

Protective Activity of *Moringa Oleifera* Leaf Extract on Lindane Induced Testicular Damage in Rats

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Abstract: Exposure of the body organs and systems to pesticides including lindane has found been to cause adverse effects, which affect functionality. Hence, this study was aimed at determining the ability, potency and nature of *Moringa oleifera* leaf in the protecting testis against lindane induced testicular damage based on some biochemical and histopathological parameters. Adult male rats were orally administered with lindane at a dose of 5.0mg/kg body weight per day for 30 days, the rat were killed using anesthetic ether, plasma was collected and testis were removed histopathological studies. Male albino rats were treated with lindane, decreased the fertility hormone such as testosterone, LH, FSH and estradiol. Lindane alter fertility hormone and altered the biochemical parameter such as protein, cholesterol, lipid profile and histopathological disorganization. Administration of *Moringa oleifera* to lindane induced tooxiicty rats restored the altered parameters. Results showed that exposure to lindane testicular and concurrent administration of *Moringa oleifera* leaf extract possess the protective activity of lindane induced testicular toxicity in rats.

Keywords: Lindane, Rat, Testis, Fertility hormones, *Moringa oleifera* leaf

1. INTRODUCTION

Infertility is defined as the inability of a couple to conceive after 12 months of unprotected regular sexual intercourse and it is estimated to affect 10%–15% of all couples. In almost half of such cases, a male factor is involved, but 15%–24% have unexplained etiology [1].

Many chemicals, such as pesticides, industrial chemicals, plastics, plasticizers, pharmaceuticals and others present in the environment, have been shown to cause disruptive endocrine effects, yet currently, for many of them, there is no known structure/function relationship [2]. Like other persistent organic pollutants, lindane can enter the food chain and lipophylicity facilitates its accumulation in the various tissues of living organisms where, after absorption and distribution, it can easily reach the essential tissues of the reproductive system [3,4,5].

Lindane has been reported to cause impairment to many biological functions, including reproduction in humans and animals. It has adverse effects on various hormone dependent reactions in the male reproductive system. The testes have been found to be highly sensitive target organs for lindane, which has been shown to disrupt testicular morphology [6,7,4,8] and induce epididymal cellular degeneration [7]. It causes alterations in Leydig and Sertoli cells and impairs their functions [4,9,10]. Investigations have revealed [7] that exogenous lindane treatment diminishes serum testosterone level, and it has been confirmed that lindane acts as an inhibitor on testicular steroidogenesis [11,12,10].

Moringa oleifera is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan where it is used in folk medicine [11] it is now widely distributed all over the world. *oleifera* is referred to as a “miracle tree” or a “wonder tree” [13] of significant socio economic

importance because of its several nutritional, pharmacological [14,13] and industrial applications [15,16]. The leaves of this plant contain a profile of important trace elements, and are a good source of proteins, vitamins, beta-carotene, amino acids and various phenolics [17]. Hence, this study was aimed at determining the ability, potency and nature of *Moringa oleifera* leaf in the protecting testis against lindane induced testicular damage based on some biochemical and histopathological parameters.

2. MATERIAL AND METHODS

2.1. Animals

Male Wistar rat (10-12 weeks of age) were obtained 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.

2.2. Collection and Identification of Plant

The *Moringa oleifera* leaves were collected from Thanjavur, Tamil Nadu, India. The collected leaves were identified and authenticated by a Botanist Dr. M. Jegadeesan, Prof. and Head, Department of Environmental and Herbal Sciences, Tamil University, Thanjavur, Tamil Nadu. A Voucher specimen (TU: 294) has been deposited at Tamil University Herbarium. The leaves were cut into small pieces and shade dried and powdered finely then used for extraction.

2.3. Preparation of Plant Extract

The collected plant materials were washed, sliced and completely dried in a hot-air oven at 37°C. The dried materials was ground into make a fine powder and used for extraction. Three hundred grams (300g) of the powdered plants were extracted with methanol using "Soxhlet Apparatus" for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used

2.4. Experimental Design

The rats are divided into three groups, 1st group served as a control which received only olive oil and 2nd group is treated with lindane at a dose of 5mg/kg. Group 3rd *Moringa oleifera* leaf treated to lindane intoxicated rats. After 30 days of treatment animals were sacrificed, blood was collected and plasma was separated and reproductive organs were weighed.

2.5. Biochemical Analysis

Plasma was analyzed for hormones such as testosterone, LH, FSH and estradiol [18]. Triglycerides by Werner *et al.*, [19] Cholesterol Allain *et al.*, [20] phospholipids by Zilversmit *et al.* [21] LDL and VLDL cholesterol were calculated as per Friedewald's [22] equation. Protein was estimated by the method of Lowry *et al* [23].

2.6. Tissue Homogenate

Immediately after blood collecting, the animals were sacrificed by cervical dislocation and the testis were dissected out, washed with ice-cold physiological saline. The required amount was weighed and homogenized using a Teflon homogenizer. Tissue homogenate was prepared in 0.1 M Tris Hcl buffer (pH 7.4) and used for the estimation of various biochemical parameters.

2.7. Histopathological Studies

Testis of each rat was fixed in Bouins fluid passed through xylene and embedded in paraffin wax tissues were sectioned at the thickness of 5 µm and stained with haematoxyline and eosin. Spermatogenesis was observed in 100x.

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.

3. RESULTS AND DISCUSSION

Like other organochlorine pesticides, lindane persists in environment and bioaccumulates in human tissues [24]. Lindane dosage was selected from the previous report by Etim *et al* [25]. Lindane induces oxidative stress in blood and tissue of rats by decreasing the activities of antioxidant enzymes and increasing free radical generation [25]. It was reported that lindane accumulates in the adipose tissue. Adipose tissues are found surrounding the visceral organs which includes liver, muscle and heart. In recent years, the positive effects of flavonoids on human health have attracted more attention. Hence the protective effect of *Moringa oleifera* leaf extract against lindane induced alterations in hormones and lipid profile has been taken up for the present investigation.

Table1. Change in the reproductive hormones in Male albino rat after Indiana and *Moringa oleifera* treatment

S.No.	Parameters	Control	Lindane Treated	<i>Moringa oleifera</i> treated
1.	Testosterone	2.920 ± 0.08	1.820 ± 0.07*	2.880± 0.03
2.	FSH (ng/ml)	3.080 ± 0.08	2.880 ± 0.06*	3.340 ± 0.05
3.	LH (ng/ml)	4.420 ± 0.08	3.680 ± 0.13*	4.020 ± 0.08
4.	Estradiol (pg/ml)	8.050 ± 0.08	5.050 ± 0.07*	7.920 ± 0.86

Values are expressed as Mean ± SD for six rats

*Significantly different from control and *Moringa oleifera* treated rats. $p < 0.05$

Table2. Changes in the lipid profile in Male albino rat testis after Indiana and *Moringa oleifera* treatment

S.No.	Parameters	Control	Lindane Treated	<i>Moringa oleifera</i> treated
1.	Protein (mg/gm)	126.454±0.19	106.25± 0.12*	113.79± 0.16
2.	Cholesterol (mg/ gm)	15.898± 0.02	14.792± 0.14*	14.792± 0.03
3.	Triglycerides (mg/ gm)	77.364± 0.53	78.076± 0.30*	77.456± 0.39
4.	Phospholipids (mg/ gm)	9.346± 0.09	5.892± 0.29*	8.345± 0.02
5	Total Lipids (mg/ gm)	165.38± 0.20	141.85± 0.19*	150.78± 0.20

Values are expressed as Mean ± SD for six rats

*Significantly different from control and *Moringa oleifera* treated rats. $p < 0.05$

Table3. Changes in the biochemical profile in Male albino rat serum after Indiana and *Moringa oleifera* treatment

S.No.	Parameters	Control	Lindane Treated	<i>Moringa oleifera</i> treated
1.	Protein (g/dl)	8.32± 0.09	5.73± 0.01*	7.24± 0.03
2.	Cholesterol (mg/dl)	144.27± 0.03	112.52± 0.01*	128.65± 0.014
3.	Triglycerides(mg/dl)	81.144± 0.49	63.25± 0.51*	73.49± 0.39
4.	Phospholipids (mg/dl)	9.34± 0.09	5.59± 0.29*	8.20± 0.09
5.	HDL (mg/dl)	32.80± 0.47	19.20± 0.37*	24.80±0.37
6.	VLDL (mg/dl)	9.44± 0.31	4.64± 0.20*	6.79±0.04
7.	LDL (mgd/l)	16.60± 0.33	11.84± 0.21*	15.56± 0.21

Values are expressed as Mean ± SD for six rats

*Significantly different from control and *Moringa oleifera* treated rats. $p < 0.05$

Both *in vitro* and *in vivo* lindane exposure interfere with androgen metabolism and with the formation of a 5 α -dihydrotestosterone receptor complex in the prostate of rats [26]as the consequences of exposure to lindane .Furthermore, as an endocrine disrupting chemical, it may interfere with male reproductive performance and fertility. Reports suggest [27]. That lindane readily penetrate the blood testis barrier, directly affecting spermatogenesis. The accumulation of lindane and its isomers in target sites may possibly be responsible for various biochemical alterations, resulting in reduced spermatogenesis, leading to decrease in hormones, and an

increase in morphological abnormalities [28]. In the present study, we also observe the decreased levels of testosterone, estradiol, LH and FSH (Table 1) on lindane treated rats. Administration of *Moringa oleifera* leaf extract to lindane treated rats restored the altered levels of hormones. The results of the present study agreement with the previous study where *Moringa oleifera* treated rats restored the hormonal status and improve the male sexual function [29, 30].

Testis and serum total cholesterol and triglyceride levels were significantly increased ($P < 0.05$) in lindane administered rats compared to that of control animals. In rats co-treated with *Moringa oleifera* leaf along with lindane the serum cholesterol and triglyceride levels followed by a significant decrease in the phospholipid level was significantly reduced ($P < 0.05$) compared to rats treated with lindane alone (Table 2 and 3). Serum HDL and protein level was decreased significantly ($P < 0.05$) and that of LDL, VLDL levels were found to be increased significantly ($P < 0.05$) in lindane treated rats compared to control animals. In rats which received *Moringa oleifera* leaf co-treatment along with lindane, the HDL fraction was increased significantly ($P < 0.05$) with significant reduction in LDL, VLDL fraction compared to animals treated with lindane alone

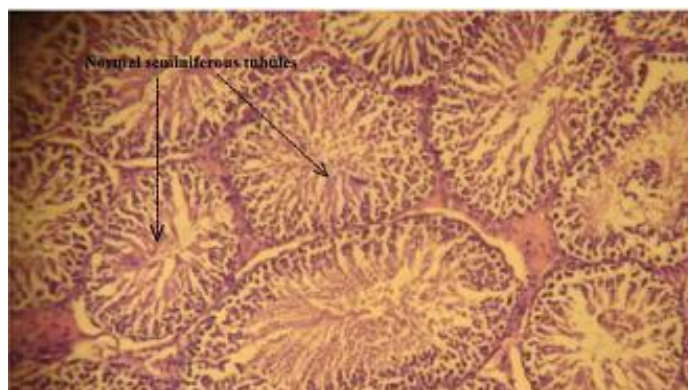


Fig 1A shows histological observation of normal rat testis

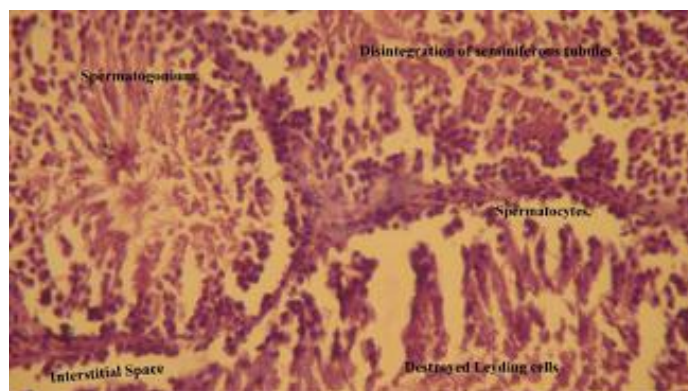


Fig 1B shows histological observation of lindane treated rat testis

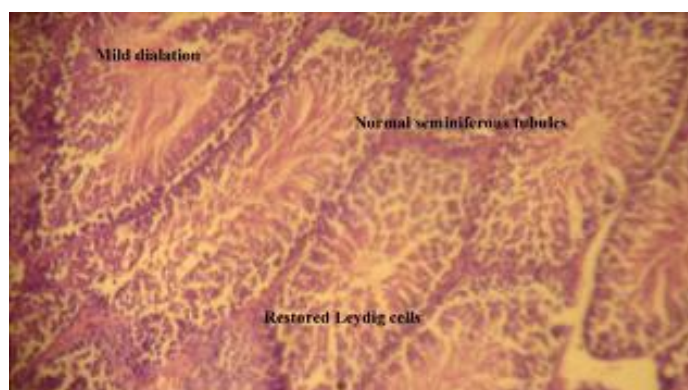


Fig 1C. Shows histological observation of lindane and moringa oleifera leaf treated rat testis

A significant increase in the cholesterol, triglyceride LDL and VLDL values were observed in the serum of lindane treated rats compared to control animals which is an indication of severe lindane induced hyperlipidemia. Lindane induced hepatotoxicity was reported earlier by Sharma *et al.*[31]. Previous studies have reported elevated serum levels of triglycerides and cholesterol in rats and mice that were on diet contaminated with lindane [32,33]. This is in accordance with the previous study where *Moringa oleifera* presented the reduction of cholesterol in the high-fat diet fed induced hyperlipidemic rats[34].

Group I show that testicular tissues stain normal, seminiferous tubules are well defined, normal and prominent (Fig 1A). Group II animal testicular tissues administered with lindane only. Leydig cells appear destroyed and the remnants quite dispersed. Seminiferous tubules appear quite ill defined as the endothelial cells appear destroyed and or dispersed. Lindane thus had deleterious effect on testicular tissues (Fig 1B). Group III animals treated with lindane along with moringa, the number and morphological integrity of Leydig cells have been restored (Fig 1C) relative to group 1. More Leydig cells are being preserved. Observation indicates that the toxic effects lindane was reduced by moringa administration. The histopathological observation of Present finding is in agreement with Priyadarshani and Varm, [35] and Prabsattroo *et al.*, [29] studies.

In conclusion, it may be mentioned that the altered hormonal and biochemical profiles due to lindane exposure is reversed towards normalization by *Moringa oleifera* leaf extract. *Moringa oleifera* leaf not only protects the integrity of plasma membrane but at the same time increase the regenerative and reparative capacity of the testis. These results suggest that the phytochemical compounds of *Moringa oleifera* leaf efficiently protects the testis from lindane toxicity by minimizing cell membrane disturbances and helps in normal functioning of testis.

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