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Histopathological changes induced by Malathion in wistar rats and its reversal by *Emblica officinalis*

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Abstract
Malathion, an organophosphate insecticide has been used in agriculture and domestic practices since mid-1940s. Present study aimed to analyze the preventive effect of *Emblica officinalis* juice against Malathion induced toxicity in histological structures of liver of male wistar rats. Mature Wistar rats were divided into 7 groups fed orally with different formulations of Malathion and *Emblica officinalis* juice (equivalent to 5 mg/kg dose of vitamin C) for the duration of 10 days. Group A serves as control. Group B and C administered with 100 mg/kg and 500 mg/kg of Malathion respectively. Recovery groups (Group D supplemented with 100 mg/kg of malathion along with *Emblica officinalis* juice (EOJ); Group E tested with 500 mg/kg of malathion along with EOJ; Group F administered with for 5 days and then supplemented with EOJ till 10th day; Group G treated with 500 mg/kg of malathion for 5 days and then supplemented with EOJ till 10th day). Present study revealed Malathion induced groups showed Sign of pyknosis in Hepatocytes, alteration in normal morphology of liver, depletion in organelles, congestion of blood vessels with hemorrhage, increased vacuolization in cytoplasm of hepatocytes in dose dependent manner. EOJ supplemented groups showed normal cellular arrangement, reduced signs of necrosis, significant decrease in number of vacuoles and intracellular spaces, elevated level of hepatocytes. Present study concluded that Malathion induced devastating effects on liver morphology and *Emblica officinalis* juice reverse the deleterious effects of Malathion.

Keywords: *Emblica officinalis*, hepatotoxicity, histopathology, Malathion, organophosphates

Introduction
Organophosphates compounds (OPCs) are one of the important class of pesticide, widely in use since mid-1940s [1]. There are more than 100 different OPCs widely used as insecticide as well as herbicides [2]. Two third portion of this insecticide is used in agriculture practices. In 1990, a WHO report on pesticides in agriculture estimated 2, 20, 000 deaths due to pesticide poisoning, 99% of these were in the developing countries and 20,000 were unintentional [3]. This report also revealed that organophosphate pesticides were accumulated in the environment and residues has been detected in the soil, water bodies, vegetables, grains and other foods products [4]. Organophosphate (OP) pesticides are known to cause inhibition of acetylcholinesterase and pseudo cholinesterase activity in the targeted species [5]. Other systems that could be affected by OP intoxicant are immune system, haematological system, [6] pancreas [7], liver [8] and the reproductive system [9].
Malathion [O,O-dimethyl-S-(1,2-dicarcethoxyethyl) phosphorodithioate] is most popular commercially used organophosphate pesticide. It has wide spectrum uses cross the world, especially in developing countries for protecting ornamental plants, greenhouses, livestock, stored grains, forests, buildings, gardens, agriculture from pests. Epidemiological research into the acute and chronic toxicity indicates that Malathion is highly toxic to mammals [10]. Mammals were exposed to Malathion through oral, dermal and inhalation routs [11-13]. The extensive use of Malathion has led to the exposure of numerous non-target animals, birds, invertebrates thus contaminated food chain [14-16]. The toxicity of Malathion is subjected by its metabolites. The metabolites which were primary source of toxicity, produced by the oxidation of Malathion in mammals, insects, and plants. They were 40 times more acutely toxic than Malaoxon [17].
Emblica officinalis commonly known as Amla belongs to family *Euphorbiaceae*. Fruit extract of *Emblica officinalis* is an important constituent of many Ayurvedic formulation such as chyvanprash and triphala. Traditionally, the fruit is useful as astringent, cardio tonic, laxative, diuretic, liver tonic, stomachic, antipyretic, anti-inflammatory, abnormalities of urine, leucorrhea, liver complications and cough [18].

It was also reported to have antioxidant, antimutagenic, cytoprotective, hepatoprotective, antitumor, antifungal, antimicrobial, hypolipidemics effects [19].

Present study aimed to assess the protective effect of *Emblica officinalis* juice (EOJ) in attenuating Malathion induced hepatotoxicity.

Materials and Methods

Chemicals

Commercial grade Malathion (52.5% w/w) obtained from Shivalik Agro Chemicals, Samba (Jammu and Kashmir), India. *Emblica officinalis* purchased from local market and authenticated by a pharmacognosist and a voucher specimen was deposited in department for future reference and its juice was further extracted. EOJ having 5 mg/kg of Vitamin C was taken for the study.

Test Animal

Mature Wistar rats (weighing approximately 150–200 g) were obtained from disease free Animal House, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra. The animals were housed in plastic cages (5 per cage), fed on standard laboratory diet and water *ad libitum*, exposed to a 12 h light/dark cycle, and maintained at a laboratory temperature of 25 ± 2°C. The animals were quarantined for 10 days before beginning of the experiments. The experimental protocol was approved by the Institutional Animal Ethics Committee, (Animal Ethics Approval No-CPCSEA/IAEC/IPS-120). All experiments were carried out in accordance with 'Guidelines for care and use of animals in scientific research (Indian National Science Academy 1998, Revised 2000)'.

Treatment Schedule

LD_{50} of Malathion for rats was observed approximately 1500 mg/kg [20]. Two doses of Malathion selected for the study were 100 mg/kg ($1/15^{th}$of LD_{50}) and 500 mg/kg ($1/3^{rd}$ of LD_{50}) doses was selected. Mature Wistar rats were divided into 7 groups having 6 rats each fed orally. Experimental design for different groups were as follows:

Control Group

Group A: serves as control; normal saline (1ml/kg) was provided for ten days.

Treatment groups

Group B: administered with 100 mg/kg of Malathion for ten days.

Group C: tested with 500 mg/kg of Malathion for ten days.

Recovery groups

Group D: having EOJ along with 100 mg/kg of Malathion for ten days.

Group E: EOJ and 500 mg/kg of Malathion was administered for ten days

Group F: administered with 100 mg/kg of Malathion for 5 days and EOJ was given on 6^{th} day till 10^{th} day.

Group G: tested for 500 mg/kg of Malathion for 5 days and EOJ was given on 6^{th} day till 10^{th} day

On 10^{th} Day, liver samples were collected from each group for histopathological evaluation.

Histopathological Analysis

Histopathological studies of liver tissues were carried out by using standard techniques given by Pearse [21]. Tissues were fixed in Bouin's fixative for 24 hours and washed for 2-3 hours in running tap water. After dehydration through alcoholic grades, the tissues were embedded in paraffin wax (60^0C). The sections were cut at 5 µm thickness. Then, dewaxing was carried in xylene for 15-20 min. Slides were processed through descending alcoholic grades and stained with hematoxylin and eosin. Sections were studied and photographed using Olympus digital camera.

Results and Discussion

The door to door usage of organophosphorus (OP) compounds leads to their poisonings to non- targeted species [22]. Liver is among the targets of Malathion toxicity. Liver is the major site for the biotransformation, accumulation, and excretion of xenobiotics [23]. Present histoarchitectural studies on Malathion intoxicated groups showed devastating effect on normal morphology of liver with no mortality and EOJ have attenuating effects against Malathion in dose dependent manner. Control group (Group A) showing normal histoarchitecture of liver with hepatic lobules surrounding the central vein. (Fig. 1). Each lobule consisted of hepatic cords. The hepatocytes depict the hepatic cords and the morphology of hepatocyte was found to be healthy with intact plasma membrane. They have polygonal cells with centrally basophilic nuclei. In treated groups, at 100mg/kg dose of Malathion (Group B), alterations in liver parenchyma, signs of hepatocyte hypertrophy and cellular vacuolization were also observed as compared to control group (Fig. 2). Whereas at higher dose (Group C) i.e. 500mg/kg of Malathion showed devastating effects. Hepatocytes were found to be condensed with disrupted plasma membrane. Sign of pyknosis were observed in nuclei of hepatocyte (Fig. 5). Higher centrilobular and sinusoidal congestions, anucleated hepatocytes, depletion in organelles, congestion of blood vessels with hemorrhage and increased vacuolization in cytoplasm of hepatocytes were clearly visible as compared to lower dose (Fig. 2, 5). Present studies in consistent with earlier studies showed that Malathion by adversely affecting hepatocytes may disturb the cytochrome P450 system or the mitochondrial membrane transport system of liver [24]. Congestion in centrilobular and sinusoid revealed signs of liver inflammation induced by Malathion [25]. Previous studies documented that liver damage caused by Malathion May upsets the detoxification mechanisms of the liver. Present study strongly supports the findings of Rezg *et al.* [25], who observed depletion in number of from cytoplasmic organelles effecting normal histology of liver. Studies also documented hemorrhage, inflammatory cell infiltration [26-27], tissue damage, and necrosis [5] which could provoke increased WBC counts. It was also reported that rats tested with 100 mg/kg of Malathion intragastrical, exhibit hepatic damage [25]. Recovery groups (Group D, E, F, G) showing restoring effects against Malathion damage. EOJ treatment group (Group D) showing normal cellular arrangement around the central vein (Fig. 3). Signs of necrosis appeared to be reduced. Significant decrease in number of vacuoles and intracellular spaces were observed (Fig. 3, 4). The elevated hepatocytes concentration in recovery groups, is due to their increased metabolism for providing sufficient energy to repair the catastrophe. It showed increased vascularity in the liver. Group E, showing enlargement in the sinusoids, some degree of vacuolization in hepatocytes and congestion of blood vessels with hemorrhage were observed at 500 mg/kg of Malathion along with EOJ

(Fig. 6, 7). In group F and G, hypertrophy was observed showing the scavenging action of EOJ against liver damages in dose dependent manner. Attenuating effect were less significant than Group D and E.

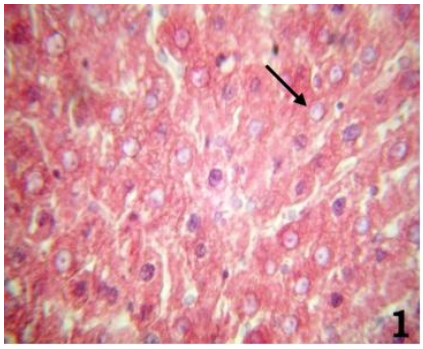

Fig 1: A microphotograph of transverse section (T.S.) of liver of control group, revealing normal hepatocytes with well defined spherical nucleus. Note the placement of cells in rows (Arrow) (×400).

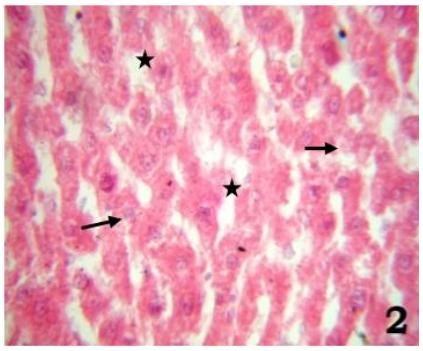

Fig 2: A portion of liver after Malathion (100 mg/kg) treatment depicting gaps between cords (Star) and coarse granules in cytoplasm. Note mild distortion in cell to cell adhesion (Arrow) (×400).

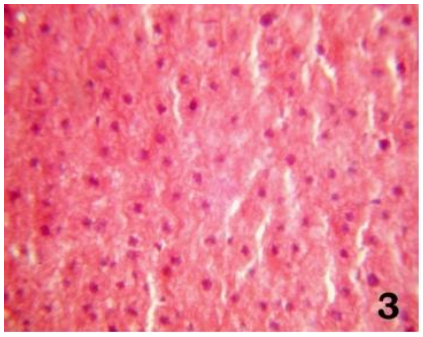

Fig 3: Microphotograph of Malathion (100 mg/kg) + EOJ (5 mg/kg of Vitamin C) treated liver revealing better consistency and signs of recovery in hepatic cords (×400).

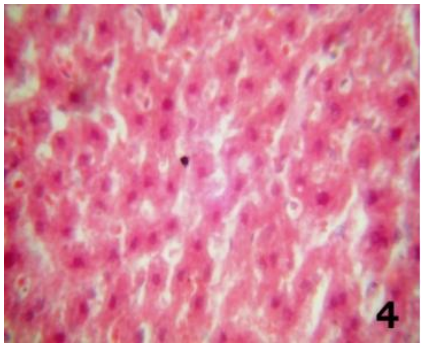

Fig 4: Microphotograph of Malathion (100 mg/kg) + EOJ (5 mg/kg of Vitamin C) treated liver revealing better consistency and signs of recovery in hepatic cords (×400).

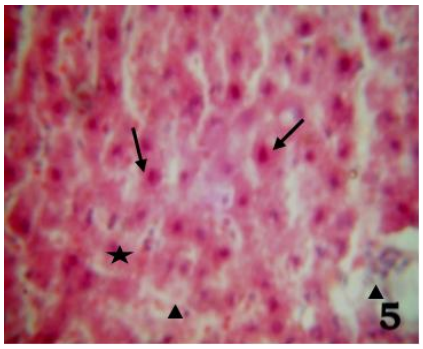

Fig 5: T.S. of liver of Malathion (500 mg/kg) treated rat showing pycnosis (Arrow) and hyalinization of cytoplasm. The condensed nucleus (Star) and large sinusoids with hemorrhaged blood cells (Triangle) (×400).

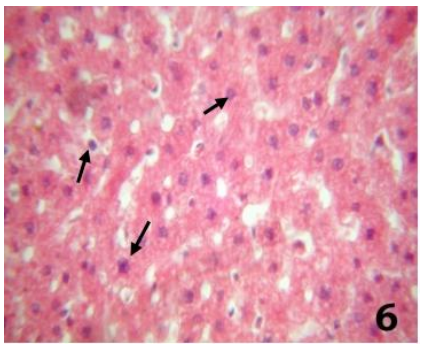

Fig 6: Malathion (500mg/kg) + EOJ (5 mg/kg of Vitamin C) treated rat liver showing recovery sign in general histology but pycnotic nuclei surrounded by hyaline cytosol (Arrow) (×400).

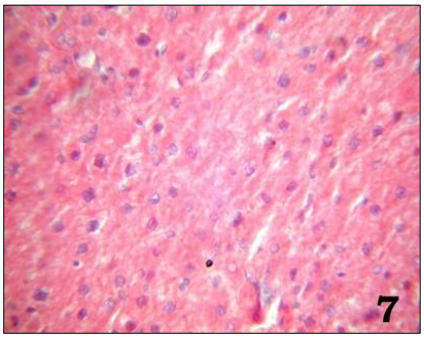

Fig 7: Malathion (500 mg/kg) and EOJ for 5 days treated liver showing little recovery in general histology (×400).

Conclusion

Present study concluded that malathion produces severe changes in hepatic tissue in dose dependent manner and *Emblica officinalis* being a rich source of ascorbic acid, gallic acid, and many hydrolysable tannins including emblicanin A and B, is a potential antioxidant, in mitigating toxic effects induced by malathion in hepatic tissue. It is advisable for the farmers and workers dealing with organophosphate pesticides, to take *Emblica officinalis* juice on regular basis to prevent toxic effect of Malathion.

Conflict of Interest

Authors declare no conflict of interest.

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