Malathion is an organophosphorus insecticide used in public health, residential, and agricultural practices since early as 1950. Over 100 food crops can be treated with Malathion and about half of total applications in the United States (U.S.) are on alfalfa, cotton, rice, sorghum, and wheat. In 2004, the Department of Pesticide Regulation (DPR) reported that 492,307 pounds of Malathion were used in California. Malathion has a broad range of use with target pests belonging to orders like mainly dipterans, lepidoptera, hemiptera and coleoptera. Malathion is a slightly toxic compound in EPA toxicity class III carrying the signal word “CAUTION”.

Histological alterations in different organisms have been reported in different non target animals other than mice exposed to different organochlorine and organophosphate pesticides has resulted in damaged tissues in terms of anatomical changes. In this study, we investigated the reproductive histological alterations in gonads (ovary) caused by Malathion in orally administered female mice as ovary is an organ sensitive to external factors and has direct bearing on the next generation.

**MATERIAL AND METHODS**

Swiss albino female mice (*Mus musculus*) were obtained from disease free small animal house of the Lala Lajpat Rai University of Veterinary Science, Hisar, Haryana, India. Ethical clearance was taken for the use of mice as experimental animal from the Institutional Animal Ethics Committee, Maharshi Dayanand University, Rohtak, Haryana, India. Mice were acclimatized to the laboratory conditions prior to the beginning of the experiments.

At the time of dosing each mouse was between 20 - 25 g b.w. and 8 to 12 weeks old and were divided into three main groups, group I mice serve as negative control received only the standard diet and distilled water, group II (positive control) mice injected cyclophosphamide (40 mg/kg of b.w.) and group III (treatment group) mice received Malathion (761.5 mg/kg b.w.). All mice were administered constant volume of specified doses to study histological alteration in ovary of mice. All mice were examined immediately after each dose, approximately 1h for sign symptoms of toxicity and sacrificed at 24h for histological changes. The female gonad was removed from the mice and transferred to fresh 10% neutral buffered formalin and left in it for 24 hrs. The tissue was then removed carefully and kept under tap water for some times. Dehydration was done in the ascending grades of alcohol (50%, 70%, 90% and absolute alcohol) for 30 minutes in each grade and transferred to xylene. The tissue was then embedded in paraffin wax, with the help of a rotary microtome and thin sections of 5-6 microns were cut, spreaded, allowed to stand for a day and deparaffinized in
REPRODUCTIVE HISTOLOGICAL ALTERATIONS INDUCED

xylene for 10 to 15 minutes and then hydrated by passing through a descending alcoholic series and then in distilled water and stained with Haris Haematoxylin solution and counter–stained with eosin stain, mounted and observed under microscope.

RESULTS AND DISCUSSION

In present study, histological alterations caused by orally administered Malathion in female mice ovary which is a sensitive organ to external factors, which might induce histopathological change as well as functional deficit. The ovary of negative control mouse showed developing follicles (primordial, primary and secondary follicles) corpus luteum and graafian follicles were observed in the cortex of ovum. Primordial follicles are composed of an oocyte surrounded by a small number of granulosa cells(Fig.-1). Our results revealed that the Malathion treated mice showed a significant loss of primordial and primary follicles and a significant reduction of follicles(Fig.-2). The development and maturation of ovarian follicles involved several stages, including primordial, primary, secondary, and antral. With the loss of primordial and primary follicles, all growing follicles (secondary and antral) were eventually depleted due to the lack of the precursor follicle populations for recruitment, with impairment to granulosa cells

Histopathology is the gold standard when defining toxicological effects. Using biomarkers linked to distinct, defined cell types and tissues may provide a direct link to histopathology without its drawbacks and it also provides increased sensitivity and specificity.

Histopathological biomarkers are closely related to stress since many pollutants either toxic or non toxic have to undergo metabolic activation in order to be able to culminate cellular change in the affected organism. The mechanism of action of several xenobiotics could initiate the formation of a specific enzyme activity that causes changes in metabolism, further leading to cellular intoxication and finally death.

Reports are not available on ovarian damage due to exposure to malathion toxicity in female mice, however, in mammals and other groups it has been reported that administration of malathion (33mg/kg/day) in rats cause atresia accompanied by a decreased number of normal antral and growing follicles in ovary, area of corpus luteum was enlarged due to dose increments. The study revealed that Malathion affects numbers of primordial, primary and prenatal follicles. In another study, exposure dependent alterations in ovary for both acute and chronic exposures were reported in fish due to sub lethal doses of Malathion where reduction in size of mature oocytes, disruption and vacuolation in cytoplasm after acute exposure, whereas chronic exposure resulted in complete loss of normal configuration of ovary, necrosis, elongated ovarian follicles, and fragmented ova with abnormal shape. The notable
microscopic changes in oocytes at different stages of development and in the nucleus of the immature oocyte of the catfish *Heteropneustes fossilis*. Malathion affects number of primordial, primary and prenatal follicles, but it increases atretic follicles and corpus luteum. It is reported that the treatment of female mice with CP (120mg/kg/bw) resulted in severe ovarian damage.

In conclusion, it is clear that the pesticide exposure causes hazardous effects at various levels to non-target organisms, including humans. The state of Haryana being agrarian state needs to take specific steps to educate farmers about its ill effects so that extent of toxicity be minimized to its inhabitants.

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