EFFECT OF LINDANE IN STEROIDOGENESIS AND TESTIS TISSUES

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Abstract
Present study to investigate the effects of lindane in stereoidogenesis and testis tissues of adult male albino rat. 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. A histopathological analysis of testicular tissue from treated rats showed cell disorganization. Cells were irregularly shaped, with marked intercellular space between the spermatogenic cells were observed. Our results imply that organochlorine insecticides like lindane can cause reproductive disorders, and therefore more attention should be directed towards understanding the affects of persistent pesticide residues on reproductive outcomes.

Keywords: Lindane, Rat, Testis, Fertility hormones.

1. INTRODUCTION
The lindane is widely used as a pesticide in many countries [7]. Lindane (γ hexachlorocyclohexane) is an organochlorine insecticide that is still employed for various purposes, including both human and veterinary medicine and as agriculture and horticulture pesticide [1]. Lindane may be taken up cutaneously [2] or orally from contaminated food[3,4] Even though the symptoms of chlorinated hydrocarbon toxicity convulsion and other [5] are evidence that chlorinated hydrocarbons exert physiological effects by their interaction with components of the nervous system, the mechanism of action of the insecticide lindane is still poorly understood. Lindane has been reported to induce reproductive abnormalities in male rat and induction of stress to play a important role in the toxicity. Lindane and other pesticide are released into the environment intentionally and exposure to such pesticide interacts with the mammalian endocrine system and may cause adverse effect on reproductive function in mammals and human [6].

Lindane is a lipophilic compound that can be accommodated in lipid bilayers [19] and this accommodation could induce a variation in membrane fluidity by modifying the cholesterol content. Biological events such as transformation, growth, cell cycle and differention [20] as well as modification in the accessibility to some hormone receptor [21] or the activities of certain enzymes [22, 23] have been shown to be accompanied by alteration in membrane fluidity.

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 [26,27,28,29]and induce epididymal cellular degeneration [30]. It causes alterations in Leydig and Sertoli cells and impairs their functions [31,32]. Investigations have revealed that exogenous lindane treatment diminishes serum testosterone level, and it has been confirmed that lindane acts as an inhibitor on testicular stereoidogenesis [33, 34]. This study was conducted to examine the effects of lindane on male reproductive parameters related to hormones and lipid profile of testis and plasma.

2. MATERIAL AND METHOD
2.1 Animals
Male wistar rat (10-12 weeks of age) were obtained Venkateshswara 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 Experimental design

The rats are divided into two 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. After 30 days of treatment animals were sacrificed, blood was collected and plasma was separated and reproductive organs were weighed.

2.3 Biochemical analysis

Plasma was analyzed for hormones such as testosterone, LH, FSH and estradiol [39]. Plasma was used to analyze protein by Lowry et al., [40], Triglycerides by Werner et al., [41] Cholesterol Alien et al.[42], phospholipids by Zilversmit et al.[43] and lipid profile. One testis from each rat was kept at -20c until assayed for cholesterol, protein and lipid profile.

2.4 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 100 x.

2.5 Statistical Analysis:

Values were expressed as mean ± 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

3.1 Abnormal spermatogenesis due to Lindane:

Seminiferous tubules are cleaved, sertoli cells are affected as a result spermatogenesis is affected. Junction between the tubules is affected [29, 30, 31].

3.2 Histopathology of albino rat testis

Sertoli cells are normal Junction between tubules are normal Seminiferous tubules are normal (Fig 1). Sertoli cells are affected due to lindane effect in testis. There is no junction between tubules seminiferous tubules are cleaved due to toxicity of lindane (Fig 2).

Fig 1 shows the normal architecture of testis

Fig 2 shows the Lindane exposed of testis

Treatment of lindane at 5mg/kg for 30days cause a hormonal disorder by decreased value of testosterone, estradiol, LH and FSH (Table 1). Blood variable such as protein and cholesterol level was decreased (Table 2 and 3). The decrease value due to lindane [44, 45, 46] in total lipid can be attributed to the reduction in cholesterol, phospholipids and neutral lipids are the major lipid classes in the leydig cells [47]. Further the decreased level of total lipid, total cholesterol, triglycerides and phospholipids in the present study may be due to the lipid peroxidative damage caused by lindane.

On the 30th day after the beginning of lindane exposure, the rats were destroyed and the tissues of the anterior pituitary, ventral prostate. Reprotoxicity of lindane testes and epididymides were immediately removed. Changes in the relative weights of the tests and epididymides were not recorded. However, significantly lower relative weights of the anterior pituitaries vs controls were found in the group treated with the higher dose of lindane (P<0.05). In both groups tested, a significant (P<0.01) decrease in the relative mass of the ventral prostate was also found.

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 [24]. 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 [25].

It is generally accepted that testosterone is converted into 5α-reduced metabolites, which interact with their specific receptors to become fully active [35, 36]. Both in vitro and in vivo lindane exposure interfere with androgen metabolism and with the formation of a 5α-dihydrotestosterone receptor complex in the prostate of rats [37, 38] as the consequences of exposure to lindane. Furthermore, as an endocrine disrupting chemical, it may interfere with male reproductive performance and fertility.

The reduction in serum testosterone (Table 1) level could also be due to the diminished responsiveness of
Table 1 Effect of lindane on hormones of experimental rats

<table>
<thead>
<tr>
<th>S.No</th>
<th>Hormones</th>
<th>Control</th>
<th>Lindane Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Testosterone (ng/ml)</td>
<td>2.92±0.08</td>
<td>1.82±0.07*</td>
</tr>
<tr>
<td>2</td>
<td>FSH (ng/ml)</td>
<td>3.08±0.08</td>
<td>2.88±0.06*</td>
</tr>
<tr>
<td>3</td>
<td>LH (ng/ml)</td>
<td>4.42±0.08</td>
<td>3.68±0.13*</td>
</tr>
<tr>
<td>4</td>
<td>Estradiol (pg/ml)</td>
<td>8.05±0.86</td>
<td>5.05±0.078*</td>
</tr>
</tbody>
</table>

*Significantly different from control (p<0.05)

Values were expressed as Mean± SD for six rats

Table 2 Effect of lindane on testis parameters of experimental rats

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Control</th>
<th>Lindane Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protein (mg/g)</td>
<td>126.45±0.42</td>
<td>106.25±0.28*</td>
</tr>
<tr>
<td>2</td>
<td>Cholesterol (mg/g)</td>
<td>15.89±0.075</td>
<td>14.79±0.33*</td>
</tr>
<tr>
<td>3</td>
<td>Triglycerides (mg/g)</td>
<td>77.36±1.20</td>
<td>78.07±0.67*</td>
</tr>
<tr>
<td>4</td>
<td>Phospholipid (mg/g)</td>
<td>9.34±0.65</td>
<td>5.89±0.20*</td>
</tr>
<tr>
<td>5</td>
<td>Total lipid (mg/g)</td>
<td>165.38±0.43</td>
<td>141.85±0.43*</td>
</tr>
</tbody>
</table>

*Significantly different from control (p<0.05)

Values were expressed as Mean± SD for six rats

Table 3 Effect of lindane on biochemical parameters of experimental rats

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Control</th>
<th>Lindane Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protein (mg/L)</td>
<td>8.32±0.20</td>
<td>5.73±0.03*</td>
</tr>
<tr>
<td>2</td>
<td>Cholesterol (mg/dl)</td>
<td>144.27±0.18</td>
<td>112.52±0.03*</td>
</tr>
<tr>
<td>3</td>
<td>Triglycerides (mg/dl)</td>
<td>81.14±0.91</td>
<td>63.25±1.29*</td>
</tr>
<tr>
<td>4</td>
<td>HDL (mg/dl)</td>
<td>32.80±0.97</td>
<td>19.20±0.83*</td>
</tr>
<tr>
<td>5</td>
<td>VLDL(mg/dl)</td>
<td>9.44±0.45</td>
<td>4.64±0.10*</td>
</tr>
<tr>
<td>6</td>
<td>LDL (mg/dl)</td>
<td>16.60±0.74</td>
<td>11.84±0.48*</td>
</tr>
</tbody>
</table>

*Significantly different from control (p<0.05)

Values were expressed as Mean± SD for six rats

Table 2 Effect of lindane on testis parameters of experimental rats

lindane to LH and the direct inhibition of testicular steroidogenesis [48]. It is well established that LH is the prime regulator of testosterone production by the Leydig cells. The present result indicate that lindane induce change in lipid profile (Table 2 and 3) may lead to the alteration in Leydig cellular structure and function. Lindane posses lipophilic character resulting in bioaccumulation in body tissue. Exposure to technical grade hexachlorocyclohexane by dermal exposure, resulted in a significant accumulation of its isomers, including lindane, in the testes and E sperm of treated rats [49]. In rats dosed orally either with 6 mg kg-1 body mass for 5 days, or with a single dose of 30 mg kg-1 body mass, lindane was still detected in the testes 2 weeks after treatment [29]. Reports suggest [50] 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 (Table 1), and an increase in morphological abnormalities [49]. Lindane has been reported to intercalate into the sperm membrane and alter the molecular dynamics of the bilayer [51]. A significant association between hexachloro-cyclohexane isomers and some DDT metabolites in the semen of infertile humans with semen quality problems has been established. The biochemical and histological effects in the testes of rats following treatment with lindane have been reported [52, 53]. Seminiferous tubules and Leydig cells degenerated during treatment with doses of 5 mg kg-1 daily, over a 30-day period [54]. The atrophy of somniferous tubules carrying necrosis spermatogenic cells was observed after lindane-treatment. 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.

According to the present results and previously published reports [49, 29] much more attention should be paid to the possible effects of environmentally persistent pesticides, even if they are banned in most developed countries, in view of the fact that they may induce changes at the cellular level to crucial stages in the reproductive processes.

4. Acknowledgement
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References

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