ejpmr, 2016,3(9), 600-603

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

ENDOSULFAN INDUCED OXIDATIVE STRESS AND BIOCHEMICAL CHANGES IN TESTES OF MICE

A. Nath[1], Aseem Kumar Anshu[1*], Priyanka[1], Chandan Kumar Singh[1], Sachidananda Behera[1], J. K. Singh[2]

[1]Research Insitute, S S Hospital and Research Institute, Patna.
[2]Department of Oncology, S S Hospital and Research Institute, Patna.

***Correspondence for Author: Aseem Kumar Anshu**

Research Insitute, S S Hospital and Research Institute, Patna.

Article Received on 19/07/2016 **Article Revised on 10/08/2016** **Article Accepted on 31/08/2016**

ABSTRACT

Endosulfan is potent toxicogen and carcinogen. It is widely used in agriculture and detected in soil, surface water, and underground water, fruits, vegetables and milk. A number of studies have been performed to investigate the effect of endosulfan on reproductive system. Exposure to endosulfan leads to degeneration of spermatogenesis, lower testosterone level and induce oxidative stress. Swisss albino male mice were grouped as control and endosulfan treated for 4, 8, 12 weeks at animal house of S S Hospital and Research Institute, Patna. Sperm count was performed by routine process, testosterone level was determined by ELISA kit method, and lipid peroxidation was done by measuring MDA level in blood serum. Decrease in testicular weight, sperm count, testosterone level and increase in MDA level were observed when compared with control upon administration of endosulfan at the rate of 3 mg/kg/body weight with significance of (p-value<0.0001). The effect of endosufan on reproductive system is deleterious and insidious. Endosulfan has been used in agriculture widely and enters human body system through food chain to cause toxicity. Hence more studies and investigations are warranted in this field the mechanism by which endosulfan impairs the reproductive system.

KEYWORDS: Endosulfan, toxicogen, carcinogen.

INTRODUCTION

Numerous new chemicals have been introduced in recent years and are still being evaluated for their potentiality to cause toxicity. A large number of chemicals which has been used and studied extensively are currently re-evaluated for their toxic effects on reproductive system[1]. India is an agro-based country and unrestrained used of pesticide 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) which is used extensively in agriculture as pesticide to protect cotton, vegetables and fruit crops. Endosulfan and its metabolites have been detected in soil, surface water, and underground water[2], milk[3, 4], fruits and vegetables[5,6]. Endosulfan enters in our body through various routs like ingestion, inhalation, or skin contact and has toxic effect on the body depending upon routs of administration, species, vehicle, and sex[7].

Endosulfan, being an endocrine disruptor, has been observed to induce alteration in the process of spermatogenesis in young and adult rats[8,9]. Decreased sperm count, intratesticular sperrmatids number, altered sperm morphology, and activity of testicular marker enzymes. Several reports demonstrate the effect of different compounds including endosulfan suppressing development of prenatal gonads and spermatogenesis in male offspring[10]. A detailed study revealed that 2.5, 5, and 10 mg/kg/body weight of endosulfan for 10 weeks reduce the intratesticular sperrmids count, sperm abnormalities, and changes in activities of marker enzymes in testes like lactate dehydrogenase, sorbitol dehydrogenase, y-glutamyl transpeptidase, and glucose-6-phosphate dehydrogenase which corroborate the detrimental effect of endosulfan on spermatogenesis [11].

The human population has been exposed to the harmful compounds like endosulfan. Our aim of the study is to Demonstate the effect of endosulfan on the reproductive system by testing on mice models. In this article, we report the effect of endosulfan on male reproductive system of mice.

MATERIALS AND METHODS

Animals: Swiss albino mice *(Mus musculus)* were reared in the animal house of S.S. Hospital and Research Institute, Patna. The male mice selected for the study were 12 weeks old and their weight was measured as 30±2 grams. Mice were kept in the polypropylene cages with paddy husk at room temperature 28±2ºC and humidity 50±5% in a controlled light (12 hrs light and 12 hrs dark). Animals were maintained in ideal conditions

as per animal ethical guidelines of the CPCSEA, (CPCSEA Regd. No. 1840/PO/ReBi/S/5/CPCSEA) Gov. of India and Institutional Animal Ethics Committee (IAEC).

Study Groups and Sampling: 6 male mice of control group were given distilled water as drinking water and 'treated group' (n=24) were administered 3mg/kg body weight of endosulfan for 4 weeks, 8 weeks and 12 weeks. Blood samples were collected from the sacrificed mice and centrifuged to obtain serum samples for estimation of Lipid Peroxidation and Testosteron level. Testes from all the animals were removed and washed three times in isotonic saline solution (0.85 v/w%) and fixed in 10% formalin for histological study.

Body and Organ weight: Body weight of each group of male mice was measured before and after the administration of endosulfan. Each group of male mice were sacrificed after 4 weeks, 8 weeks and 12 weeks and testes were cut out and weighed.

Sperm Count: The epididymis was removed and washed in isotonic saline. The semen was collected by puncturing epididymis at several places in 1ml physiological saline and then sperm count was performed with help of Neubauer's chamber under light microscope.

Lipid Peroxidation: For the estimation of MDA level, blood samples were centrifuged at 3000rpm for 110 minutes to collect serum. Level of MDA was determined by standard TBARs method with slight modification by UV spectrophotometer[12].

Estimation of Testosterone level: Testosterone level of all groups of mice in the serum samples were determined by using testosterone ELISA kit of LILAC Medicare (P) ltd.

Statistical Analysis: Mean±SD and p-value were obtained using SPSS software (Statistical Package for Social Sciences, version 16.0). A p-value less than 0.05 were considered significant. Statistical analysis was performed with the help of one way ANOVA.

RESULTS

Testis of the mice was weighed and significant changes in the weight were observed upon endosulfan treatment. The highest mean was observed in control group (0.113g), followed by 4 weeks (0.101g), 8 weeks (0.086g), 12 Weeks (0.075g) endosulfan treated mice groups. Similarly, sperm count decreased noticeably upon treatment with endosulfan in swiss albino mice. Highest mean value of sperm count was recorded in control group (5 million/µl) whereas mean sperm count in endosufan treated mice were 0.412 million/µl (4 weeks treated mice group), 0.0938 million/µl (8 weeks treated mice group), 0.0434 million/µl (12 weeks treated mice group) with significance (p-value<0.0001).

Male testosterone level was detected highest in control group (2.62 ng/ml) and 1.98 ng/ml, 1.83 ng/ml, and 1.22 ng/ml in endosulfan treated mice for 4 weeks, 8 weeks, 12 weeks respectively. Moreover, while assessing the lipid peroxidation, the mean MDA level was measured highest in endosulfan treated group for 12 weeks (46.62 nMol/ml), followed by 8 weeks (42.11 nMol/ml), 4 weeks (30.01nMol/ml) and control (22nMol/ml) with significance (p-value<0.0001).

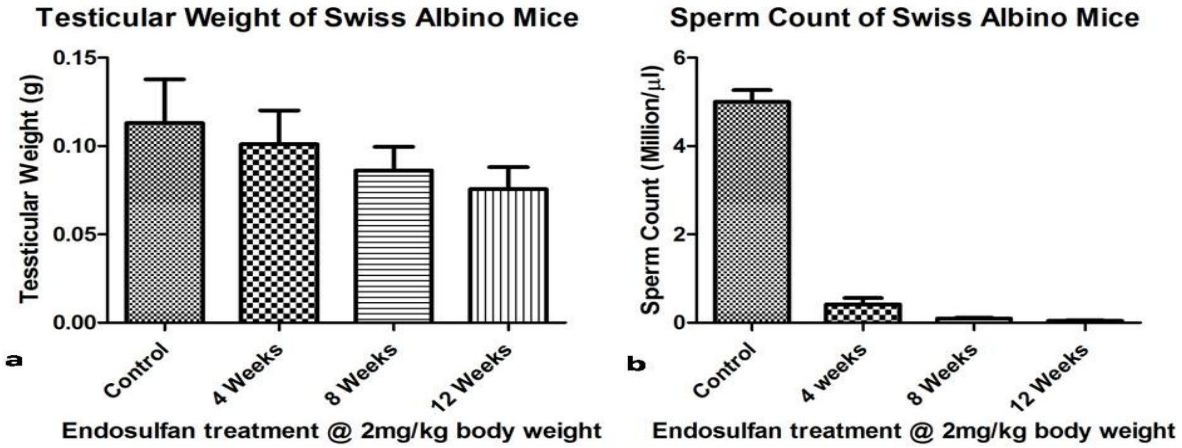

Fig 01: Testicular weight (a) and sperm count (b) in control and endosulfan treated mice for 4 weeks, 8 weeks, and 12 weeks.

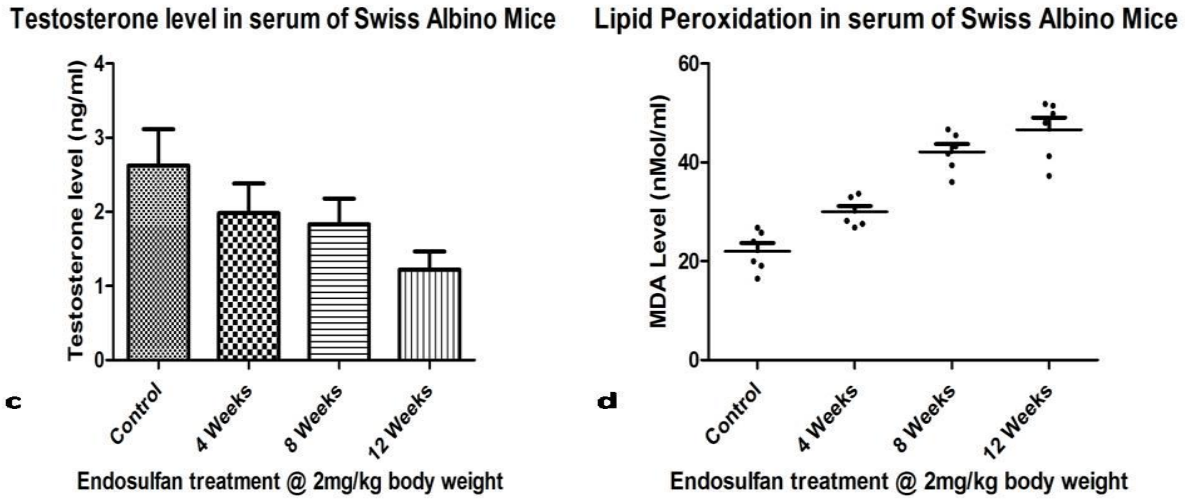

Fig 02: Testosterone level (c) and MDA level (b) in control and endosulfan treated male mice for 4 weeks, 8 weeks and 12 weeks.

Table 01: Mean±S.D of Testicular weight, Sperm Count, Testosterone Level, MDA level of control and endosulfan treated mice for 4 weeks, 8 weeks and 12 weeks.

	Control	4 Weeks	8 Weeks	12 Weeks
Testicular weight (g)	0.113±0.055	0.101±0.042	0.086±0.029	0.075±0.027
Sperm Count (Million/µl)	5±0.603	0.412±0.322	0.0938±0.027	0.0434±0.025
Testosterone level (ng/ml)	2.626±1.198	1.98±0.98	1.83±0.855	1.22±0.596
MDA level (nMol/ml)	22.0±4.106	30.013±2.898	42.117±3.943	46.62±5.980

DISCUSSION

The present investigation reflects the changes in physio-chemical mechanisms involved in reproductive system in male mice due to administration of endosulfan. The impairment of spermatogenesis in pubertal and prepubertal rat induced by endosulfan has been reported[13, 14]. The results clearly indicate that endosulfan affects spermatogenesis when administered at the rate of 2 mg/kg/body weight. It has been reported that testicular weight, enzyme activity, and sperm count significantly diminished in rats after the administration of endosulfan at the rate of 2 mg/kg/body weight[15]. Similarly, our results demonstrate quite similar pattern of derangement in the values of parameters like testicular weight, sperm count etc. Endosulfan has also been associated with the degeneration of seminiferous epithelium, reduced number of leydig cells, and immotile spermatozoa.

According to a study, endosulfan was observed to increase the biotransformation of testosterone leading to increased elimination through urine[16]. Our results shows decrease in the testosterone level in male mice upon endosulfan administration. Endosulfan has been demonstrated to induce oxidative stress. It has been reported that the endosulfan reduces the free radical scavenging enzymes like SOD, Glutathione peroxidise, catalases etc[17, 18]. Genotoxic and cytotoxic effects of pesticides on plant tissue have been affirmed[19]. Higher toxicity level is directly correlated with greater lipid peroxidation, due to malfunction developed by endosufan accumulated in cells.

However, effect of endosulfan on male reproductive system needs to be studied extensively. Moreover, data on enzyme activity, gene expression, and changes in antioxidant system could unravel prominent features as how endosulfan affects the reproductive system in male mice.

CONCLUSION

Endosulfan acts as xenoestrogen and toxic agent. An effort was made to study the effect of endosulfan of male reproductive system. Endosulfan decreases the testicular weight, spermatogenesis, testosterone level and increases the oxidative stress. Endosulfan is still used in agriculture of India widely and unrestrainedly. Ultimately, endosulfan is consumed by humans unknowingly which leads to the complications in reproductive system.

ACKNOWLEDGEMENT

Authors greatly acknowledge financial assistance from ICMR, New Delhi, Government of India. Authors are indebted to Prof. M. Rajalakshami and Dr. R.S Sharma for their kind support. We are thankful to S S Hospital and Research Institute for providing infrastructure facility.

REFERENCE

1. Foster PMD. Testicular organisation and biochemical function. In: Lamb, J.C., Foster, P.M.D. (Eds.), Physiology and Toxicology of Male Reproduction. Academic Press, New York, USA: 1988, p7.
2. HazDat. Endosulfan. Agency for toxic substances and disease registry (ATSDR), Atlanta, GA, 1998.
3. Bennett DA, Chung AC, Lee SM. Multiresidue method for analysis of pesticides in liquid whole milk. J AOAC Int, 1997; 80(5): 1065–77.
4. Nath A, Vendan SE, Priyanka, Singh JK, Singh CK, Kumar S. Carcinogenic Pesticides Residue Detection in Cow Milk and Water Samples from Patna, India. Current Trends in Biotechnology and Chemical Research, 2013; 3(1): 1-7.
5. Pokharkar DS, Dethe MD. Gas–liquid chromatographic studies on residues of endosulfan on chilli fruits. J Environ Sci Health, 1981; B16: 439–51.
6. FDA. Pesticide analytical manual, vol. 1. 3rd ed. Multiresidue methods, Method 302: method for non-fatty foods: Method II for non-fatty foods, Method 304: method for fatty foods. FDA, US Department of Health and Human Services, 1994.
7. Dikshith TSS, Raizada RB, Kumar SN. Effect of repeated dermal application of endosulfan to rats. Vet Hum Toxicol, 1988; 30: 219–24.
8. Sinha N, Narayan R, Shanker R, Saxena DK. Endosulfan induced biochemical changes in the testis of rats. Vet. Hum Toxicol, 1995; 37: 547.
9. Sinha N, Narayan R, Saxena DK. Effect of endosulfan on the testis of growing rats. Bull. Environ Contam Toxicol, 1997; 58: 79.
10. Theobald HM, Peterso RE. In utero and lactational exposure to 2,3,7,8-tetrachloro-dibenzo-p-dioxin: effects on development of the male and female reproductive system of mouse. Toxicol Appl Pharmacol, 1997; 145: 124.
11. Khan PK, Sinha SP. Ameliorating effect of vitamin C on murine sperm toxicity induced by three pesticides (endosulfan, phosphamidon and mancozeb). Mutagenesis, 1996; 11: 33–36.
12. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 1979; 95: 351-8.
13. Sinha N, Narayan R, Shanker R, Saxena DK. Endosulfan induced biochemical changes in the testis of rats. Vet Hum Toxicol, 1995; 37: 547.
14. Sinha N, Narayan R, Saxena DK. Effect of endosulfan on the testis of growing rats. Bull Environ Contam Toxicol 1997; 58: 79.
15. Sinha N, Adhikari N, Saxena DK. Effect of endosulfan during fetal gonadal differentiation on spermatogenesis in rats. Environmental Toxicology and Pharmacology, 2001; 10: 29–32.
16. Wilson VS, LeBlanc GA. Endosulfan Elevates Testosterone Biotransformation and Clearance in CD-1 Mice. Toxicology and Applied Pharmacology, 1998; 148(1): 158-168.
17. Dorval J, Leblond VS, Hontela A. Oxidative stress and loss of cortisol secretion in adrenocortical cells of rainbow trout (Oncorhynchus mykiss) exposed in vitro to endosulfan, an organochlorine pesticide. Aquatic Toxicology, 2003; 63: 229-241.
18. Dorval J and Hontela A. Role of glutathione redox cycle and catalase in defense against oxidative stress induced by endosulfan in adrenocortical cells of rainbow trout (Oncorhynchus mykiss). Toxicology and Applied Pharmacology, 2003; 192: 191–200
19. Asita AO and Makhalemele R. Genotoxicity of Chlorpyrifos, Alpha-thrin, Efekto virikop and Springbok to onion root tip cells. African Journal of Biotechnology, 2008; 7(2): 4244-4250.