

RESEARCH ARTICLE

## Pesticide Effect in Male Hormones and Antioxidant Status in Male Albino Rats

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### Abstract

Reproductive health problem is more common nowadays and various causes like nutritional defect, socio-economic problems, life style changes are reason for that, among those reason, one important factor is environmental factor. In this study, the pesticide, Lindane is used to induce reproductive toxicity and the levels of male reproductive hormone, antioxidant and histopathology of rat testis was evaluated. Ten healthy rats were treated with Lindane (5 mg/kg) for 30 d. Lindane treated rats showed decreased male hormone and antioxidant levels when compared to control. Histopathology of rat testis showed cleavage of seminiferous tubules, with detached germ cells from the basement membrane and the intracellular space between seminiferous tubule is abnormal. Result implies Lindane reproductive toxicity.

**Keywords:** Lindane, pesticide toxicity, male hormones, antioxidant, testis, histopathology.

### Introduction

Infertility is the inability to conceive after at least one or two years of unprotected sex. Since most people are able to conceive within this time, male infertility is a multi-factorial disease process with a number of potential contributing causes. Male factors contribute to almost 50% of cases of infertility; in the remainder, infertility may be due to either a female factor or a combination of male and female factors (Gopalkrisnan, 1996). Defective in sperm production is the most important cause of male infertility (Parlaktas, 2008). Experimental studies suggest that the pesticide has ability to interact with the endocrine system and reproductive capacity (Chedrese, 2008).

Pesticides interact with the endocrine system and the reproductive system through various mechanisms. In an Iranian study, about 50% of the products used in agriculture could potentially interfere with the endocrine system, 33% of them leading to male infertility, 8% having estrogenic activity, 4.5% antiandrogenic and 22% thyreostatic (Ebrahimi and Shamabadi, 2007). Herbicides, insecticides and fungicides are the main endocrine disruptor chemicals (EDCs) likely to be encountered (carbofuran, chlpyrifos, dimethoate, lindane, trillate, triflurarin 2,4-D and penttchlorophenol, linuron). Organophosphate pesticides have a small effect on male reproductive hormones, suggestive of a secondary hormonal disturbance after testicular damage (Padungtod, 1998). This was confirmed by Recio (2005) who found that out of 64 agricultural workers (48%) had FSH outside the normal range, with values substantially higher than normal during the periods of highest pesticide use; LH was also slightly elevated, but no abnormal findings LH were recorded for testosterone.

Exposure to organophosphorus pesticides has been associated with changes in the chromatin structure of sperm, which may raise the proportion of cells highly susceptible to DNA denaturation; these compounds also interfere with spermatid chromosomal segregation, again raising the risk of genetic damage (such as Turner's syndrome) because of aneuploidy. Significant associations have been found between serum levels of organochlorides, immune and endocrine alterations, indicative of the risk that these products interfere in the course of gestation (Gerhard, 1998). Human are frequently exposed to pesticides in various form among those lindane is most important for the onset of reproductive toxicity. The estrogenic properties of lindane have been demonstrated in several systems, including the production of vitellogenin (egg yolk protein) and zona radiata (egg shell protein) in primary hepatocytes (liver cells) from Atlantic salmon (*Salmo salar* L.). Lindane has also been shown to damage human spermatozoa at concentrations as low as those found in female genital tract secretions (Silvestroni, 1999). Testosterone is the most significant hormone among male sex hormones (androgen). Testosterone also acts on cells in the testes to make sperm. LH acts on specialized cells within the testes and stimulates release of the male sex hormone, testosterone, FSH acts on a separate population of cells within the testes to stimulate the production of sperm. Decreased testosterone values combined with an insufficient FSH and LH indicate reproductive toxicity. An intimate structural and functional relationship exists between the two separate compartments of the testis i.e. the seminiferous tubule and the interstitium between the tubules.

LH indirectly stimulates androgenous testosterone production in spermatogenesis, so testosterone and FSH are the hormones that are directed at the seminiferous tubule (Campagn, 2002). Oxidative stress contributes to defective spermatogenesis by reducing the antioxidant level in blood and testis that leads to male infertility. Glutathione (GSH) has a protective effect on sperm motility. Superoxide dismutase (SOD) and catalase are enzymatic antioxidants that protect spermatozoa from superoxide anion and hydrogen peroxide ( $H_2O_2$ ). Considering the above facts in view, this study was aimed to find out the effect of male hormone and antioxidant status in pesticide induced male albino rat.

### Materials and methods

**Experimental animals:** Male rats were obtained from the Sri Venkateshwara Enterprises, Bangalore, India. The animals were housed in polypropylene cages. Rats were fed with pelleted food and water was provided through plastic bottles.

**Collection of samples:** After 24 h of the last treatment, the rats were weighed and sacrificed. Blood samples were collected in heparinized vials. Testis was dissected and transferred in sterile container containing cold normal saline. One side of testis of 30 d treatment was preserved in Bouin's fixatives for histological observation. Proper ethical guidelines were followed in the present study (Approval No. 791/03/B/ CPCSEA).

**Quantitative determination of plasma hormone levels:** Plasma levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, testosterone and estradiol were measured by enzyme linked immunosorbent assay (Alford *et al.*, 1973).

**Antioxidant determination assay:** Superoxide Dismutase (SOD) was assayed according to the method of Marklund and Marklund (1974). Reduced glutathione was determined by the method of Mannervik *et al.* (1979). The activity of glutathione peroxidase (GPx) was determined by the method of Rotruck *et al.* (1973). The activity of catalase was assayed by the method of Sinha (1972).

**Histopathology of testis:** Testis tissues were fixed in Bouin's fixative for histological studies. After dehydration in alcoholic series and cleaning in xylol, the tissues were embedded in paraffin wax. Sections were made with 5  $\mu$ m thickness. The sections were stained with haematoxylin followed by eosin and examined under a light microscope.

### Results and discussion

Several pesticides induce reproductive toxicity by disturbing the endocrine system especially reducing the androgen level in human and animals.

Lindane is an organochlorine pesticide which has been used for many years in the treatment of lice and scabies and its exposure induces reproductive toxicity by reducing male reproductive hormone and testis antioxidant level. Spermatogenesis is maintained by male reproductive hormones, among that testosterone plays a very important role. The pituitary hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH) are responsible for the stimulation of spermatogenesis and steroidogenesis, respectively (Greep and Fevold, 1937). LH binds to Leydig cells to stimulate testosterone secretion and the testes to produce androgen. In the present study, lindane treated rats showed decreased values of testosterone along with that FSH, LH and estradiol also showed decreased values because lindane has anti-androgenic activity and it binds to the androgen receptor that will reduce the secretion of these hormones (Table 1). The reduction in the testosterone level also is due to diminished responsiveness of cells in testis and that have inhibition in testicular steroidogenesis, estradiol level is decreased due to impaired synthesis of estradiol from testosterone (Anita and Geri, 1995).

Administration of lindane decreased the activities of superoxide dismutase, catalase, glutathione and glutathione peroxidase in crude homogenate of testis as compared with control (Table 2). Superoxide dismutase, catalase, glutathione and glutathione peroxidase are antioxidant enzymes distributed in different sub-cellular fractions of testis as well as in the epididymal sperm and epididymis (Sujatha *et al.*, 2001; Chitra *et al.*, 2001; Latchoumycandane *et al.*, 2002). Superoxide dismutase is considered to be the first line of defense against deleterious effects of oxyradicals in cell by catalyzing dismutation of superoxide anion to hydrogen peroxide and molecular oxygen. Catalase, glutathione reductase and glutathione peroxidase protect cells from highly toxic hydrogen peroxide by catalyzing hydrogen peroxide into water and oxygen. Reduction in the GSH level may be due to indirect conjugation with excess electrophiles produced due to lindane exposure. The reduction in the GSH level in this study may be due to direct conjugation of GSH with electrophilic species which are produced increasingly by lindane exposure or due to inhibition of enzymes GPx, etc. which are involved in GSH synthesis and regeneration. The reduction in the SOD and CAT level may be due to the excessive production of superoxide anions after exposure of lindane. GPx is a GSH using enzyme and plays an important role in maintaining GSH homeostasis and tissue detoxification. Lindane exposure leads to decrease in GPx activity in our study, which may be due to the depleted level of GSH. Superoxide radicals are deleterious to PUFA (polyunsaturated fatty acid) and structural proteins of plasma membrane and the disposition is saturated by SOD. Decrease in SOD favours the accumulation of superoxide radical, which in turn inhibits CAT. The decreased CAT activity results in augmentation of  $H_2O_2$  generation (Chitra *et al.*, 2001).

Table 1. Effect of lindane on male reproductive hormones.

Parameters	Normal	Lindane
Testosterone (ng/mL)	2.920±0.0807	1.820±0.0748
Follicle stimulating hormone (ng/mL)	3.080±0.0805	2.880±0.0652
Luteinizing hormone (ng/mL)	4.420±0.0800	3.680±0.1300
Estradiol (pg/mL)	8.050±0.0860	5.054±0.0789

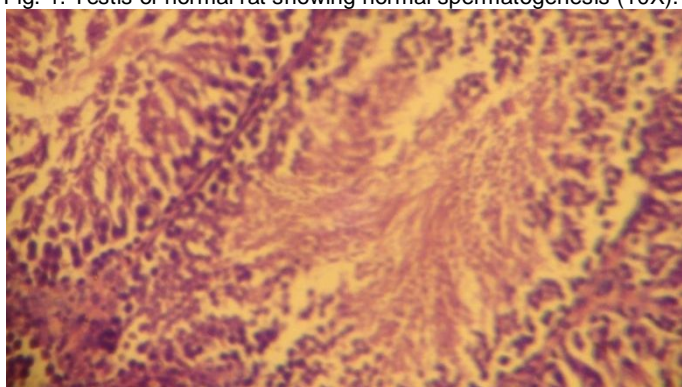
Values are expressed as Mean±SD for five rats.

Table 2. Effect of lindane on antioxidants.

Parameters	Normal	Lindane
GSH (kmol H <sub>2</sub> O <sub>2</sub> consumed/min/mg)	5.1660±0.132	2.9180±0.4195
SOD (nmol/min/mg)	27.8040±0.6072	20.2900±0.4782
Catalase (µmol H <sub>2</sub> O <sub>2</sub> consumed/ min/mg)	0.7500±0.0100	0.5440±0.0089
GPX (nmol NADPH oxidized/min/mg)	43.6700±0.5438	34.0400±0.1140

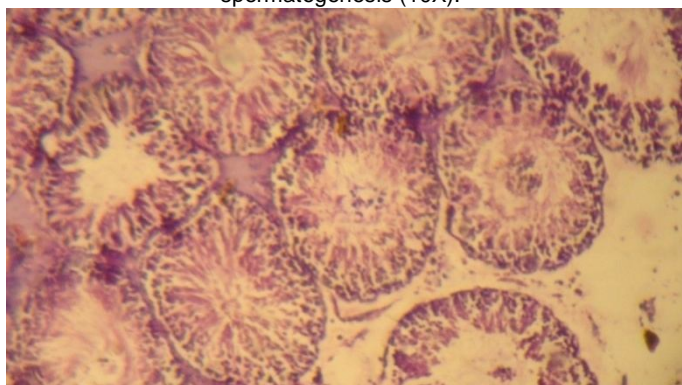
Values are expressed as Mean±SD for five rats. Mean values within the row followed by different letters are significantly different from each other at P<0.05 level comparison by Duncan's multiple range test (DMRT).

Fig. 1. Testis of normal rat showing normal spermatogenesis (10X).



Histopathology sectioning showing normal seminiferous tubule, spermatogonia and interstitial space.

Fig. 2. Lindane treated rat testis showing affected spermatogenesis (10X).



Histopathology sectioning showing depressed spermatogenesis, loss of sperms, increased interstitial space and cleavage of seminiferous tubule.

In this study, control rat showed normal seminiferous tubule with epithelium consist of two distinct populations of cells, normal spermatogenic cell, called sertoli cell which support and nourish the developing spermatozoa, in the intertubular space, leydig cell and connective tissue cells were present (Fig. 1). The space between seminiferous tubule is normal and there is no cleavage in the tubules. Lindane treated rats showed cleavage of seminiferous tubule with disturbed spermatogenesis with detached germ cells from the basement membrane and leydig cell and connective tissue also degenerated.

The primary function of the leydig cell is the production of testosterone. As this leydig cell is degenerated, the testosterone level is decreased in lindane treated rats (Fig. 2). Seminiferous tubules cleavage may be due to a direct action of lindane on the seminiferous tubules or an indirect effect through inhibition of testosterone production. The reduction in the tubular diameter has been correlated with decreased spermatogenic activity of the testis (Hikim *et al.*, 1989).

### Conclusion

From the present study, male hormones are decreased in pesticide induced rat than normal because pesticide has the effect to affect the androgen receptor by having nature to androgen and also the pesticide used has anti-androgensterone activity. Antioxidant also showed decreased value because pesticide results in excessive production of superoxide radicals and Ros.

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