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Histological alterations induced due to malathion and cyclophosphamide exposure in mice

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Abstract
The present study was carried out to assess the histological alterations on the hepatic tissues of mice caused due to malathion and cyclophosphamide administered orally and intra-peritoneal, respectively. For this purpose, malathion (761.5mg/kg of body weight. i.e. 50% of LD50) and cyclophosphomide (40mg/kg of body weight) were administered into Swiss albino mice before sacrificing them for histological analysis alongwith control. Malathion and cyclophosphamide exposure displayed cell injury, hepatocytes swelling, hepatocytes degeneration results in dilated central vein and sinusoids between hepatocytes with pyknotic nuclei as compared to negative control reflecting the radially arranged hepatic cords around the central vein.

Keywords: histology, malathion, liver, hepatocytes, organophosphates

1. Introduction
In recent years, marked increase in intensive agricultural activities has resulted into massive use of pesticides. Although under the umbrella of pesticides agricultural productivity has increased, yet the negative impacts of pesticide exposure on non-target organisms can't be neglected. Marked by excellent hydrophobicity and persistence, organophosphates have become the most widely used pesticides [1]. Malathion (Diethyl 2-[(dimethoxy phosphorothioyl) sulfanyl] butanedioate) being an organothiophosphate contact pesticide has been recommended for control of various pests *viz.* rice hispa, pod borer, leaf weevil, jassids, aphids, mites, white fly and beetle in field crops like paddy, pea, soyabean, castor, okra, brinjal, tomato and grapes, respectively [2]. Cyclophosphamide (N, N-bis(2-chloroethyl) tetrahydro-2H- 1,3,2-oxazaphosphorin-2-amine 2-oxide, CP), is an oxazaphosphorine derivative of alkylating agent, nitrogen mustard known for its significant immunosuppressive activity. Hence, it has been widely used for the treatment of auto-immune diseases, transplantations [3] and cancer treatments [4]. But phosphoramide mustard and acroline are the results of cyclophosphamide metabolism. While acroline is a highly reactive metabolite it may be responsible for liver injuries [5]. Few studies have suggested the role of reactive oxidant species for the depletion of anti-oxidant mechanism in liver [6, 7]. Hepatic injuries caused by pollutants, pesticides, metals, carcinogens and drugs in fishes and mammals have also been previously reported [8, 9]. As liver is involved in detoxification process, it may bioaccumulate various contaminants or metabolites, resulting in hepato-toxicity. Thus, for understanding the toxicity caused by various chemicals it becomes mandatory to analyze the histological alterations upon their exposure. Various studies stamp the incidences of pesticide and metabolites' induced hepato-toxicity in mice [10, 11]. Hence, the present study was carried out to envisage the histological alterations induced by malathion and cyclophosphamide exposure in mice.

2. Materials and methods
2.1 Collection of test animal: The study was carried out in February, 2012 in Department of Zoology, Maharshi Dayanand University, Rohtak, Haryana. 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 body weight and 8 to 12 weeks old.

Table 1: Treatments given to mice for analysis of histological alterations

Group No.	Treatment	Mode of administration
GroupI	Distilled water	Oral
GroupII	Cyclophosphamide(40 mg/kg of body weight)	Intra-peritoneal
Group III	Malathion (761.5 mg/kg of body weight)	Oral

2.2 Experimental set up: All mice were administered a constant volume of specified doses alongwith control to study histological alteration in liver of mice. Triplicates were maintained for each treatment. All mice were examined immediately after each dose, approximately 1h for sign symptoms of toxicity, and sacrificed after 24h for histological changes.

2.3 Histological analysis: The liver was removed from mice and transferred to fresh 10% neutral buffered formalin for 24 hrs. The tissue was then carefully removed and kept under tap water for some time. Ascending grades of alcohol (50%, 70%, 90% and absolute alcohol) were used for dehydration purpose for 30 minutes per grade and transferred to xylene thereafter. The tissue was then embedded in paraffinated wax. After that thin sections of 5 -6 microns were cut, with the help of a rotary microtome. Then they were deparaffinized in xylene for 10 to 15 minutes and hydrated by passing through a descending alcoholic series followed by passing through distilled water and staining was performed using Haris Haematoxylin solution and counter – staining was performed with eosin stain [12]. Thereafter, the tissue was mounted and observed under microscope.

3. Result and Discussion

Histological alterations can be used as standard markers for analyzing toxicological effects of various pollutants and metabolites. The use of distinct and defined cell types not only provide the details of specified affected tissues but histological evidences are also sensitive and specific [13]. Metabolic stresses induced by various toxic or non-toxic chemicals induce changes in histology of the affected organism. Most probable cause of cellular intoxication and damage is the production of reactive oxygen species like super-oxide anion, hydroxyl-radical and hydrogen peroxide as a result of toxicants' oxidative metabolism [14-16].

In present study, the orally administered female mice with malathion resulted in functional deficit as well as histological changes. The liver of controlled mice (Fig. 1) showed the normal hepatic architecture where normal hepatocytes have cords that extend from the central vein to the portal triads. The cords were separated from each other by sinusoids. Kupffer cells were also present along the sinusoidal spaces. However, when the mice were treated with malathion the cells got injured. Malathion exposure in Group III resulted in hepatocytes swelling, hepatocyte degeneration, enlargement of central vein, mononuclear cell infiltration in the parenchymatous tissue and portal area. The results revealed that the malathion induced cell injury in treated (Fig. 2) mice as compared to control mice, displayed cell injury, The CP group showed nuclear degeneration of parenchymatous cells in addition to necrosis of hepatocytes. After exposure, the hepatocytes exhibited clumped chromatin and irregular nuclear boundaries in addition to lesions that were observed to be occurred with approximately the same frequency (Fig. 3). Mammalian toxicity via oral, dermal and inhalation administration of malathion have been previously reported [17, 18]. The formation of reactive oxygen species and toxic metabolites are the most probable cause for hepatic-toxicity induced due to malathion [19] and cyclophosphamide exposure, [7] respectively.

Contrarily, previous study reported [20] that the treatment of female mice with CP (120mg/kg/bw) no effects on the heart, liver, stomach, and pancreas. Results are in agreement with the study [21] which reported that in treated mice with CP (100mg/kg/bw), there were histological changes in the liver, pancreas, spleen, lungs, and heart, but not in the kidneys. These different results may attribute to the difference in dosage and the time of examination used in the two laboratories and also various organs have different repairing capacity after the treatment. Studies [21] reported that exposure of CP results in small sized hepatocytes, eosinophilic and showed a homogenously stained cytoplasm lacking the cytoplasmic vacuolization typical of glycogen accumulation. Accumulation of inflammatory cells adjacent to particularly central veins without apparent changes in the endothelium or vascular integrity was observed. Microscopic examination also revealed the dose and time dependent increase in damage caused to mice. After two days of treatment, vacuolation of hepatocytes, necrosis, portal mononuclear cell infiltration, and microgranuloma formation was also observed. Rats treated by gavage with 130 mg malathion/kg/day (unspecified purity) for 1–2 weeks resulted in diffused hydropic degeneration of the liver and those treated with 390 mg/kg/day had focal necrosis, vacuolar degeneration in the portal hepatocytes, Kupffer cell hyperplasia and microgranuloma [22].

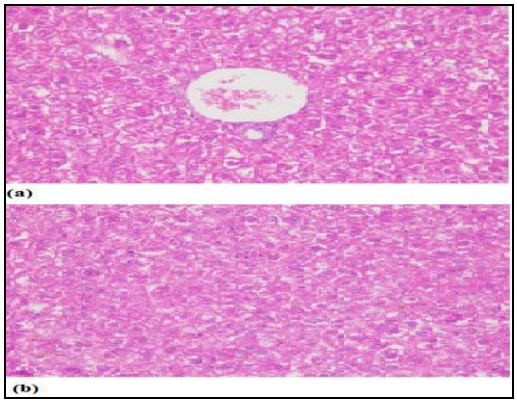

Fig 1: Section through the liver of negative control mice showing normal structure of central vein and portal vein

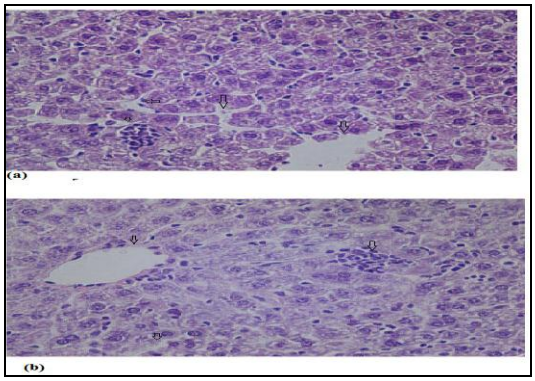

Fig 2: Section through the liver of mice exposed to 761.5mg/Kg malathion showing cell injury, hepatocytes swelling, hepatocyte degeneration, and enlargement of central vein.

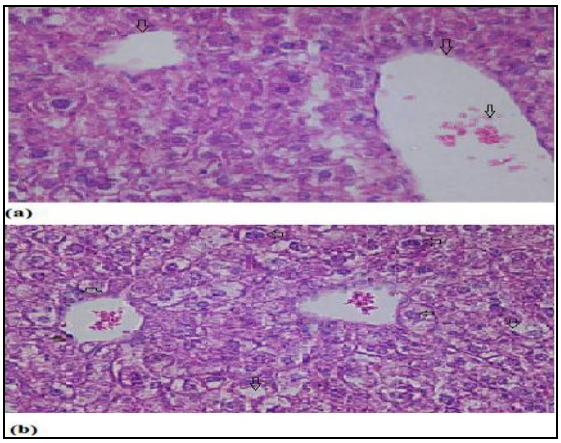

Fig 3: Section through the liver of mice exposed to 40mg/Kg cyclophosphamide showing enlargement of central vein and hepatocyte degeneration.

4. Conclusion

It can be concluded that malathion and cyclophosphamide cause damage to liver in swiss mice. Dose and time dependent damage due to malathion exposure has been noticed. Judicious agronomic use of malathion and medical use of cyclophosphamide must be ensured.

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6. References

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