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The Effect of Endosulfan on Ovaries of *Mus musculus*: A Histological Study

Arti Kumari[1*] and Preety Sinha[1]

[1]Department of Zoology, A. N. College, Patna – 800013, Bihar, India.

Authors' contributions

This work was carried out in collaboration between both authors. Authors PS and AK jointly designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors PS and AK managed the analyses of the study. Author AK managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Today pesticidal poisoning is an important health issue. The term "pesticides" covers a wide range of substances of differing chemical composition. Although we know these are useful in agricultural sector. However, it's increased and unprotected use in agricultural practices has greatly enhanced the chances of its exposure to animals and man. This increases the risk of health disorders.
Objective: The aim of present study was designed to examine the effect of pesticide an Endosulfan on the ovary of female *Mus musculus*.
Materials and Methods: They were administered orally according to their body weight with Endosulfan @ 3 mg/kg body weight for 2 weeks, 4 weeks, 6 weeks and 12 weeks. The animals were sacrificed on day next to the last day of treatment and ovaries were extirpated. Ovaries were studied histologically after haematoxylin - eosin staining.
Results and Discussion: Observations showed structural defects during histological examinations of Graafian follicles after exposure of Endosulfan. Degeneration in follicular cells was seen and ova were often detached. Degeneration of ova occured in some follicles, irrespective of the stage of

**Corresponding author: E-mail: artikumari.singh@gmail.com;*

their development. The organization of granulosa cells was also disturbed in longer duration of treatment. The abnormalities gradually increased with dose duration time. Such types of abnormalities have not been seen in the control ovary.
Conclusion: It may be concluded from this study, Endosulfan has adverse effects on ovary at cellular level. Damaged cells of ovarian follicles, destroys steroid hormone production, which can result into improper ovarian function.

Keywords: Endosulfan; histological changes; ovary; Mus musculus.

1. INTRODUCTION

India depends mainly upon agriculture for its economy and food requirement. Therefore, in India the application of pesticide is in fairly large quantities for the control of a wide variety of pests that would diminish the quantity and quality of food produce. Unfortunately, with the benefits of pesticides have also come drawbacks. Some drawback so serious, that they now threaten the long-term survival of major ecosystems by disruption of predator-prey relationships and loss of biodiversity.

Endosulfan is an organochlorine group of insecticides and acaricide [1], classified as a 'Class II moderately hazardous' chemical [2]. The US Environmental Protection Agency [3] classifies it category Ib (highly hazardous). It is a semi-volatile cyclodine pesticide that can migrate over a long distance through various environmental media such as air, water and sediment. It can accumulate in top species even human through bioaccumulation [4]. There are some major health concerns due to their prolonged persistence in the body. There is substantial evidence which suggests that Endosulfan causes reproductive and developmental damage. It is endocrine disruptor [5], can affect steroid production of follicular cells [6]. Saiyed have reported the effect of Endosulfan with male reproductive deformities [7]. Many other studies have demonstrated its reproductive and developmental toxicity, especially among males [3]. Endosulfan causing reproductive defects has also been reported [8-10]. It caused deleterious effect at cellular and subcellular levels on animal model [11,12]. It may also cause Infertility [13]. Higher levels of Endosulfan may be associated with Preterm Delivery and increased oxidative stress [14].

Therefore, the present study is an attempt to demonstrate the changes in Graafian follicles by different exposure time of Endosulfan in mice. And we know, the dose and duration of exposure are important to determine the cellular and biochemical toxicity by Endosulfan [15].

2. MATERIALS AND METHODS

2.1 Pesticide

Endosulfan (EC = 35%, w/v) obtained from local market (manufactured by Excel Industries LTD, Mumbai, India).

2.2 Experimental Model

In the present investigation, experiments were performed on 12 weeks old and sexually matured healthy female Swiss albino mice, *Mus musculus* weighing 28-32 g body weight. The animals were housed at controlled environmental conditions 22±2℃, relative humidity 50±10%, and 12 hr dark-light cycle. Animals were housed and allowed to free access to food and water. All experimental procedures were conducted as per the guide lines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

2.3 Experimental Design

30 mice were divided into 5 groups with 6 mice in each group. Group – 1 was treated as Control mice fed distilled water/kg body weight. Group - 2, Group – 3, Group – 4 & Group – 5 were fed Endosulfan (3.0 mg/kg body weight) daily by oral gavage method for 2, 4, 6 and 12 weeks respectively.

2.4 Histological Studies

After Twenty-four hours of the last treatment, mice of control and experimental groups were sacrificed and their ovaries were removed. The ovaries were weighed after cleaning the fat tissue and wiping them dry with blotting paper. Digital balance was used for this purpose, which had a minimum count of 0.0l mg. Ovaries were fixed in 10% formaldehyde solution for 24 hours.

Fixed tissue was dehydrated, cleared with xylene and completely embedded in paraffin wax. The tissue was then sectioned at a thickness of 4 µm, mounted and affixed to slides. The tissue sections were stained with hematoxylin - eosin stain and examined with a light microscope.

2.5 Statistical Analysis

Data are expressed as mean ± SD. All data analyzed by using the software "Graph Pad 5.0". The p-value was considered significant when p≤0.05.

3. RESULTS

3.1 Weight of Ovaries

Ovaries collected from controlled mice, had an average weight of 16.64±0.88 mg, while in Endosulfan treated mice weight of ovary reduces with shrinkage in size. More frequent reduction was observed in 6 weeks and 12 weeks Endosulfan treatment. In 6 weeks Endosulfan treated mice, weight of ovary was 11.48±0.87 mg with a range from 10.0 - 12.4 mg and in 12 weeks Endosulfan treated mice, weight of ovary was 8.02±1.18 mg with a range from 6.0 – 9.2 mg. This reduction in weight could be ascribed to degenerative changes in treated ovaries.

3.2 Histological Studies

3.2.1 Observations of control ovary

Control group (Untreated group) of mice shows normal ovary with continuous germinal epithelium with well defined different stages of ovarian follicle, well defined ovarian cortex and medulla region and mature Graafian follicles (Fig. 1). In structural morphology of Graafian follicle, oocyte is tightly surrounded by granulosa cell layer (Fig. 1).

Table 1. Weight of ovary in control and endosulfan treated mice

Group	Number of mice	Dose duration	Ovary weight		% Decrease	Comparison between the groups
			Range (mg)	Mean ± SD		
Control	6	-	15.4 – 18.0	16.64±0.88		p < 0.0001
Treated	6	6 weeks	10.0 – 12.4	11.48±0.87	31	
	6	12 weeks	6.0 – 9.2	8.02±1.18	51.80	

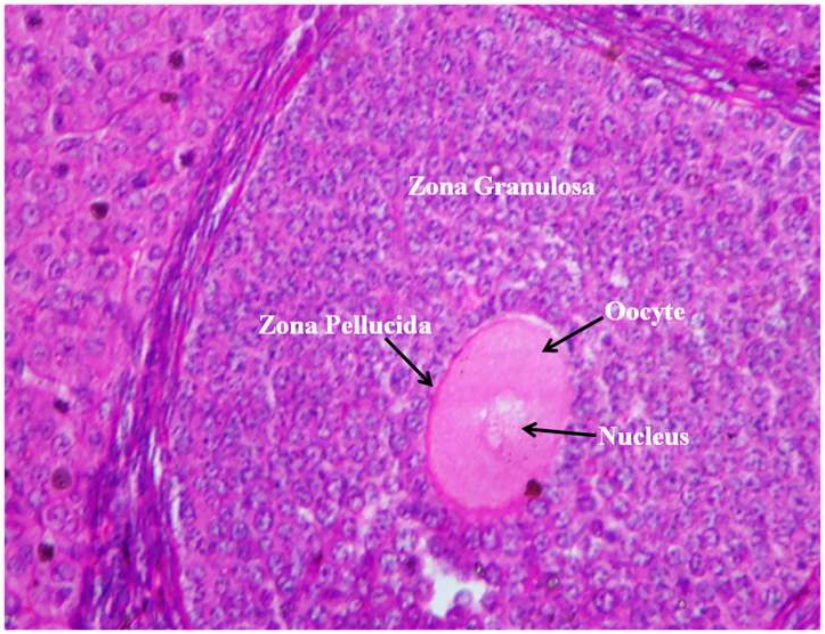

Fig. 1. Photomicrograph of control ovary showing mature graafian follicles (40 X)

3.2.2 Observations of endosulfan treated ovary

In ovaries of Endosulfan treated mice, examination revealed that the most remarkable changes were seen.

Ovaries of 2 weeks Endosulfan administered mice showed degeneration in primary follicles. Germinal epithelium was in degenerated condition (Blue arrow, Fig. 2). Cytoplasmic materials were not seen around germinal epithelium cells. Degenerated granulosa cells were also observed (Black arrow, Fig. 2).

Ovaries of 4 weeks Endosulfan administered mice showed degeneration in follicular stages and Graafian follicle. Vacuolated follicles were also observed in ovary. Degenerated ova in Graafian follicle with degenerated granulosa cells were observed. Vacuolated spaces in granulosa cells were also observed in Graafian follicle (Fig. 3).

Degeneration of some follicular cells (Yellow arrow, Fig. 4) and increased vacuolization on either side of ova of primary follicle (Black arrow, Fig. 4) were observed in 6 weeks Endosulfan administered mice.

In Ovaries of 12 weeks Endosulfan administered mice, the oocyte was in degenerative condition (Blue arrow, Fig. 5) and often appeared detached from the surrounding cells. Granulosa cells were also in degenerative condition and loosely bounded (Red arrow, Fig. 5).

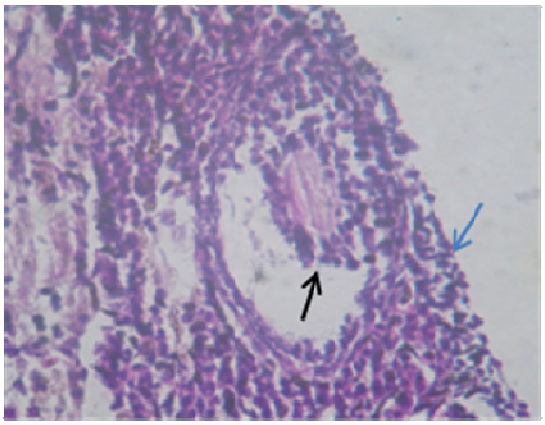

Fig. 2. Photomicrograph of graafian follicle of 2 weeks endosulfan treated mice @ 3 mg/kg body weight. (80 x)

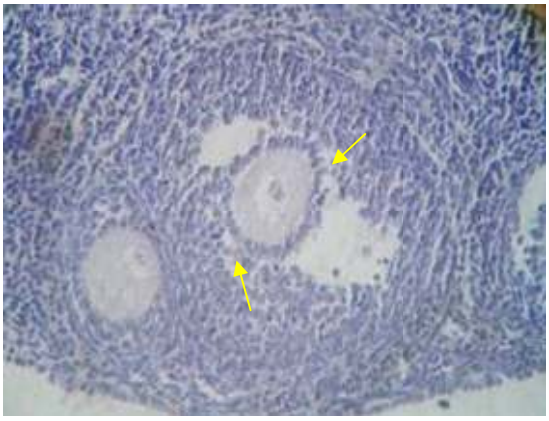

Fig. 3. Photomicrograph of graafian follicles of 4 weeks endosulfan treated mice @ 3 mg/kg body weight. (80 x)

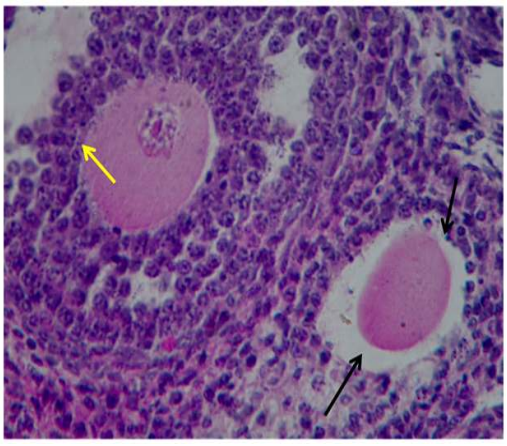

Fig. 4. Photomicrograph of graafian follicles of 6 weeks endosulfan treated mice @ 3 mg/kg body weight. (80 x)

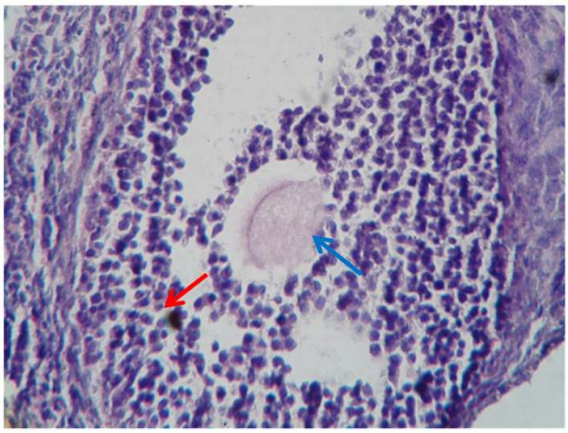

Fig. 5. Photomicrograph of graafian follicles of 12 weeks endosulfan treated mice @ 3 mg/kg body weight. (80 x)

4. DISCUSSION

Use of pesticides has a major impact on environment. Endosulfan is an organochlorine insecticide and acaricide used in a variety of field, fruit, and vegetable crops. Endosulfan accumulates in these foods. Hence, the most likely way for people to be exposed to Endosulfan is eating the contaminated food with it. Its metabolites have been reported in human and domestic animals' milk [16-18], chilli fruits [19], fruit and vegetable [19,20]. Paul and Balasubramaniam studied several cases of poisoning that have been reported in work place where men were exposed to Endosulfan over long periods [21]. It is quite evident that Endosulfan causes deleterious effect [10]. It has been also well established that Endosulfan causes deleterious effect on reproductive physiology. In male it retards the spermatogenic cycle [8,22,23]. In the last fifty years it has been observed Endosulfan may also cause the disease in the quality of semen, an increase in testicular and prostate cancer, an increase in defects in male sex organs, and increases incidence of breast cancer [7,24-26]. Oral administration of the pesticides Endosulfan, Methyl parathion and Mancozeb, showed that they inhibited compensatory ovarian hypertrophy, decreased the number of healthy follicles, increased the number of atretic follicles, and affected the oestrous cycle in rats [27,28]. This study exhibited changes in the weight of the ovaries. Ovary weight reduced at 31 percent in 6 weeks and at 51.8 percent in 12 weeks Endosulfan treated mice as compared with control group. Loss of weight of ovary may indicate degeneration occurs in ovary. Here effect of Endosulfan is directly proportional with time of exposure. It has been reported that much lower doses of toxicants Endosulfan may result functional or organic disorders in later life if the exposure takes place during the early developmental phase [29].

Folliculogenesis is the process in which a recruited primordial follicle grows and develops into a specialized Graafian follicle with the potential to either ovulate its egg into the oviduct to be fertilized or to die by atresia. Ovary also produces steroid hormones estrogen and progesterone. Estrogens stimulate the development and proliferation of uterine endometrium during the Estrous cycle in mammals and also have anabolic effects on skin, bone and muscles [30]. Moreover, the ovarian follicle has also been shown to be a direct target for xenoestrogens [31]. Comparative study of control and treated ovary of mice showed degenerative changes in all the ovarian follicles including Graafian follicles. This type of deleterious impact on gonads will adversely affect the hormone synthesis and hence effect reproduction. Hiremath and Kaliwal studied the effect of Endosulfan on ovarian functions in hemi castrated virgin Swiss albino mice (80–120 days old; 10/dose; for 15 days) [15]. He reported decreased healthy ovarian follicles and increased number of atretic follicles. Observations showed detached and degenerated or destructed ova in treated animals after exposure of Endosulfan. The organization of granulosa cells was disturbed in prolonged Endosulfan treatment in mice. This is because of degeneration in cytoplasmic material of follicular cells. Degenerated oocyte damages ovarian follicles and, in turn, destroys steroid hormone production, which can result in ovarian failure. Moreover, it is the way of leading sign of infertility.

5. CONCLUSION

Our studies suggest a gonadotoxic potential of Endosulfan. Histological views of Graafian follicle clearly show adverse effects of Endosulfan and it increases with time of exposure. It may be a cause of improper function of ovary in mice. Hence, Future agricultural practice must aim to reduce pesticide use to a minimum. After the Stockholm Convention, Endosulfan is banned in more than 75 countries. India completely banned the Endosulfan in May 2013 and agreed to phase out use of Endosulfan by 2017.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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