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RESEARCH ARTICLE

PESTICIDE CAUSED DISRUPTION OF CORPUS LUTEUM LEADING ELEVATED LEVEL OF ESTROGEN IN MICE

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ABSTRACT

Over the past several decades, awareness has been growing regarding the reproductive health effects of exposures to certain chemicals. Some pesticides may interfere with the female hormonal function, which may lead to negative effects on the reproductive system through disruption of the hormonal balance necessary for proper functioning. Toxicants that interfere with ovarian function can act directly on the ovary or indirectly through demonstrating their influence at the hormonal level. In the present study, the effects of endosulfan, an oranochlorine pesticide, were investigated after its long-term treatment in low residual level doses on ovaries of albino mice in co-relation with estrogen level in the serum. Endosulfan was orally administered at 3mg/kg body weight to swiss albino female mice for 2, 4 and 6 weeks. Mice were sacrificed and blood were collected for hormonal assay and histological examination of ovaries. Elevated level of estrogen was observed with increase in dose duration. Degenerated corpus luteum was also observed after endosulfan administration. Degeneration were increased with increased duration of dose of endosulfan which causes gradually elevated estrogen level in mice due to which ovulation of mice does not takes place in such high level of estrogen and mice become infertile.

Key words: Endosulfan, Ovary, Estrogen, infertile, hormone

INTRODUCTION

Organochlorines are a diverse group of synthetic chemicals, many of which were released into the environment in past decades through their use as pesticides or industrial products. Organochlorine compounds degrade slowly, lipid soluble, bioaccumulate in the food chain, and may be found in human adipose tissue[1], blood[2], serum[3] and breast milk[4]. Small amounts of some of these chemicals cause death[5]; disrupt hormones and reduce the ability to successfully reproduce[6]; and have been associated with specific cancers[7]. Endosulfan is one of the most abundant organochlorine pesticides in the global atmosphere and is capable of undergoing long range transport to remote locations such as the Arctic[8]. Endosulfan causes oxidative stress[9], which is implicated in its neurotoxic effects [10, 11], damage to the adrenal gland[12]. The mode of action of endosulfan is to bind and inhibit γ -amino-butyric acid (GABA)-gated chloride channel receptor, thereby inhibiting GABA-induced chloride flux across membranes[13]. Endosulfan also acts as an endocrine disruptor and cause damage to testes of male fish–-including the Leydig cells, Sertoli cells, and seminiferous tubules–-that is likely to have a negative effect on male fertility[14]. Significantly lowered levels of testosterone and oestradiol have been found in fish with elevated residues of endosulfan[3]. It also induces the activation and proliferation of progesterone receptors—another oestrogen-mimicking effect—in human breast cancer cells[15], and it decreases the activity of progesterone[16]. As well as mimicking oestrogen, endosulfan is also antiandrogenic[16]. Relatively recent discoveries of the hormone disrupting properties of some pesticides at low exposures [17] have raised interest in how low-dose acute or chronic pesticide exposures—the types of exposures that can currently occur both occupationally and environmentally – impact reproductive fertility. Exposure to endosulfan is high. Apart from occupational exposure which has resulted in many poisonings, residues in food and drinking water are widespread globally at sufficiently high levels to constitute a threat to human health, and to result in consistent findings of human body burdens. Although a substantial amount of research has been conducted to associate occupational exposure to endosulfan with fertility problems in men, studies among women are scarce. Hence the present study was undertaken to investigate the effects of endosulfan on corpus luteum of albino mice in co-relation with estrogen level in the serum.

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MATERIALS AND METHODS

Chemicals

Pesticide Endosulfan, manufactured by Excel India Pvt. Ltd., Mumbai with EC 35% was used for the experiment. Doses were given according to the body weight of mice per day as it makes direct impact on them. The concentration of Endosulfan administered orally to the animals was 3 mg/kg body weight.

Animals

Adult (10-12 weeks old; 30-32 gm body weight) swiss albino female mice were housed in groups of 10 mice per cage, maintained under controlled conditions of temperature (22 ± 2°C) and light (12 :12 :: L : D) and provided with feed pellets (as recommended by Templeton, 1945) prepared in the laboratory and water *ad libitum*. All mice received humane care.

Study Groups

The mice were set in two groups (n =10 per group) as following: The 'control group' received distilled water as drinking water. The 'treatment group' was administered Endosulfan (3 mg/kg body weight) by oral gavage method for four weeks.

Sampling

The animals were sacrificed after the scheduled treatment. The mice were anesthetized with mild ether for the purpose. The blood and tissues from the control and treated mice were collected as a sample to test and collect the data. Blood sample of individual mice of each group was taken in separate RIA tube by orbital sinus puncture. After separation of the serum it was collected in separate small, labeled vials at 4 ^{0}C for the biochemical test. The ovaries from the sacrificed mice were removed and washed three times in isotonic saline (0.85 gm/ml) and fixed for the subsequent histological studies under light & electon microscopy.

RESULTS

In control group of mice estrogen level was 18.6 pg/ml. while estrogen level gradually increases after 2 weeks, 4 weeks and 6 weeks of endosulfan administration and it will became 31.4 pg/ml, 40.1 pg/ml and 46.7 pg/ml (Text Figure 1).Control mice show normal graffian follicle with different primordial follicles (Plate – I, Fig: A). Degeneration in mature graffian follicles were observed after 4 weeks of endosulfan while vacuolization were clearly observed in corpus luteum. Deshaped corpus luteum were also visible (Plate – I, Fig: B). Vacuolization were observed around corpus luteum. Many vacuolated spaces were observed in cortex region of ovary. Primary follicles were devoid of ova (Plate – I, Fig: C). Corpus luteum were observed in degenerated condition. Germinal epithelium was also fragmented. Granular ova were observed on degenerated condition (Plate – I, Fig: D).

DISCUSSION

Environmental fate of Endosulfan, its occurrence in soil and water in the Arctic region was reported by [8]. Sharpe [18] have

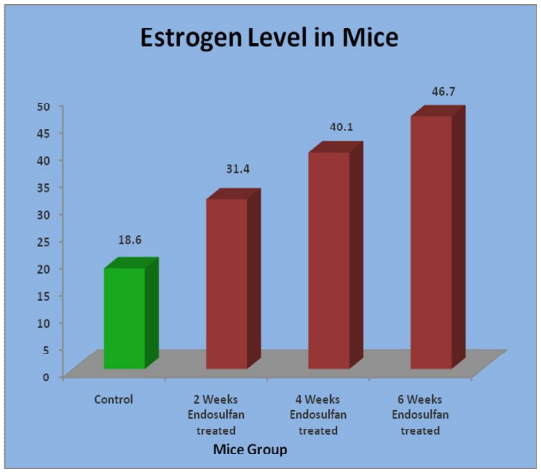

Fig. 1.

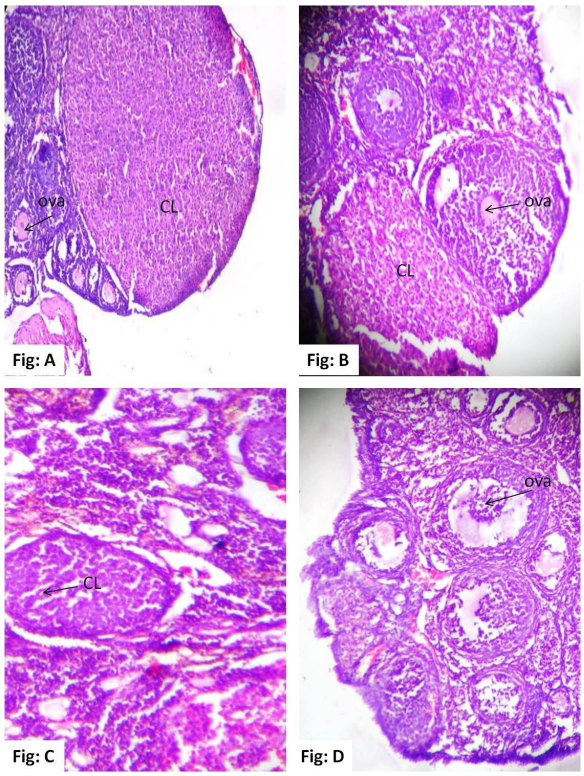

Plate No. 1.

bserved evidence of a link between environmental chemicals and adverse effects on human reproductive health. Acute toxic effects of Endosulfan and Diazinon pesticides on adult amphibians were explored by [11]Lawrence. Role of glutathione redox cycle and catalase in defense against oxidative stress induced by endosulfan in adrenocortical cells of rainbow trout were explored by [12]Dorval. Reactive oxygen species in vitro pesticide induced neuronal cell (SH-SY5Y) cytotoxic role of NF kappa B and caspase-3in pesticide induced cells[10]. Sharma[19] Studies on the genotoxicity of endosulfan in different tissues of fresh water fish *Mystus vittatus* using the Comet assay. It was observed that endosulfan causes elevated level of estrogen in mice. Amount of estrogen increases with increased

duration of dose of endosulfan, which finally causes abnormal development of secondary sex organs as well as corpus luteum. Endosulfan and its metabolites in fertile women, placenta, cord blood, and human milk were studied in detailed by [20]Cerrillo, he find elevated level of endosulfan in cord blood and human milk as well as placenta. Effect of environmental and lifestyle factors for organochlorine exposure among women living in Southern Spain were analysed by Cerrillo[1] and find many cases of infertility due to this.

Screening for estrogen and androgen receptor activities in 200 pesticides by *in vitro* reporter gene assays using Chinese hamster ovary cells were studied by Kojima[21]. The effects of endosulfan on the testes of bluegill fish, *Lepomis macrochirus* were studied in details with histopathological study by Dutta[14]. Pesticide residues and reproductive dysfunction in different vertebrates from north India were reported by Singh [3]. Different stages of degeneration of primary and secondary follicles were also observed. Degeneration in corpus luteum was observed which finally alters estrogen synthesis in mice. Due to degeneration of corpus luteum ovulation of mice does not takes place in such elevated level of estrogen which finally causes infertility in mice. Thus it is concluded from study that endosulfan causes degenerated corpus luteum due to endosulfan administration. Degeneration were increased with increased duration of dose of endosulfan which causes gradually elevated estrogen level in mice due to which ovulation of mice does not takes place in such high level of estrogen and mice become infertile.

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