminiferous tubules. It is very significant to note that the testes in endosulfan treated mice. They were found to get affected at an early stage (from spermatids to 1st stage of spermiogenesis), as compared to any other organochlorine pesticide. The present study also confirms that only oral administration (5 mg/gm body wt.) for 20 days shows effect on spermiogenesis in 30% of mice. Furthermore, in 20 days treated mice, notable changes were seen during the 1st stage of spermiogenesis. Significant decrease in testosterone level ($P \leq 0.0001$) and sperm count ($P \leq 0.001$) was observed after endosulfan exposure. Abnormal spermatozoa in the lumen of seminiferous tubules were found to be very few in number and with a non-motile tail. Approximately 30% of the heads of spermatozoa were found with rounded acrosome, which will lead to infertility.

Keywords: Endosulfan; Toxicity; Spermiogenesis; Infertility; Mice

Introduction

India is an agro based country and unrestrained use of pesticides is inevitable. One of the broad spectrum insecticide, Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6, 9-methano-2,4,3-benzodioxathiepin-3-oxide) is an organo-chlorine insecticide and acaricide.

Endosulfan is used to control agriculture insects and mite pests on a variety of fields, fruits, and vegetables. It causes acute toxicity in animal and human beings due to over exposure.

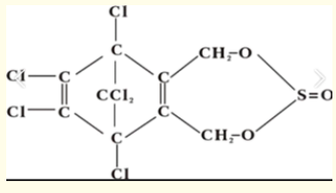

Figure 1: Chemical structure of endosulfan.

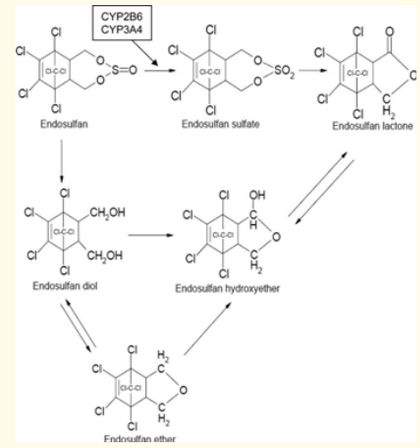

Figure 2: Metabolism of endosulfan

Endosulfan is absorbed by the human via stomach, lungs, and through skin. The previous studies performed elucidate the stereoselective metabolism of endosulfan in different organs and characterized the cytochrome p450 enzyme that is involved in me-

tabolism of endosulfan. The CYP3A enzymes are major enzymes contributing to steroselective disposition of endosulfan. CNS is the main target of endosulfan toxicity [1]. Endosulfan and its metabolites have been found in both tissue and serum samples. Endosulfan affects kidney, liver, immune system, ovary, and testes. Endosulfan acts on the testes, causing problems in spermatogenesis and spermiogenesis. Exposure to sublethal doses of endosulfan and its metabolites induce DNA damage and Mutation. Spermiogenesis is the third phase of spermatogenesis, in this phase the spermatocyte undergoes structural changes. First the acrosome is formed, then the tail develops and additional a majority of the superflows cytoplasm is removed and which taken up by sertoli cells. Finally, mature sperm is developed [2]. During the cap phase the acrosomal undergo structural changes and gets its final shape the maturation phases [3]. Endosulfan affects shaping of sperm head; acrosome and nuclear condensation the shape of a sperm head is species-specific and in the sickle shaped [3,4]. The acrosome is a bag of enzymes which sits at the anterior pole of the sperm head. The acrosome contains enzymes required for the sperm to penetrate the surrounding layers of the two sites. The formation of acrosome begins with the production of proacrosomal granules from the golgi apparatus [5]. The re-shapping of the head and acrosome nuclear condensation occur in parallel. Due to endosulfan during spermiogenesis, the size of the spermatids had decreased to ~5% of a somatic cell nucleous. The campation occur through dramatic changes in the way the DNA is packed and falls under the broad banner of epigenetic changes in chromatid structure that affect transcription. Endosulfan causes a disturbance in spermiogenesis, leading to low sperm count, production of abnormal sperm, deshaped acrosomal head, tails of elongated spermatids and decrease in the quality of serum, impairment of sperm motility, reduction of fertilization ability. Endosulfan can directly injure the testes. A testicular toxin and various derived compounds were shown to induce severe damage to the spermatogenic epithelium in mice model [6]. The effect of endosulfan on the testes appears to be manifested mainly in the Sertoli cells, presenting more morphological changes under scanning electron microscopy. Endosulfan can also interfere with the normal functioning of mitochondrial enzymes. The endosulfan alters the activity of some marker enzymes such as-glucose-6-phosphate dehydrogenase, lactate dehydrogenase, y-glutamyl transpeptides and alkaline phosphate, that decrease mitochondrial energy production in swiss albino mice [7]. Endosulfan exposure cause over-production of reactive oxygen species (ROS), resulting in a decline of sperm count and infertility in wildlife and human [8]. The antioxidant system plays an protective role in testes and other biological tissues and ROS has been known to damage macromolecules, including membrane bound polyunsaturated fatty acid (PUFA), causing impairment of cellular function. Spermatozoa are rich in PUFA, and, therefore, could be highly susceptible to oxidative stress. Pesticides affect spermatogenesis through hormonal or genotoxic pathways. Testicular toxicity of endosulfan manifested as decreased spermiogenesis and testicular hormones synthetics (steroid-genesis) [9]. And also profound decrease in the level of plasma LH, FSH and Testosterone associated with decrease in mice exposed to endosulfan for 30 days. Endosulfan exposure may delay sexual maturity and interfere with hormone synthesis in male mice. Administration of endosulfan led to a dose-dependent reduction of testicular weight and the number of motile spermatozoa in the epididymis. Testicular histological observations also revealed a marked loss of gametes in the lumen of seminiferous tubules [10]. In endosulfan treated swiss albino mice, testosterone production by testes decreased after pesticide exposure. Oral administration of endosulfan disturbs and alters the process of spermiogenesis that leads to male infertility.

Materials and Methods

Animal and treatment

Swiss albino mice were reared in the animal house of S.S Hospital and Research Institute, Patna CPCSEA Regd.No.1840/PO/ReBi/s/5/CPCSEA Govt. of India. The male mice selected for the study were of 2 weeks, 4 weeks, 6 weeks old and their weight was measured as 27 ± 2 gm. Mice were kept in polypropylene cages with paddy hucks at room temperature of 28°C and humidity (50 ± 5°) in a controlled light (12 hrs light and 12 hrs dark).

Body and organ weight

Body weight of each group of male mice was measured before and after the administration of endosulfan. Male mice were sacrificed after 2 weeks, 4 weeks, and 6 weeks of endosulfan treatment. Testes were cut out and weighed then fixed in 10% neutral formalin.

Sperm count

The cauda epididymis were removed and washed in isotonic saline. The semen was collected by puncturing cauda epididymis at several places in 1 ml physiological saline and then sperm were stained with 1% eosin dye. Then, sperm count was performed with the help of Neubauer's chamber under a light microscope. To minimize error, count was repeated at least five times for each swiss albino mouse.

Statistical analysis

Mean ± SD were calculated for the present study p-value was calculated by using one way ANOVA.

Measurement of testosterone level

The quantitative measurement of testosterone level in the serum samples of all groups of endosulfan administered Swiss albino mice was determined by using the testosterone ELISA kit of DRG Instruments Gm6H, Germany.

Histological study

For histopathology studies of endosulfan administered mice, tissue samples were processed and stained by routine procedure (H&E stain) and slides were observed under light microscope.

Results

In 20 days treated mice, the small and non-motile sperms were seen in the lumen of seminiferous tubules. About 30% of the heads of spermatozoa showed rounded acrosomes, which will lead to infertility (Figure 3 and 4). Significant decrease in testosterone ($P \leq 0.0001$) and sperm count ($P \leq 0.001$) was observed after endosulfan administered Swiss albino mice 3.0 mg/kg body wt. for 20 days (Figure 5 and table 1 and 2). Histopathology observation of testes of control Swiss albino mice shows normal process of spermiogenesis.

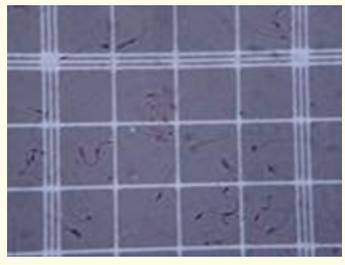

Figure 3: Coiling of sperm.

Figure 4: Endosulfan treated mice sperm.

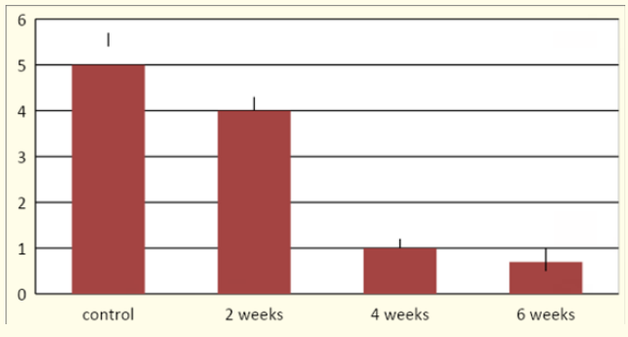

Figure 5: Level of the number of sperms/ml in suspension of control and treated mice (2W, 4W, 6W).

Group no	Age of swiss albino mice	No of weeks	Vector	No of sperm/ml
A	14 - 16 Weeks	Control	D.W	5×10^8
B	10 - 12 Weeks	2W	Endosulfan	$.4125 \times 10^8$
C	14 - 16 Weeks	4W	Endosulfan	$.0937 \times 10^8$
D	14 - 16 Weeks	6W	Endosulfan	$.0925 \times 10^8$

Table 1: Sperm density (no. of sperms/ml) - in control and treated mices (2W, 4W, 6W).

Treatment	Sperm motility (%) (Cauda)	Spermdensity (Millions/ml^3)	
		(Testes)	(Cauda)
Control IA	69.61 (± 3.58)	4.15 (± 0.06)	21.70 (± 0.37)
Endosulfan 5 mg/ kg.b.wt./day IIA 15 days	59.11 (± 2.35)	2.71 (± 0.37)	20.69 (± 20.69)
Endosulfan 5 mg/ kg.b.wt./day IIB 30 days	45.21* (± 2.7)	0.87* (± 0.13)	12.14* (± 1.80)

Table 2: Effect of Endosulfan on sperm motility and sperm density of swiss albino mice.

Value given is the mean of the result obtained from 5 animals. Figure in parathesis indicates ± SE of mean *= significant ($P < 0.001$).

After 2 weeks of endosulfan administration increased intercellular space between seminiferous tubules has been observed at some places and normal spermiogenesis was observed, architecture of seminiferous tubules was almost normal.

Deformed architecture and degradation in leydig cells increased Inter seminiferous tubules have been observed after 4 weeks of endosulfan administered Swiss albino mice.

Thin wall of seminiferous tubules and abnormalities in shape of nuclei of leydig cells were observed in 6 weeks endosulfan administered mice.

Biochemical changes in levels of glycogen, sialic acid protein, and cholesterol were also observed in endosulfan administered mice. A decrease in levels of Glycogen and Sialic acid was reported in endosulfan treated mice as compared to the control. However, the level of protein was found to increase and no significant change in cholesterol level was reported (Table 3).

Our work confirms the only oral administration (3 mg/gm body wt.) for 20 days showed an effect on 30% of mice on spermiogenesis. This is parallel to unpublished data of our laboratory, work done by electromicroscopy at molecular level. Significant increase of inter-seminiferous tubular spaces and degenerated seminiferous epithelium (Figure 6).

Parameters	Control	Endosulfan 5 mg/kg.b.wt/day	
		IIA 15 days	IIB 30 days
Glycogen (Mg/gm)	2.70 (± 0.13)	1.00* (± 0.23)	0.92* (± 0.23)
Sialic acid (Mg/gm)	5.10 (± 0.19)	4.10* (± 0.18)	4.11 (± 0.10)
Protein (Mg/gm)	255.30 (± 17.20)	327.96 (± 23.02)	354.62* (± 0.44)
Cholesterol (Mg/gm)	5.92 (± 0.41)	5.46 (± 0.83)	6.26 (± 0.85)

Table 3: Biochemical changes of swiss albino mice after oral administration of Endosulfan.

Values given are the mean of result obtained from 5 animals.

Figure in parathesis indicates ± SE of mean *= significant ($P < 0.001$).

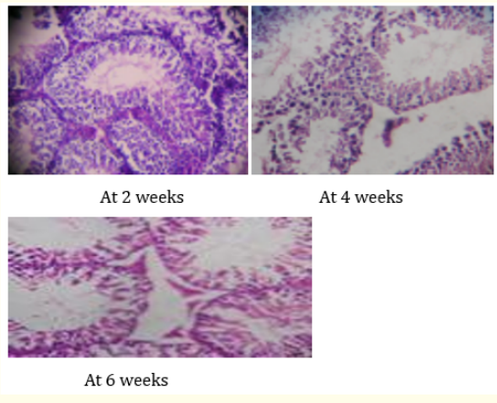

Figure 6: Endosulfan administered Swiss albino mice for 2, 4 and 6 weeks.

Note: Abnormal spermiogenesis was observed at 4 and 6 weeks.

Group no	NO of Weeks	Range (ng/ml)
A	Control	0.66 - 5.4
B	2W	1.98
C	4W	1.83
D	6W	1.22

Table 4: The level of testosterone (ng/ml) in serum of control and treated mice 2 weeks, 4 weeks and 6 weeks.

Discussion

In this research, we have described the mechanism through which endosulfan affects male fertility. Endosulfan comprises a large number of distinct substances with dissimilar structures and diverse toxicity which act through different mechanisms. Therefore, several of the aforementioned mechanisms are probably involved in the pathophysiological pathways explaining the role of endosulfan exposure in effects on sperm quality and male fertility. The decreased sperm motility and density after oral administration of endosulfan at various dose levels may be due to androgen insufficiency [11,12] which caused impairment in testicular functions by altering the activities of the enzymes responsible for spermiogenesis. The study was carried out to see the effect of endosulfan exposure during prepuberty on spermiogenesis in the testes of growing mice to see its role in inducing damage to gonads attaining sexual maturity. The acrosome of the sperm is a specialized, cap-like structure covering the anterior portion of the sperm nucleous. The activity of the acrosomal enzymes indicates the intact status of the acrosome. Endosulfan effects on DNA damage in sperm is thought to be caused by incomplete maturation during spermiogenesis and apoptosis [13]. This study reported a significant net decrease in sperm concentration in the exposure groups from 39% for fenvalerate exposure [14] to 51% for 2, 4-D [15]. The inhibitory effects of endosulfan like other chlorinated insecticides [16] on the secretion of pituitary gonadotrophins (FSH and LH) and in testis of mice.

Conclusion

It is evident from the present study that endosulfan significantly reduces the testosterone level, sperm count in swiss albino mice. It also induces histopathological alteration of testicular tissue at cellular and sub-cellular levels. Low sperm count, low sperm motility, abnormal sperm, deshaped acrosomal head, reduction of fertilization ability, after administration of endosulfan confirms effects on earlier spermiogenesis i.e. 1st stage leading to infertility.

Conflict of Interest

No conflict of interest was found between the authors.

Ethical Clearance

The current study was approved by the Institutional Animal Ethics Committee (IAEC) S.S Hospital and Research Institute, Patna, Bihar-India.

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