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Sub-Acute Toxicity of Heptachlor At Low Dose Exposure in Wistar Rats

Medical Science

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ABSTRACT

Objective: The present study was undertaken to study the effect of low dose exposure of heptachlor on haematological values, biochemical profile, reproductive system and pathological changes in some vital organs in male rats.

Methods: The present study was conducted on 30 adult healthy male wistar rats. 30 male rats were randomly divided into five groups. Each group consisted of 6 male rats. Heptachlor solution (1 mg/ml) was diluted in methanol to prepare formulation of 1, 2.5 and 5 ppm for oral administration. Blood samples were collected at day 0 (before drug administration) and on day 28 for haematological and serum biochemical analysis. All the animals were sacrificed humanly on day 29 and post mortem examination was performed in the confined disinfected laboratory to determine the presence or absence of gross and histopathological lesions.

Results: The result of haematological, biochemical evaluation, semen analysis and histopathological examination of tissues reveled that low dose exposure of heptachlor to male rats for 28 days has little detrimental effects on biochemical profile of animals treated with 5 ppm of heptachlor. However, heptachlor exposure at high dose (5 ppm) did not have any detrimental effect on parameters of male reproductive system of rats.

INTRODUCTION

Polychlorinated cyclodienes are a class of chemical compounds employed as pesticides. The compounds like Chlordane components (CHLs) and their metabolites (heptachlor, cis-heptachlor epoxide, U82, MC4, trans-chlordane, MC5, cis-chlordane, MC7, oxychlordane, MC6 and trans-and cis- nonachlor) and aldrin, dieldrin, endrin, isodrin and endosulfan were being used extensively in most parts of the world. As lipophilic molecules, they have a strong tendency to accumulate in milk and in fatty tissues of both animals and human beings [1]. Heptachlor is highly toxic to humans and can be absorbed through the skin, lungs and gastrointestinal tract. It causes hyperexcitation of the central nervous system and damage to the liver. Poisoning symptoms observed in laboratory animals include lethargy, incoordination, tremors, convulsions, stomach cramps or pain, and coma. In humans exposed to chlordane, a closely related organochlorine insecticide which usually contains 10% heptachlor, signs of neurotoxicity such as irritability, salivation, lethargy, dizziness, labored respiration, muscle tremors and convulsions have been observed. In severe cases, death may occur due to respiratory failure. Persons with convulsive disorders or liver damage are at increased risk from exposure [2]. Oral exposure of heptachlor to laboratory animals results in a variety of adverse effects including liver effects, neurological effects, reproductive system dysfunction and developmental effects. There is very little information available about the short-term exposure to high doses of heptachlor to humans. But animal studies show that heptachlor is very toxic to animals and humans. Animals that were fed high levels of heptachlor during a short period of time experienced tremors and convulsions. The animals studied have shown enlarged livers, damage to liver and kidney tissue, and increased red blood cells. Animals that were fed heptachlor developed liver cancer. Studies show that female rats exposed to heptachlor were less likely to become pregnant. Those that did become pregnant had smaller litters or the offspring showed developmental problems. When baby rats were fed heptachlor, they developed cataracts just after their eyes opened. Other studies show that heptachlor fed to animals caused cancer. The U.S. Environmental Protection Agency believes heptachlor is a probable cancer causing agent because of the results of a number of studies [3]. Low level exposure to heptachlor may cause detrimental effect on various systems of body. Side effect on male reproductive system at high dose has been reported. However, data on alteration in haematological, biochemical and parameters related to male reproductive system after low dose exposure of heptachlor in rats have not been previously reported. Looking to this fact, the present study was therefore undertaken to determine the sub-acute toxicity of heptachlor at low dose exposure with attention to male reproductive system in wistar rats.

MATERIAL AND METHODS

The present study was conducted on 30 adult healthy male wistar rats. Healthy male rats of 6-8 weeks old were selected after physical and behavioural examination. The live body weight range was within ± 20 % of the mean body weight at the time of randomization. The rats were procured and housed in standard laboratory conditions in polysulphone in individual ventilated caging system at animal experimental room and provided with food and water ad libitum. The experimental protocol for general procedures and use of animals for conducting the present study was reviewed and approved by the institutional animal ethics committee (IAEC).

All animals were acclimatized to experimental conditions for minimum period of five days before grouping and dosing. 30 male rats were randomly divided into five groups. Each group consisted of 6 male rats. (Table 01).

Heptachlor solution (1 mg/ml) was diluted in methanol to prepare formulation of 1, 2.5 and 5 ppm for oral administration. The concentration was made in such a way that only 0.5 ml volume of formulation was to be administered to all rats in test groups. The rats of vehicle control group were administered with 0.5 ml methanol by oral route, whereas rats of negative control group were not dosed.

Table 01 Experimental Design of the Study

Group	Cage no.	No. of animals	Treatment	Animal ID
I	1, 2	6	5 ppm Heptachlor	1-6
II	3, 4	6	2.5 ppm Heptachlor	7-12

III	5, 6	6	1 ppm Heptachlor	13-18
IV	7, 8	6	Vehicle	19-24
V	9, 10	6	Control	25-30

Blood samples were collected from retro-orbital plexus into clean sterilized plain and EDTA added centrifuge tube at day 0 (before drug administration) and on day 28 for haematological and serum biochemical analysis.

All the animals were sacrificed humanly on day 29 and post mortem examination was performed in the confined disinfected laboratory to determine the presence or absence of gross and histopathological lesions.

RESULTS AND DISCUSSION

Effect of daily oral administration of heptachlor for 28 days on clinical symptoms and mortality:

All animals were observed daily for physical and behavioural changes till day 28 of experiment. Animals of any group did not reveal any symptoms attributable to 28 days of oral administration of heptachlor and methanol in vehicle control group. One animal from group I and II were found dull depressed and emaciated during the study period.

Effect of daily oral administration of heptachlor for 28 days on various hematological parameters:

The mean values of all the haematological parameters (total WBC count, total RBC count, haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelets and differential leukocyte count) in treatment groups (I, II, III and IV) were not significantly altered compared to control group except MCV and MCH. These two parameters were significantly decreased in all four groups compared to control which is indicative of effect of vehicle on haematological profile in the present study.

Effect of daily oral administration of heptachlor for 28 days on various biochemical parameters:

The biochemical parameters glucose, AST, ALT, ALP, total bilirubin, total protein, albumin, globulin, urea, creatinine and uric acid were studied before and after daily oral administration of heptachlor in rats.

The mean value of glucose in animals of group II was significantly lower compared to control group, whereas non significance decrease in glucose level has been noticed in group II, III and IV compared to control. The mean level of AST in animals of group I, II and III were non significantly decreased compared to respective value found in control animals. The AST level was also observed non significantly lower in methanol treated animals co compared to control. Thus, the decreased level of AST observed in observed in the present study may be of vehicle related effect. The ALT level in group II has been found significantly higher than the level found in control group, nevertheless the same was found non-significantly increased in animals of group I, III and group IV.

The mean values of ALP in animals of group I, II and III were significantly decreased when compared to control group. The ALP level in animals of group IV were not significantly altered which indicates the treatment related alteration on ALP level in rats. All other biochemical parameters of animals of treatment groups were not significantly altered compared control group. Decrease in testicular ALP was observed in endosulfan treated rats [4]. Decrease in serum level of ALP has been also observed following oral administration of Mancozeb, a fungicide in rats [5]. Decrease in activity of alkaline phosphatase in the present study may be due to due to adverse effects of the pesticide; the liking mechanism may be inhibition of testicular steroideogenesis by the destruction of cytochrome p-450, which is the result from decrease steroid hydroxylases and decline lysosomal enzyme alkaline phosphatase, in the presence of NADPH and oxygen which might result from decline the enzyme.

Effect of daily oral administration of heptachlor for 28 days on gross lesions in organs:

Gross pathological examination liver, kidney, stomach, duodenum, spleen, and testes of all animals did not reveal any abnormal lesions attributable to oral administration of heptachlor for 28 days in rats.

Effect of daily oral administration of heptachlor for 28 days on male reproductive system:

Left testes and epididymis were collected and processed for sperm motility, testicular spermatid count, epididymal sperm count and sperm morphology [6]. Alterations in sperm motility, spermatids per gram of testis, sperm per gram of cauda have not been observed in all treatment groups when compared to control group. In observation of head, mid piece and tail abnormalities (Sperm morphology) in the present study revealed no significant alterations compared to control group. However, a dose-dependent significant decrease in the sperm count and coagulating gland fructose levels was reported, with marginal degeneration of seminiferous tubules of male rats after administration of high dose of heptachlor [7]. Wango et al. (1999) also reported significant suppression of plasma testosterone levels ($P < 0.05$) and cortisol ($P < 0.02$) levels in heptachlor-treated rats as compared to corn oil–treated controls [8]. The finding in the present study indicated that heptachlor did not cause adverse effects on male reproductive system after low dose exposure for 28 days to rats.

Histopathology examination:

Tissues of adrenals, liver, kidney, spleen, testes and epididymis were collected from all the animals of treated and control groups during necropsy for observations of microscopic alteration. No histopathological alterations were observed in any of the organ in treatment and control group.

CONCLUSION

The result of haematological, biochemical evaluation, semen analysis and histopathological examination of tissues reveled that low dose exposure of heptachlor to male rats for 28 days has little detrimental effects on biochemical profile of animals treated with 5 ppm of heptachlor. However, heptachlor exposure at high dose (5 ppm) did not have any detrimental effect on parameters of male reproductive system of rats.

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