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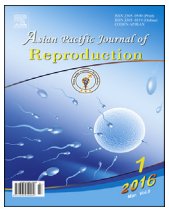

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Evaluation of Deltamethrin induced reproductive toxicity in male Swiss Albino mice

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ABSTRACT

Objective: To assess the adverse effect of Deltamethrin (DM) (technical grade) on reproductive organs and fertility indices of male Swiss albino mice, *Mus musculus*.
Methods: Forty male mice were divided into four experimental groups: control, vehicle control (peanut oil), high dose DM treated and low dose DM treated group. An oral dose of 3 mg/kg b.wt (low dose) and 6 mg/kg b.wt (high dose) of Deltamethrin was administered for a period of 45 days to male Swiss albino mice.
Results: DM caused a significant reduction in body and organ weights, sperm count, sperm motility percent, sperm viability, serum testosterone level, sialic acid content of cauda epididymis and fructose level of seminal vesicle. DM-treated groups also showed a significant decline in testicular 3β and 17β Hydroxysteroid Dehydrogenase (HSD) activities. Histological examinations revealed significant alterations in the testes of dosed groups.
Conclusion: Deltamethrin is a toxic pyrethroid pesticide that produced significant reproductive toxicity in treated male mice as revealed by the severely affected parameters and the altered gravimetric indices.

1. Introduction

Under the pretext of demographic growth with all its consequences, agricultural production resorts to the use of a varied and a large quantity of insecticides to improve the production and preservation of foodstuffs. Thus, the use of insecticides has increased rapidly and is now widespread to the lowest level of agricultural production. The increasing release of chemicals into the environment dictates attention to a better understanding of their toxicity in human and ecotoxicological effects. Several currently used pesticides are known to adversely impair reproductive competence of males under laboratory, field, clinical or occupational settings. Published studies have reported that pyrethroids can impair fertility, deteriorate semen quality, and cause testicular degeneration, male reproductive failure and malformations in the fetus of rodents following repeated exposure [1,2]. Synthetic pyrethroids are modified derivatives of pyrethins, natural substances obtained from flowers of pyrethrum species. Concerning to their high bio-efficacy at low concentrations, enhanced photo-stability and relatively low mammalian and avian toxicity, pyrethroid insecticides are widely used in agriculture, domestic and veterinary applications than other insecticides, particularly organochlorine, organophosphate and carbamate insecticides.

Deltamethrin [(R, S)] is a type-II pyrethroid synthetic insecticide, which has been widely used to control noxious insects in agriculture, forestry and horticulture. A number of studies have demonstrated genotoxic and tumorogenic effects of deltamethrin in mammalian and non-mammalian species [3]. During pyrethroid metabolism, reactive oxygen species (ROS) are generated and result in oxidative stress in intoxicated animals. In mammals, sperm plasma membranes have extremely high concentration of polyunsaturated fatty acids and insufficient antioxidant defenses; hence they are highly susceptible to lipid peroxidation. The production of ROS is a normal physiological event in various organs including the testis controlling sperm capacitation, acrosome reaction and sperm–oocyte fusion. However, overproduction of ROS can be harmful to sperm and subsequently to male fertility. Hence, the present study was carried out in order to assess the deleterious effect of technical

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grade Deltamethrin on various aspects of male reproduction to have an overall understanding of male infertility induced by this rampantly used pyrethroid. Open literature studies reveals several flaws including lack of appropriate control group, no data on the purity of test material, use of commercial mixtures containing Deltamethrin as a minor constituent, inadequate number of animals per group i.e. as low as only 3 animals per group, use of one dose level only, questionable route of administration (intraperitoneal or subcutaneous injection), no body and organ weight data, etc. A number of studies used commercial mixture containing Deltamethrin and other substances, such as Xylene, as the test material, making it impossible to attribute any effects to Deltamethrin or any other single component of the mixture since the controls received an entirely different vehicle. Keeping in view the above facts, present study has been designed accordingly so as to minimize the source of discrepancies in providing the evidence of reproductive toxicity of Deltamethrin.

2. Materials and methods

2.1. Animals & chemicals

Healthy, adult, pathogen free, colony bred male albino mice (*Mus musculus*) of Swiss strain weighing between 30 and 40 g obtained from IAEC recognized supplier were used for the experiments. The experimental protocol and the number of animals used for the experiments were mentioned in a detailed proposal and approval was obtained as per the guidelines of the institutional animal ethics committee, under registration No. 167/1999/CPCSEA from the Ministry of Social Justice and Empowerment, Government of India and Committee for the purpose of Control and Supervision of Experiments on Animals, Chennai, India. All the animals were acclimatized for seven days prior to the commencement of experiment. The animals were housed in an air-conditioned animal house at a temperature of 26 ± 20 °C and exposed to 10–12 h of day light and relative humidity of 40%–50%. Animals were randomized into control and treated groups and were caged separately. Standard chow (obtained from Amrut laboratory, Baroda, India) and water was provided *ad libitum*. Test chemical Deltamethrin (technical grade) of 98.11% purity was generously gifted from Meghmani Organics Limited, Ahmadabad (India). All the other chemicals used were procured from Himedia Laboratories, India and Sigma Aldrich (UK). All the chemicals used were of analytical grade.

2.2. Experimental design

Deltamethrin is considered to be readily absorbed when given orally as all pyrethroids are lipophilic; and absorption through gastrointestinal tract is higher than other routes. Hence, oral route of administration was selected for the treatments. Deltamethrin was administered via oral gavage dissolved in peanut oil at a dose level of 3 mg/kg body wt. (1/10th of LD_{50}) and 6 mg/kg body wt. (1/5th of LD_{50}). The doses were determined on the basis of LD_{50} of deltamethrin in peanut oil i.e. 30 mg/kg body weight [4].

Animals were divided into following groups (8 animals per group):

Group I: Control (given distilled water and food *ad libitum*); Group II (VC): Vehicle Control (given only peanut oil); Group III (HD): Deltamethrin HD treated (given 6 mg/kg body weight deltamethrin dissolved in peanut oil); Group IV (LD): Deltamethrin LD treated (given 3 mg/kg body weight deltamethrin dissolved in peanut oil).

All the groups were treated for 45 days and at the end of experiment, animals were weighed and sacrificed using light ether anesthesia.

2.3. Tissue collection

At the termination of experiment, animals were dissected and testis, cauda epididymis and seminal vesicle were dissected out carefully. Tissues were weighed, processed and homogenates were prepared accordingly.

2.4. Body and organ weight

The body weight of control and all treated groups of mice were recorded to the nearest milligram on a digital balance (Reptech). The animals were weighed before and at the end of each week prior to autopsy. Similarly, weights of organs were recorded to the nearest milligram on digital balance (Citizen, Japan).

2.5. Fructose

Fructose level was estimated in seminal vesicle of control and treated mice by the method of Foreman *et al.* [5]. The concentration of fructose was calculated using the regression formula obtained from the standard graph.

2.6. Sialic acid

Periodate resorcinol method was used for quantitative determination of free and glycosidically bound sialic acids [6]. The method involves oxidation of total sialic acid by treatment with periodic acid which forms a chromogen with resorcinol reagent. This chromogen is then extracted in an organic solvent and compared with standard at 630 nm.

2.7. Sperm count and sperm motility

Sperm count and motility in cauda epididymis of control and treated mice was determined using the Neubauer chamber of hemocytometer according to the method of Prasad *et al.* [7].

2.8. Sperm viability

Live: Dead ratio of cauda epididymal sperms was estimated by using the method of Talbot and Chacon [8].

2.9. Serum testosterone level

Serum testosterone levels were assayed using a solid phase enzyme immunoassay (ELISA) utilizing the competitive binding principle. Testosterone present in the sample competes with enzyme labeled testosterone for binding with anti testosterone antibody immobilized on the microwell surface. The amount of conjugate bound to the microwell surface decreased in proportion to the concentration of testosterone in