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TOXIC INFLUENCE OF ENDOSULFAN (AN ORGANOCHLORINE PESTICIDE) ON CAUDA EPIDIDYMIS STRUCTURE AND FUNCTION OF MALE RAT

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ABSTRACT

Endosulfan insecticide is a polychlorinated compound used for controlling a variety of insects. It is practically water-insoluble, but readily adheres to clay particles and persists in soil and water for several years. The present study sought to investigate the toxic influence of endosulfan on epididymal structure and function of male rat. Wistar male rats were administered endosulfan at dose level of 10 mg/kg/b.wt per day for 30 and 60 days. Cauda epididymis were subjected for sperm dynamics, biochemical and histopathological analysis. A significant decrease in the weight, sperm motility and sperm density in epididymis was observed. Statistically increase in protein content and decrease in sialic acid, glycogen content was observed at various dose levels of endosulfan treated rats. The activities of epidiymal marker enzymes i.e. phosphodiesterase and adenylate cyclase was decreased. Serum testosterone, Leutinizing Hormone (LH), and Follicle Stimulating Hormone (FSH) were also found to be decreased. Histoarchitecture of cauda epididymis showed various degenerative changes. From the above mentioned findings it has been concluded that the epididymal function and structure was seriously affected by toxic effect of endosulfan.

KEYWORDS: Endosulfan, sperm motility and density, Testosterone.

INTRODUCTION

Sperm are man's immediate and personal connection to the future of our species and the disappearance of half of this connection is hard to ignore. Sperm content in healthy men around the world have fallen about fifty percent in the last fifty years and exposure to pesticide is an important cause of this decline.[1] Toxicology of the epididymis has received

less attention than other **re**gions of the male reproductive system. A search of the literature shows that the testis has been the major emphasis in toxicology while the number of manuscripts focused on toxicity associated with the epididymis is fewer. Endosulfan is a pesticide belonging to the organochlorine group of pesticides, under the Cyclodiene subgroup. It was introduced in the 1950's and it emerged as a leading chemical used against a broad spectrum of insects and mites in agriculture and allied sectors. It is used in vegetables, fruits, paddy, cotton, cashew, tea, coffee, tobacco and timber crops. It is also used as a wood preservative and to control tse-tse flies and termites This pesticide is classified as a Highly Hazardous chemical by the US Environmental Protection Agency (EPA) United Nations Environmental Programme (UNEP), World Health Organization (WHO), Industrial Toxicological Research Centre (ITRC) in India.[2,3] However, India is the largest producer, consumer and exporter of Endosulfan. In rats and mice studies suggests its teratogenic and carcinogenic properties.[4] It directly affects the central nervous system, causes liver and kidney (chronic glumerulonephrosis) damage.[5] It also impairs the reproductory system.[6,7] Behavioural and neurological changes have also been noticed.[8] In humans also Congenital birth defects, reproductive health problems, Cancers, loss of immunity, neurological and mental diseases were reported.[9,10,11,12] The present study was therefore aimed to find out the toxic effect of endosulfan on epididymal function and structure of male rat.

MATERIALS AND METHODS

Animal Model

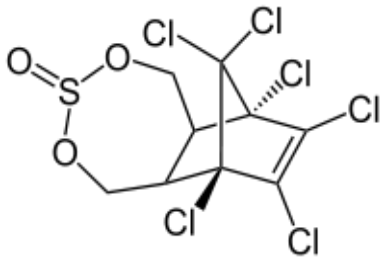
Healthy and fertile albino rats (Wistar) weighing 150-200 gm were used for experimentation. The animals were housed in polypropyline cages, measuring 430 x 270 x 150 mm. The animals were fed on pelleted standard rat chow supplemented with soaked grams and wheat. Water was provided *ad-libitum*. They were acclimatized for 7 days to the laboratory conditions at 22-240 C with provision 12 h light: 12h dark cycle. The "guidelines for the care and use of animals for scientific research" was strictly followed.[13]

TEST MATERIAL

Chemical Name: (6,7,8,9,10,10- hexachloro- 1,5,5a,6,9,9a- hexahydro – 6,9- methano- 2,4,3- benzodioxathiepine-3-oxide)

Chemical Formula: C9H6Cl6O3S

Chemical Structure: Endosulfan



Technical endosulfan (α and β isomers in the ratio of 70:30) obtained from Hoechest, Mumbai (India) was used for the experimentation. It is brownish crystals with slight odour of sulphur dioxide and endosulfan sulfate.

Median lethal dose (LD50) of Endosulfan

The LD50 is statistically derived single dose of a substance that can be expected to cause death in 50% of the animals. In this investigation various calculated doses (mg/kg.b.wt.) of insecticide was given orally. Ten animals were tested for each dose level. Poisoning symptoms and mortality was observed daily for three days following the treatment. Results of the toxicity were analyzed statistically for the determination of LD50 of the endosulfan.[14]

Experimental Procedure

Proven fertile male rats were divided in three groups of 10 animals each. Group I animals were kept as control and were administered only the vehicle (olive oil), by gavage whereas the animals of Groups II and III were treated with 10 mg/kg b.wt/day of test compound for 30 and 60 days. At the end of the experimentation, the rats were weighed, sacrificed under light ether anesthesia. The Epididymis and other male reproductive organs were removed, weighed and processed for detailed sperm dynamical, biochemical, hormonal and histopathological studies. Blood was collected from heart in preheparinized tubes. The serum was separated from the blood by centrifugation at 3000 rpm and stored at –20^{O}C.

Spermdynamics

(I) Sperm motility

Sperm motility was assayed by the method of Prasad et al. (1972).[15] The epididymis was removed immediately after anaesthesia and known weight of cauda epididymis was gently teased in a specific volume of physiological saline (0.9% NaCl) to release the spermatozoa from the tubules. The sperm suspension was examined within five minutes after their isolation from epididymis. The results were determined by counting both motile and immotile

sperms in at least ten separate and randomly selected fields. The results were finally expressed as percent motility.

(ii) Sperm density

Sperm density was assayed by the method of Prasad *et.al.* (1972) [15] Briefly total number of sperms were counted using haematocytometer after further diluted the sperm suspension from cauda epididymis. The sperm density was calculated in million/ ml as per the dilution.

(III) Biochemical Parameters

The total protein.[16] sialic acid.[17] and glycogen,[18] were assayed. The activities of epidiymal marker enzymes, phosphodiesterase.[19] and adenylate cyclase,[20] were also estimated.

(IV) Histology

Cauda epididymis was fixed in Bouin's fixative and cut into pieces and processedthrough ethanol-xylene series. It was then embedded in paraffin and bee wax (3:1) (M.P. 55-62 0º C).Sections were cut at 5 um thick and stained with Harris haematoxylene and eosin (H&E)

(V) Hormonal analysis

Testosterone, Leutinizing Hormone (LH), and Follicle Stimulating Hormone (FSH) were estimated through chemiluminescence in fully automatic Advia Cemtaus Immuno Assay System. The commercially available kit was purchased from My Bio Source LLc San Diego, USA.

Statistical analysis

The data obtained from the above experiments were subjected to statistical analysis. Data were represented as mean ± S.E.M. The differences were compared for statistical significance by "t- test" by using SPSS software (16.0 version) and they were considered non significant at $P \leq 0.05$, significant at $P \leq 0.01$ and highly significant at $P \leq 0.001$. Graphical representation of data has been done using Microsoft Excel 2010.

RESULT AND DISCUSSION

The results obtained after oral administration of endosulfan are shown in the figures 1-10 and figures A-C. A significant reduction in the weight of epididymis was observed in endosulfan treated animals. The weight of epididymis is largely dependent on the mass of differentiated spermatogenic cells and the reduction in the weight of epididymis may be due to reduced tubule size, decreased number of germ cells and elongated spermatids.[21,22] Also

spermatogenic arrest and inhibition of steroid biosynthesis of Leydig cells, a site of steroid biosynthesis may contribute to the decline of epididymis weight.[23] The spermatozoa motility and density (figure.1-2) in cauda epididymis was significantly decreased.

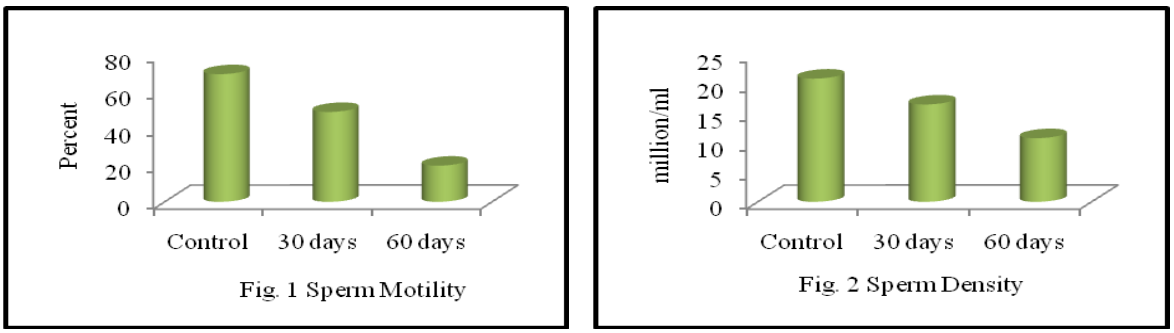


Figure 1-2: Sperm dynamics in cauda epididymis after endosulfan treatment in rats.

Sperm motility is affected by altered enzymatic activities of oxidative phosphorylation. Full ATP pool is crucial for normal spermatozoal movement and a slight derivation of ATP leads to reduction in motility,which may cause infertility.[24] Decreased sperm density in the cauda epididymides is an indicator of reduced spermatogenesis as a result of the toxicity of any agent.[25] Biologically active gonadotropins are essential for normal sperm production, growth, development and maturation of testes and cauda epididymis.[26] Reduction in sperm density may be due to alteration in androgen gonadotrophin. The epididymal spermatozoa are highly dependent on testosterone and epididymal protein for their final maturation and development of progressive motility and fertilizing capacity. The decreased motility of sperm in cauda epididymis indicates less ability of sperm to interact with the oocyte plasma membrane.[27] Cyclic nucleotides, especially cAMP have been shown to be an intrinsic regulator of sperm motility. Moreover, the acquisition of sperm motility when they traverse the epididymis is reported to be correlated with an increase in the intracellular cAMP content.[28] The epididymal biochemistry showed depletion of glycogen, sialic acid and elevation of epididymal protein (Figure 3-7).

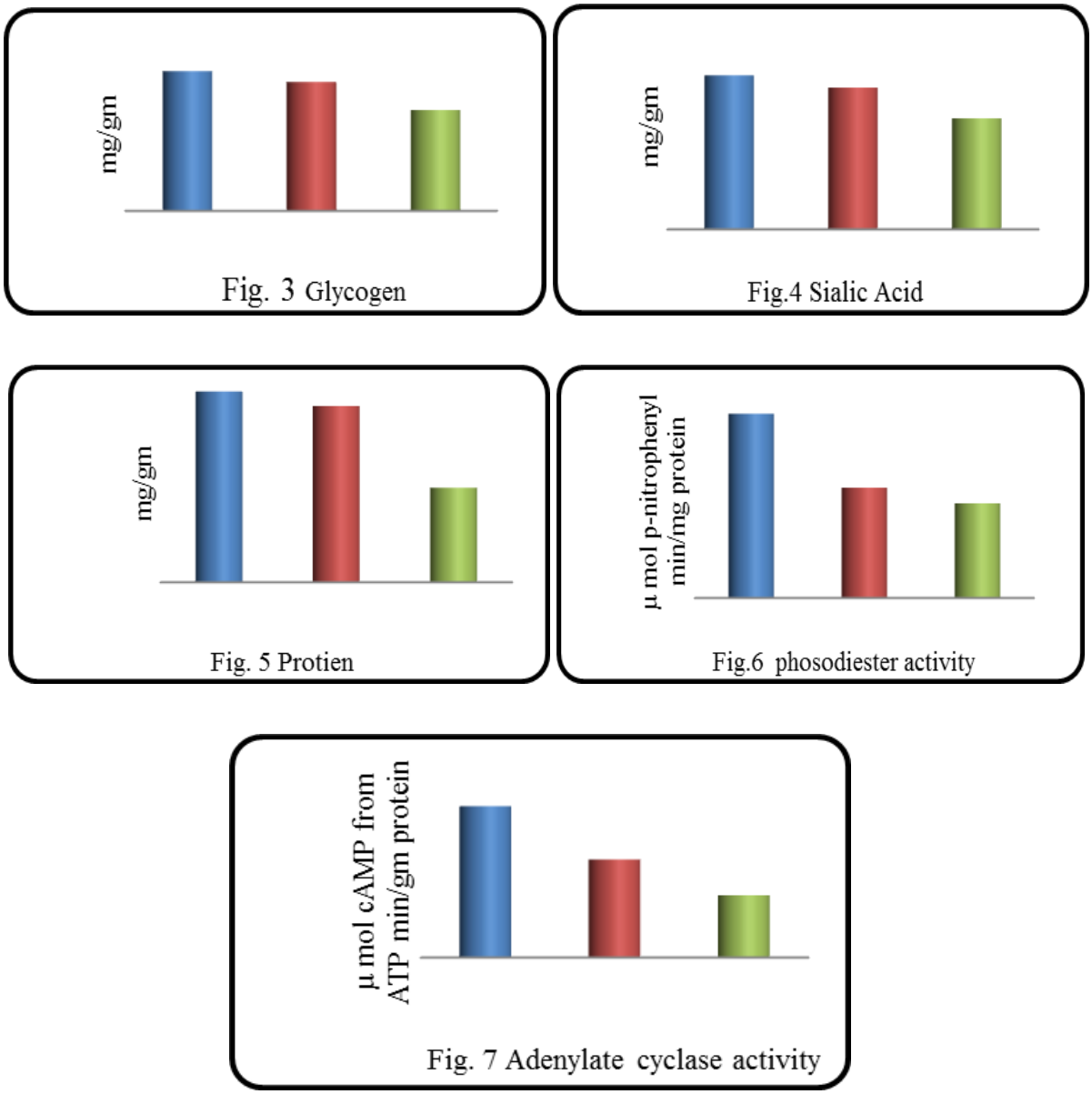
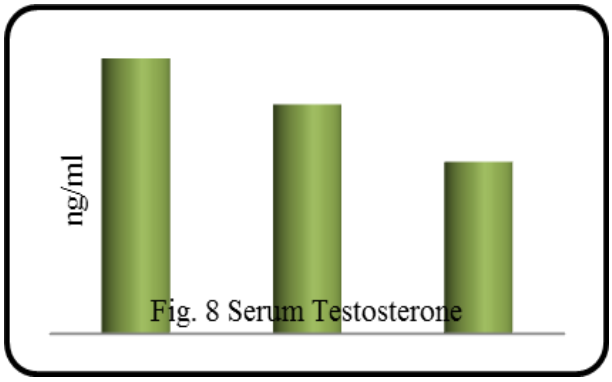


Figure 3-7: Biochemical changes in cauda epididymis of endosulfan treated rats

A fall in glycogen level may be due to interference in glycogenolysis. Since glycogen is an energy source for general metabolism and constant supply of glucose is essential for proper functioning of epididymis,[29] similarly reduction in sialic acid content may be due to absence of spermatozoa or reduced androgen production.[30] Elevation in total protein content may be due to the hepatic detoxification, which resultsin the inhibitory effect on the activities of enzymes involved in the androgen biotransformation.[31]

The activities of epidiymal marker enzymes, phosphodiesterase and adenylate cyclase as well as Testosterone, Leutinizing Hormone (LH), and Follicle Stimulating Hormone (FSH) were also found to be decreased. (fig8-10).



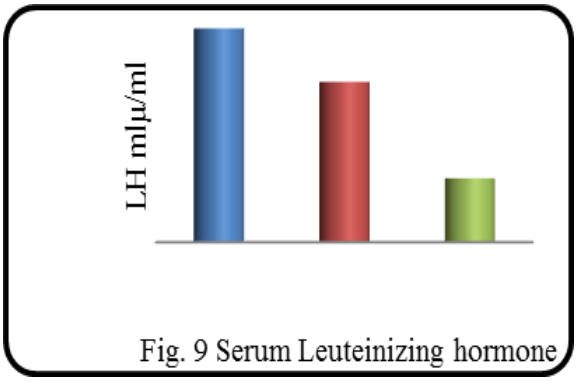
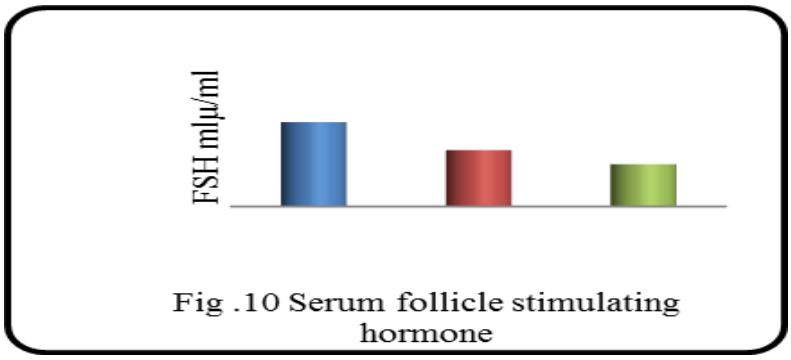


Figure 8-10: Serum hormonal profile of endosulfan treated rats



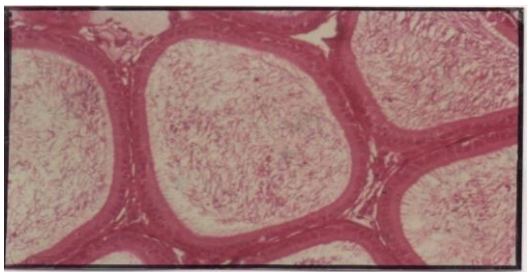
In the present study, endosulfan decreased the activity of adenylate cyclase enzyme in epididymis thereby it is clear that cAMP is not produced and ultimately resulted in the decreased sperm motility. The decline in phosphodiesterase activity observed in the present study may be triggered by similar factors in the epididymal fluid. Therefore, the decrease in the epididymal enzymes adenylate cyclase and phosphodiesterase is correlated to the poor motility of sperm in rat epididymis.

Efferent ducts respond to toxic insult by at least two different means an increased rate of fluid reabsorption or decreased secretions (i.e. Cl-); or a decreased rate of reabsorption or increased secretions. The first response leads to increased viscosity of luminal fluids, sperm stasis, ductal occlusions, granulomas and possibly fibrosis. The second response dilutes the luminal fluid, decreases sperm concentration, and leads to a decrease in sperm transit time through the epididymis. Testosterone is the principal androgen of the testes and it is essential for sperm production and maintenance.[32,33] There may be two mechanisms by which insecticides could reduce circulating levels of testosterone; first by enhancing its degradation, excretion or tissue uptake or second by depressing circulating LH levels and thereby reducing LH dependent testicular steroidogenesis.[7] It is well established that organochlorine pesticides reduce acetylcholinesterase activity and block nerve impulses. This effect may

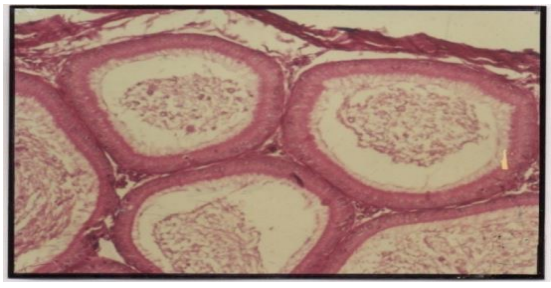
alter the release of pituitary hormones, namely FSH and LH, leading to the reduction of sperm production in the testes.

Administration of endosulfan also changes the biochemical parameters of epididymis part of the reproductive tract like other pesticides.

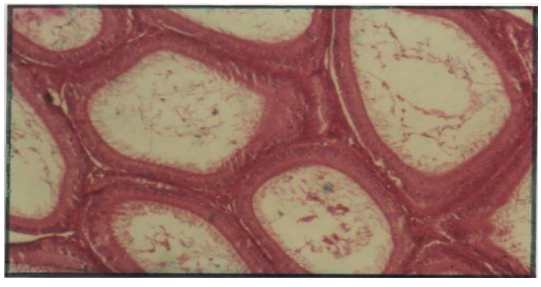
Figure A showed the cauda epididymis of control rat with normal histological features like large tubules lined with pseudostratified columnar epithelial cells with long prominent stereocilia. Inter tubular stroma contains connective tissues and blood vessels. The lumen is filled with mature spermatozoa, whereas marked histological changes were observed in the epididymis of endosulfan treated rats (figure B-C) like other pesticides,[34] Epididymis plays an integral role in male reproduction by providing a favorable fluid microenvironment for sperm maturation and storage and fluid secreted by epididymis is controlled by neurotransmitter substances that is allocrine and paracrine hormones.[35] The physiological and biochemical integrity of epididymis depend upon androgen deficiency of androgens causeda marked reduction in tubular diameters regression of epididymal epithelium, decline in spermatozoal number in cauda epididymis and change in composition of epididymal plasma.[36]



A: Control rat



B: Endosulfan (10 mg/kg. body wt. 30 days)



C: Endosulfan (10 mg/kg. body wt. for for 60 days)

Figures A- C: Microphotographs showing histological changes in cauda epididymis after endosulfan treatment

In conclusion, the result of the present study showed the toxic effect of endosulfan on epididymal structure and function of male rat.

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