



PROTECTIVE ROLE OF *MORINGA OLIFERA* SEED ON LINDANE INDUCED REPRODUCTIVE HORMONAL DISORDERS IN MALE ALBINO RATS

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ABSTRACT

The present study summarizes studies on pesticide and associated mechanism leading to adverse effects on male reproductive hormones. Lindane cause reproductive toxicity in testis, which decreased the levels of hormones such as testosterone, FSH, LH, estradiol and also related biochemical parameters such as lipid profile and protein. The protective effect of *Moringa olifera* seed against lindane induced male hormonal disorder in experimental albino rats has been observed. Albino rats treated with lindane along with *Moringa olifera* seed powder (100 mg/100 g b.wt) showed alteration in reproductive hormones, biological parameter and also histopathological changes were observed. The mechanism of therapeutic role of *moringa oliferae* in lindane induced male hormonal disorder will be discussed.

KEY WORDS: *Moringa Oliferae*, Lindane, Toxicity, Reproductive Hormones.



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INTRODUCTION

It is believed that environmental contaminants may cause infertility. Exposures to chemicals such as phthalates, polychlorinated phenyls, dioxin and pesticide are the risk factors¹. Detection of environmental contaminants in human tissues, along with human infertility rates are increasing and environmental toxicants are potentially important for reproduction problems by causing hormonal imbalance². Lindane is a suspected reproductive toxicant and its exposure has negative effect in the human reproductive system. Lindane is used as an insecticide. The severity and nature of the adverse effect is variable and it can be influenced by factors such as sex, level of exposure and individual sensitivity to lindane. Effects of lindane on the male reproductive system can cause changed in altered sexual behavior, problem in fertility. It has adverse effects on various hormone dependent reactions in the male reproductive system³. The testes is highly sensitive organs for lindane, because it changes the testicular morphology⁴. It causes alterations in Leydig and Sertoli cells so its functions are altered⁵. Investigations have revealed⁶, that exogenous lindane treatment diminishes the serum testosterone level, and it has been confirmed that lindane can inhibit testicular steroidogenesis by reducing serum testosterone concentrations. Some causes of male infertility are treatable by surgery some other treatments are taken antibiotics if it is an infection, surgical correction, if it has repaired in duct obstruction. Antibiotic treatment may cure an infection of the reproductive tract, but can't always restore fertility. Hormone replacement can be done in which infertility is caused by high or low levels of certain hormones or problems with the way the body uses hormones⁷. In men, both clomiphene and hMG can cause temporary blurred vision, breast enlargement and tenderness, and weight gain and also cause liver damage. In men, gonadotropin may cause fluid retention, breast enlargement, acne, weight gain. Evidence of the use of herbal remedies to treat problems with fertility dates all the way back to 200 A.D. Herbal remedies can be taken in several different forms, including capsules, tablets, teas and powdered infusions. Moringa is called the Miracle Tree because all parts like Moringa leaves, pods, flowers, fruits, roots, bark, and Moringa seeds can be utilized with many medicinal benefits. 300 diseases can be cured by taking this supplement along with hundreds of other health benefits because it has more than 90 nutrients, 46 different antioxidants, and all 8 essential amino acids⁸. Moringa leaves have the potential to raise the sperm count and its mobility, and also increase the opportunity for the sperm to fertilize the egg. Testosterone is the most important male sexual hormone. In males, it is mainly responsible for the development of the sex organs, the formation and maintenance of the typical male sexual characteristics, sperm production, the controlling of the male desire. Moringa provide body with sufficient amount of Vitamin D which has positive effects on testosterone levels⁹. Moringa seeds are rich in minerals, antioxidants, high protein density and vitamins and good cholesterol¹⁰.

2. MATERIALS AND METHODS

2.1 Preparation of drug

Moringa seed was collected from Thanjavur district. Mature pods are selected from that and shadow dried, powdered and stored in air tight container.

2.2 Animals

Male wistar rats [150 grams/kg] (10-12 weeks of age) were obtained from venkateshwara breeders, Bangalore. The rats were maintained under a well regulated light and dark (12h-12h) schedule and were allowed to free access to laboratory chow and tap water. The rats were divided into three groups. The first group [five rats] served as a control which received only feed and water, second group [five rats] is treated with lindane (5 mg/kg) and the third group [five rats] is treated with lindane along with moringa seed powder (100mg). After 30 days of treatment animals were sacrificed and blood plasma and testis were collected for the experiment. Proper ethical guidelines were followed in the present study (Approval No. 791/03/B/ CPCSEA).

2.3 Blood plasma analysis

The plasma was analyzed for hormones such as testosterone, LH, FSH, and estradiol was determined by enzyme linked immunosorbant assay¹¹ [Alford F. P.]. Protein by¹²[Lowry *et al.*], Triglycerides by¹³ [Werner *et al.*], Cholesterol¹⁴ [Alien *et al.*], Phospholipids by¹⁵ [Zilvermit *et al.*] and lipid profile by¹⁶ [Fring's *et al.*] was determined from plasma.

2.4 Tissue parameter

One testis from each rat was assayed for cholesterol, protein, and lipid profile.

2.5 Statistical Analysis

Values were expressed as mean \pm SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons. The results were statistically analyzed by Graphpad Instat Software version 3 was used and $p < 0.05$ was considered to be significant.

2.6 Histopathology analysis

Testis in each rat was preserved in 5% formaldehyde passed through xylene and embedded in paraffin wax. The tissue were sectioned at the thickness of 5 μ m and stained with haematoxylin and eosin. The spermatogenesis was observed in testis at 100X.

RESULTS

Testis weight were observed in three groups, normal rat kept as control showed normal weight and decreased testicular weight observed in lindane treated rat, in Moringa treated rats the testis weight seems to be elevated than lindane induced rat. Testosterone is the principle androgen secreted by the Leydig cells that is

decreased in lindane induced rats and it seems to be increased in Moringa treated rats. Along with that hormone FSH, LH and estradiol were decreased in lindane treated rat and in drug treated groups show increased male hormone level i.e. equal to normal rats. Biochemical parameters are directly involved in the mechanism of male hormonal disorders. Biochemical estimation was done for three groups - protein concentration, Triglyceride, total cholesterol, HDL and LDL. First group served as control shows normal value, Lindane treated show decreased level, where as drug treated seems to be elevated/increased. Impaired spermatogenesis is a result of male hormonal disorder, In histological studies normal rat shows the normal seminiferous tubules with normal cells but in lindane treated there is change in seminiferous tubules arrangement and cell damage also seen where as in Moringa treated rat testis recovery is seen in seminiferous tubules and cells in testis.

DISCUSSION

The reduction in serum testosterone level could also be due to the diminished responsiveness of Leydig cell to LH and the direct inhibition of testicular steroidogenesis. It is well established that LH is the prime regulator of testosterone production by the Leydig cells. The present result indicates that lindane induce changes in lipid profile may lead to the alteration in the Leydig cellular structure and function. Lindane posses lipophilic character resulting in bioaccumulation in body tissue. The phyto chemical compound saponin present in moringa seed has the ability to increase the male hormone level. It has been reported that steroid and saponin constituents found in many plants possess fertility potentiating properties, and they are useful in the treatment of impotence.¹⁷ The saponins may boost the level of testosterone in the body as well as trigger libido enhancing effect observed in this study¹⁸. Exposure to Lindane by dermal exposure resulted in a significant

accumulation of its isomers in the testes of treated rat. According to that lindane readily penetrates the blood testes barrier¹⁹, directly affecting spermatogenesis. The accumulation of lindane and its isomers in target sites (i.e.) testis, may possibly be responsible for various biochemical alterations, resulting in reduced spermatogenesis, leading to decrease in hormones, and an increase in morphological abnormalities. The biochemical and histological changes has been reported after treatment with Lindane²⁰. Semiferous tubules and Leydig cells is degenerated during treatment with lindane doses at 5 mg kg⁻¹ daily over a period of 30 days²¹. The atrophy of seminiferous tubules carrying necrosis in spermatogenic cells was observed after lindane-treatment²². The presence of flavonoids in the *M. oleifera* extracts has a role in altering androgen levels²³. The alkaloids can also cause facilitation of sexual behavior and has an effect on sexual behavior²⁴. The improvement in male sex hormone and biochemical parameter discussed in the current study might be due to the presence of such compounds in *M. oleifera* seed extracts²⁵. Testicular tissue was analyzed morphologically by light microscopy (Fig. 1). In the lindane-treated rats, the cells were irregularly shaped and there was marked intercellular space between the spermatogenic cells. Spermatogenesis was still present, but cell disorganization was found. Semiferous tubules are cleaved, Sertoli cells are affected as a result impaired spermatogenesis. Junction between the tubules has been affected (Fig. 2). The observed decreased value in the level of biochemical parameter in rat testis is due to lindane administration²⁶. Have reported accumulation of testicular lipid components along with fatty degeneration in testicular tissue of rat in response to g-HCH treatment for 45 days. In *moringa seed* treated rat the histopathology of rat tissue show recovered seminiferous tubule with normal spermatogenesis with sertoli cell, leydig cell and normal intertubular space because it is rich in phytochemicals like saponin and flavonoids which regulate normal spermatogenesis (Fig. 3).

Table 1
Changes in the reproductive hormones in male albino rat after lindane treatment (Mean ± SE)

S.NO	HARMONES	CONTROL	LINDANE TREATED	MORINGA TREATED
1	Testosterone(ng/ml)	2.920±0.08	1.820±0.07	2.520±0.02
2	FSH(ng/ml)	3.080±0.08	2.880±0.06	3.340±0.02
3	LH(ng/ml)	4.420±0.08	3.680±0.13	4.020±0.08
4	Estradiol(pg/ml)	8.05±0.86	5.050±0.07	7.920±0.13

Table 2
Changes in the biochemical profile in male albino rat testis after lindane treatment (mean ± SE)

S.NO	PARAMETERS	CONTROL	LINDANE TREATED	MORINGA TREATED
1	Protein(mg/g)	126.45±0.42	106.25±0.283	120.33± 0.0796
2	Total Cholestrol(mg/g)	15.898±0.07535	14.792±0.03394	14.92±0.1825
3	Triglycerides(mg/g)	77.364±1.2064	78.076±0.6739	76.48±0.399
4	Phospholipid(mg/g)	9.346±0.06528	5.892±0.2000	7.345±0.028
5	Total lipid(mg/g)	165.38±0.4391	141.85±0.4327	146.36±0.2091

Table 3
Changes in the biochemical profile in male albino rat serum after lindane treatment (mean ± SE)

S.NO	PARAMETERS	CONTROL	LINDANE TREATED	MORINGA TREATED
1	Protein(mg/l)	8.322±0.2065	5.734±0.03631	6.220 ± 0.0796
2	Total Cholestrol(mg/ dl)	144.27±0.1826	112.52±0.03401	130.68±0.03826
3	Triglycerides(mg/ dl)	81.144±0.4992	63.250±1.297	72.610±0.59
5	HDL(mg/dl)	32.800±0.9702	19.200±0.8374	26.000±0.3742
6	VLDL(mg/ dl)	9.440±0.4574	4.644±0.1015	7.766±0.04785
7	LDL(mg/dl)	16.600±0.7439	11.840±0.4868	14.664±0.2168

HDL: High density lipoprotein.VLDL: very low density lipoprotein. LDL: low density lipoprotein.
Mean values within the row followed by different letters (Superscript) are significantly (P < 0.05) level different from each other and same letter are non significant were comparison by Duncan's multiple range test (DMRT).

Figure 1
Histopathology of normal albino rat testis.

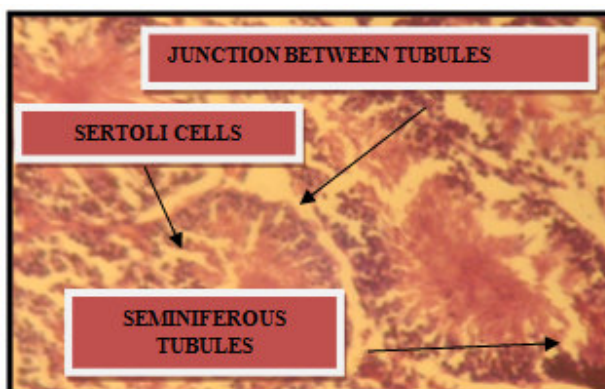


Figure 2
Histopathology changes in albino rat testis after lindane treatment. Cleaved due to lindane treatment.

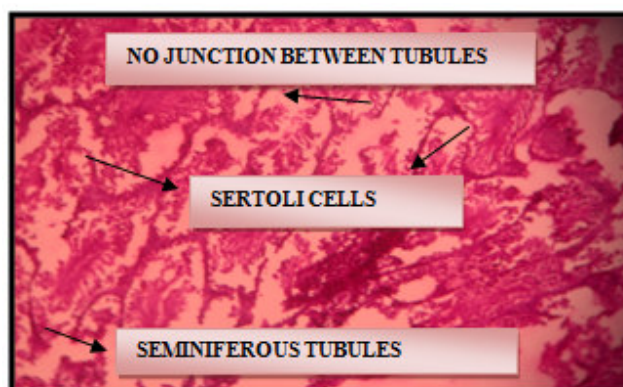
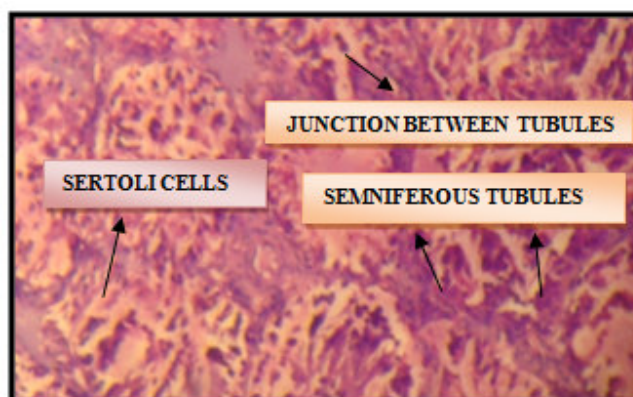


Figure 3
Histopathology changes in albino rat testis after lindane along with moringa treated. Recovered due to moringa seed administration



Sertoli cells (SC), Junction between tubules (T), Seminiferous tubules (ST),

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