



RESEARCH ARTICLE

PATHAOLGY

REPRODUCTIVE TOXICITY OF ENDOSULFAN ON MALE RATS

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ABSTRACT

Endosulfan is an organochlorine insecticide extensively used to control pests of vegetables and fruits and is well absorbed by ingestion, inhalation and skin contact. Its toxicity, depends upon route of administration, vehicle and species and sex of animal. The present study was designed to evaluate the reproductive toxicity of sub chronic dose of endosulfan on male rats. Adult male rats were exposed to 0, 1.5, 3 and 6mg endosulfan /kg body weight through oral intubation for 45 days. Decreased testicular wet weight, reduced sperm count and increased sperm abnormalities associated with histopathological alterations like edema, degenerative changes, separation of epithelium from basement membrane, were observed in all the endosulfan treated rats in a dose dependent manner.



KEYWORDS

Endosulfan, Reproductive toxicity, testicular weight, sperm abnormalities, degenerative changes.

INTRODUCTION

Endosulfan (6,7,8,9,10,10 – hexachloro - 1,5,5a,6,9,9 a – hexahydro - 6,9 – methano - 2,4,3 – benzodioxathiepin - 3 - oxide) is a pesticide belonging to the organochlorine group of pesticides, under the cyclodiene subgroup. The cyclodiene insecticides are among the most toxic and environmentally persistent insecticides known. Some of the physical and chemical properties of cyclodienes, such as low volatility, chemical stability, lipid solubility, and slow rate of biotransformation and degradation were originally seen as positive attributes for a successful pesticide. These attributes lead to bioconcentration, biomagnification in certain food chains, and persistence in the environment¹. Immunological, neurological and genotoxic effects of endosulfan in laboratory animals were well documented but reports regarding its effect on reproduction were scanty². Hence in present study we explored the dose dependent effect of endosulfan on testicular impairment in male rats.

MATERIALS AND METHODS

Seventy two male wistar rats (procured from Bros Scientific Co. Tirupati) weighing around 120 g were divided at random in to four groups of 18 animals each and were maintained under a well regulated light and dark(12h:12h) schedule at 24±3⁰ C. They were allowed free access to laboratory chow and tap water. The test group was given by oral intubation of endosulfan (95.32% purity was procured from the Hyderabad Chemicals Pvt. Ltd., Hyderabad) dissolved in groundnut oil at a dose of 0, 6, 3 and 1.5mg/kg b.wt. for 60 days. Twenty four hours after the last treatment, the rats were sacrificed by cervical dislocation. The testis were dissected free and weighed immediately and fixed in Bouins fluid for histopathological examinations.

Fixed tissues were processed by routine paraffin embedding technique. Sections of 5-6 μ thickness were cut and were stained with routine Haematoxylin and Eosin method (H & E)³.

1. Sperm count and sperm morphology:

The cauda epididymis was weighed, teased and minced into a known volume of normal saline at 37⁰ C, A drop of sperm suspension was placed on a clean slide and spread gently to make a thin film. The film was air dried and stained with 1% eosin Y and morphological changes to sperm heads and tails were assessed. The remaining sperm suspension was used for sperm counting using a Neubauer haemocytometer according to the method of Feustan⁴.

2. Statistical analysis:

The results were expressed as mean \pm S.E. All analyses were carried out using the SPSS statistical program. The effect of treatments was determined by analyzing the data using one way-ANOVA followed by Duncan's multiple comparison tests.

RESULTS

1. Testis wet weight (g): Significant (P<0.05) decrease in the wet weight of testes was noticed in groups II, III and IV when compared to the group I (control).

2. Sperm count (10⁷ /ml): Significant (P<0.05) decrease in the sperm count was observed in the endosulfan treated groups when compared to control group.

3. Sperm morphology(%): Significant (P<0.05) increase in the sperm morphological abnormalities like loose heads, bent neck (Fig. 3), bent and coiled tails were observed in the endosulfan treated groups when compared to control group

Table 1
Effect of endosulfan on testis weight, sperm count and sperm morphologies

Groups	Testis wet weight(g)	Sperm count(10^7 /ml)	Sperm abnormalities (%)
Control	2.75±0.53 ^a	20.87±2.01 ^a	4.13±0.44 ^a
Group II	2.34±0.28 ^b	11.16±0.61 ^b	16.83±1.14 ^c
Group III	2.35±0.38 ^b	12.43±1.52 ^b	13.21±0.35 ^b
Group IV	2.33±0.40 ^b	12.6±0.41 ^b	13.11±0.78 ^b

(Mean ± SE, n=6) (P<0.05)

Means bearing different superscripts (a,b,c) between the drug treatments differ significantly
Means bearing different superscripts (1, 2, 3, 4) between the days differ significantly

4. Histopathological changes in testis:

In testis congestion, interstitial edema, separation of seminiferous tubules, perivascular edema, degeneration of seminiferous tubules with desquamated cell debris within the lumen, mononuclear cells and plasma cell infiltration in the interstitial spaces, hyalinization of few seminiferous tubules and blood vessels and loss of tubular epithelium leaving only basement membranes with sertoli cells (Fig. 1 and 2) were observed.

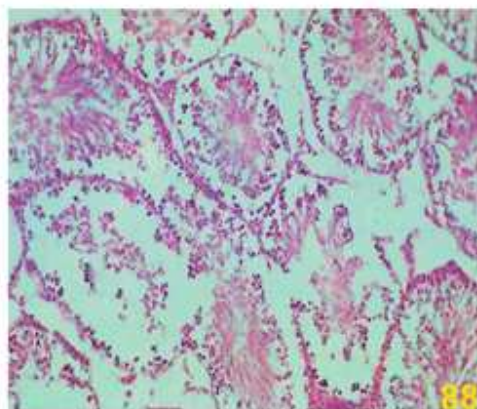


Fig 1

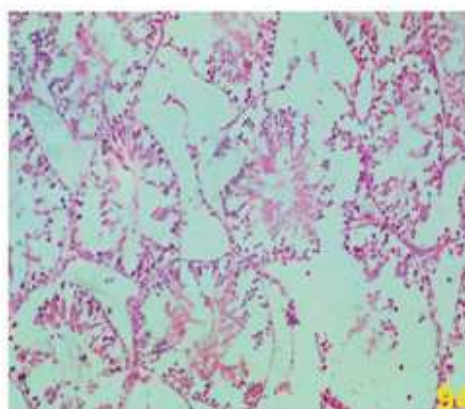


Fig 2

Fig. 1 Testis showing loss of tubular epithelium leaving only basement membrane and sertoli cells.
Fig. 2 Testis showing severe degenerative changes with loss of normal architecture.



Fig 3



Fig 4

Fig. 3 Sperm abnormalities like bent neck

Fig. 3 Sperm abnormalities like bent neck with coiled tail.

DISCUSSION

Significantly ($P < 0.05$) decreased testicular wet weights were observed in all the endosulfan treated rats when compared to the control group. The decrease in the testicular weight of testis in endosulfan treated rats may indicate impairment at testicular, pituitary or hypothalamic level. Decreased weight of the testis might be due to decreased production of the seminiferous tubular fluid as this contributes to the weight of the testis or due to reduced protein content, as the growth rate of any organ is proportional to its protein contents⁴ or might be due to loss of tubular epithelium as observed histopathologically in the present study.

Significantly ($P < 0.05$) decreased sperm count along with increased sperm morphological abnormalities were observed in all the experimental groups when compared to the control group. The decreased sperm count might be due to lowered availability of the FSH and LH which are necessary for the meiosis and development of spermatids⁵ or due to decreased intra testicular concentration of testosterone² as androgens induce meiosis, formation and development of spermatids in

response to the FSH and sperm production cannot proceed optimally without continuous supply of androgens⁶. The increased sperm abnormalities might be due to endosulfan induced abnormalities in mitotic and meiotic chromosomes of rats or due to induced alterations in testicular DNA and sperm chromatin structure⁷.

Gupta and Chandra⁸ observed microscopic changes in testis like degeneration of seminiferous tubules and interstitial edema. One-third of the tubules in sections were devoid of spermatogenic elements and were lined by a single layer of cells consisting of Sertoli cells and some spermatogonia after endosulfan treatment. Naquvi and Vaishnavi⁹ observed the degenerative changes in the seminiferous tubules, testicular atrophy of rats treated with endosulfan. These changes might be due to oxidative damage as endosulfan treatment causes decreased ascorbic acid and anti-oxidant levels in testis, as this acts as a free radical scavenging agent¹⁰. In conclusion, the present studies indicate that endosulfan causes impairment of male reproduction by reducing sperm count and increasing abnormal counts and affects the androgenicity in male rats.



CONCLUSION

The present study allows us to postulate that endosulfan impairs reproductive performance of males by reducing sperm count, increasing abnormalities of sperms in testis and causing histopathological changes like degenerative changes in the testis of male rats.

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