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HISTOLOGICAL STUDY OF THE EFFECT OF MALATHION ON LIVER AND KIDNEY TISSUES OF MICE MODEL

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ABSTRACT: Malathion is a widely used organophosphorous pesticide that a large number of populations are undesirably exposing themselves to severe health risk due to taking up the contaminated foods, water and vegetables containing malathion. The present study was carried out through histopathologic test to evaluate the extent of damage caused by malathion in the liver and kidney tissues of mice. Twenty five adult female albino mice were used and dispense mainly into two groups as control group and treatment group. The mice of control group were feed with poultry food without any ingredient of malathion. Each of the treatment groups (group1, group 2, group 3 and group 4, containing five animals in each group) were supplied everyday with the same type of food containing the following amounts of malathion as 65mg, 13mg, 2.6mg, and 0.52mg per 50gm (10gm food/mouse) of food, respectively. After fifteen days the animals of both groups were sacrificed and the organs of sacrificed animal were subjected to histopathological examination and then visualized under light microscope. Results demonstrated various histopathological alterations such as enlargement of the sinusoids and vacuole formations in hepatocytes and central vein, congestion of blood vessels of hepatic tissue, hemorrhage and necrosis in hepatic tissue and swelling appearances, highly degeneration and shrinkage of glomeruli, Bowman's capsules and related tubules structure, edema of renal tubules and rising of urinary space in renal tissue compared with their control. Therefore, we strongly recommend here for the ban of malathion for used as pesticide.

INTRODUCTION: Malathion is one of the most extensively used organophosphorous pesticide throughout the world to protect crops against different types of pest and to get more yield. It is highly effective, rapid in healing action, accactable to most conditions and relatively cost-effective. After first registered for use in the United States in 1956, it has been abundantly used in agriculture, grain storage, garden, ornamental plants, greenhouse and forests.

Inspite of its claim to low acute mammalian toxicity, it is generally functional to manage a large variety of pests, including mosquitoes, grasshoppers, gypsy moths, fruit flies, mites, aphids, spider mites, bollworms, boll weevil, chin bugs, spittle bugs, mealy bugs, loppers and scales¹.

This hazardous chemical is applied practically via ground and aerial sprays, aerosols, foggers, baits, paints, pet collars, animal dips, animal dust bags, and cattle feed blocks. It is exposed in our environment through diverse ways such as inhalation, skin contact and through ingestion¹. Annual predictable use of malathion throughout the world range from 10 to 15 million pounds of active ingredients². Besides of its deliberate use to

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control pest and insect, occasionally showing moderate to highly toxicity to wide variety of non target organism like as bird and water organism, including fish³ even humans health⁵. Malathion is basically a nerve toxin, worked by inhibiting the action of an enzyme called acetylcholinesterase (AChE) at the synaptic junction⁶, which is used to break down another chemical acetylcholine that plays a crucial role in the transmission of impulses from one nerve cell to another. Due to binding of AChE with malathion, AChE cannot break down acetylcholine, which consequently accumulates in nerve cells and causing rapid twitching, incoordination, paralysis and death^{7,8}.

Inhibition of liver acetylcholinesterase (AChE) activity is normally regarded as a practical parameter of poisoning by organophosphorous pesticides. Malathion's toxicity is enhanced by its metabolites and contaminates. It is readily oxidized into its metabolites malaaxon in mammals, insects, and plants, which is the major source of malathion's toxicity and is 40 times more toxic than malathion^{9,10}. There are various abnormalities related with malathion toxicity such as vision loss, kidney damage¹¹ and lung damage¹² and myriad negative health effects. It has been connected to child leukemia, aplastic anemia and adult leukopenia¹³.

Acute (short term) effects of malathion include headaches, nausea, dizziness, salivation, tearing, urination, diarrhea, convulsions, muscle weakness, incoordination, abdominal cramps, blurred vision, pupil constriction, abnormal eye movement, slowed heart beat, depressed respiratory system, skeletal muscle damage, paralysis and coma^{14,15,16}. Exposure of malathion can potentially cause long term damage to the nervous system, poor mental health and deficits in memory and concentration^{17,18}.

Malathion has also been related with mutagenicity, carcinogenicity and DNA abnormalities^{19,20}. Several studies showed that malathion provoke a range of physiological, biochemical, immunological, and histological alteration in experimental animals^{21,22}. Due to its massive use as pesticide in agricultural sector and causing serious environmental trouble as well as harmful effect on non target organism including human, the

policy maker of several countries are searching to apply biological and other nonpolluting methods for combating with pest. In Bangladesh, the activity of Pesticide Incident Monitoring System (PIMS) is not enough to control of marketing pesticide containing food stuff and general public undesirably consume and subjected to serious health threat. Therefore, there is an utmost need to find out safety dose of malathion that effectively destroy pest and insect, on other side is risk free to human beings and other non target organism.

MATERIALS AND METHODS:

Animals: Twenty five matured female albino mice weighing around 23-27gm (80-90 days) were collected from the Department of Pharmacy of the University of Jahangirnagar, Dhaka, Bangladesh. All mice were handled in accordance with the typical guide for care and use of laboratory animals (under the license of Institutional animal, medical ethics, bio-safety and bio-security committee (IAMEBBC) for experimentations on animal, human, microbes and living natural sources . No. 33/320/IAMEBBC/IBSC).

Chemical: Malathion (57%) was purchased from local super market of Rajshahi by the trade name of Ecomala produced by Oponin pharma. The concentration of malathion was calculated from the percentage of active ingredient of commercial formulation of malathion.

Experimental design: Mice were dispensed indiscriminately mainly into two groups as follows-

Control group: A total of five mice were feed with poultry food (10gm/mouse) without any ingredient of malathion.

Treatment groups: There were four treatment groups containing five animals in each group.

Group 1: Experimental animals were orally given malathion at a dose of 1300mg/kg body weight for a period of fifteen days, which was the LD₅₀ dose in case of female mouse.

Group 2: Experimental animals were orally given malathion at a dose of 260mg/kg body weight for a

period of fifteen days, which was the five times dilution of LD₅₀ dose.

Group 3: Experimental animals were orally given malathion at a dose level of 52mg/kg body weight (five dilution of dose 2) for a period of fifteen days.

Group 4: The experimental animals were orally given malathion at a dose of 10.5mg/kg body weight (five dilution of dose 3) for a period of fifteen days.

Histological study: After completion of treatment period, the animals were anesthetized using chloroform and sacrificed by cervical dislocation. Liver and kidney was collected carefully from the mice using standard procedure. Collected organs were washed thoroughly with 0.9% normal saline to remove any trace of blood. Fat tissues adhered to the organs were also removed carefully and afterwards organs were sliced into small tissue pieces using a surgical scalpel for allowing easy penetration of the chemicals inside the tissue.

The dissected tissue was treated with Bouin's fluid (fixative) for -16-18 hour and subsequently washed under running tap water for one hour until complete removal of most of the Bouin's fluid from the tissues. Followed by washing, dehydration of the tissues was conducted by immersing the tissue in a series of gradually increasing concentrations of alcohol (50%, 70%, 80%, 95% and absolute alcohol) and embedded into paraffin wax for making blocks.

The block was to be trimmed by removing of wax from the surface of block to expose the tissue. Sectioning of the tissue was performed by using a microtome (The microtome machine was sold from Tokyo, Japan by the trade name of SHIBUYA produced by optical. Co LTD). The microtome was pre-set to cut the tissue as thicknesses around 6 µm.

Blocks Small ribbons of tissue sections were placed on microscopic slide with help of warm distilled water containing few drops of Mayer's albumen and deparaffinized with xylene solution. Haematoxyline and eosin yellow solution was used to stain the tissue for preparing permanent slide. Histopathological changes were observed under 20x of a light microscope (The microscope was

purchased from Italy by the trade name optika) and snaps were taken.

RESULT:

Phenotypic appearance:

The animals of treated group were received food containing different concentration of malathion solution based on the number of group. First two days mice received tinny food probably due to the bad odor of malathion in the food. Nevertheless, from third day they normally took food. But their appearance clearly indicated the toxicological effect of malathion. They looked like as cadaverous and dull. However, ending treatment period they were sacrificed and prepared of histological slide from their two organ (liver and kidney). The slide clearly indicated the injurious effect of malathion on both organs.

Effect of malathion on mice liver: Light microscopic observation of liver of control mice showed regular and compact configuration with well organized hepatic cell and central vein (**Fig. 1A**). Whereas, the section of malathion treated mice showed different histopathological alteration. The slide from treatment group 1 showed severe damage in hepatic tissue including prominent enlargement of sinusoids, infiltration of mononuclear cell, dilation, hemorrhage and necrosis (**Fig. 1B**). Animal of treatment Group 2 indicated moderate toxicity of malathion moderate enlargement in the sinusoids, vacuole formations in hepatocytes and congestion of most blood vessels with hemorrhage (**Fig. 1C**). The liver section of treatment group 3 showed hepatocytes, paler, necrosis and had lost their normal shape and arrangement (**Fig. 1D**). The histological slide of treatment group 4 showed no significant changes other than generalized nodule formation in central vein (**Fig. 1E**).

Effect of malathion on mice kidney: Histopathological study on the kidney of control mice showed regular structure with capillaries, tubules, glomerulus, and Bowman's capsule (**Figure 2A**). On the other hand, the areas of renal cortex containing renal corpuscles and associated tubules expressed more pronounced changes in treated animals compared with control. In the case of treatment group 1, highly degeneration of glomeruli, Bowman's capsules and associated

tubules structure, shrinkage of glomeruli and edema of renal tubules and raising of urinary space was also noticed (**Figure 2B**). Treatment group 2 showed severe congested glomeruli, eroded Bowman's capsule, coagulative necrosis and inflammation between tubules (**Figure 2C**). Mild Swelling appearance, paler and vacuolation in the intertubular spaces were also appeared in group 3 (**Figure 2D**).

In treatment group 4, there were no significant changes in renal tissues other than just mild blood hemorrhage and vacuolation in the intertubular spaces (2E).

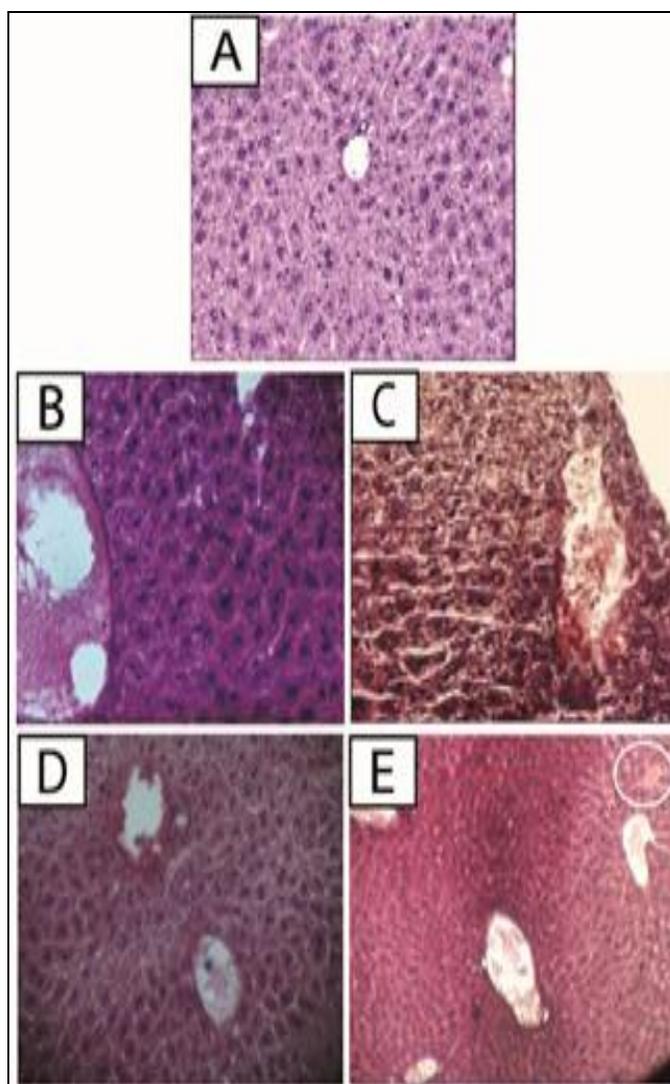


FIGURE 1: LIGHT MICROSCOPIC SLIDES OF LIVER SECTIONS OF MICE. A. LIVER SECTION OF CONTROL MICE SHOWS REGULAR STRUCTURE. B-E. LIVER SECTION OF TREATED MICE SHOWING VARIOUS HISTOLOGICAL ALTERATIONS. B REPRESENTS FOR TREATMENT GROUP 1. C STANDS FOR TREATMENT GROUP 2. D SYMBOLIZES TREATMENT GROUP 3 AND E

REPRESENTS TREATMENT GROUP 4. (IMAGES ORIGINAL EXPOSURE 20X).

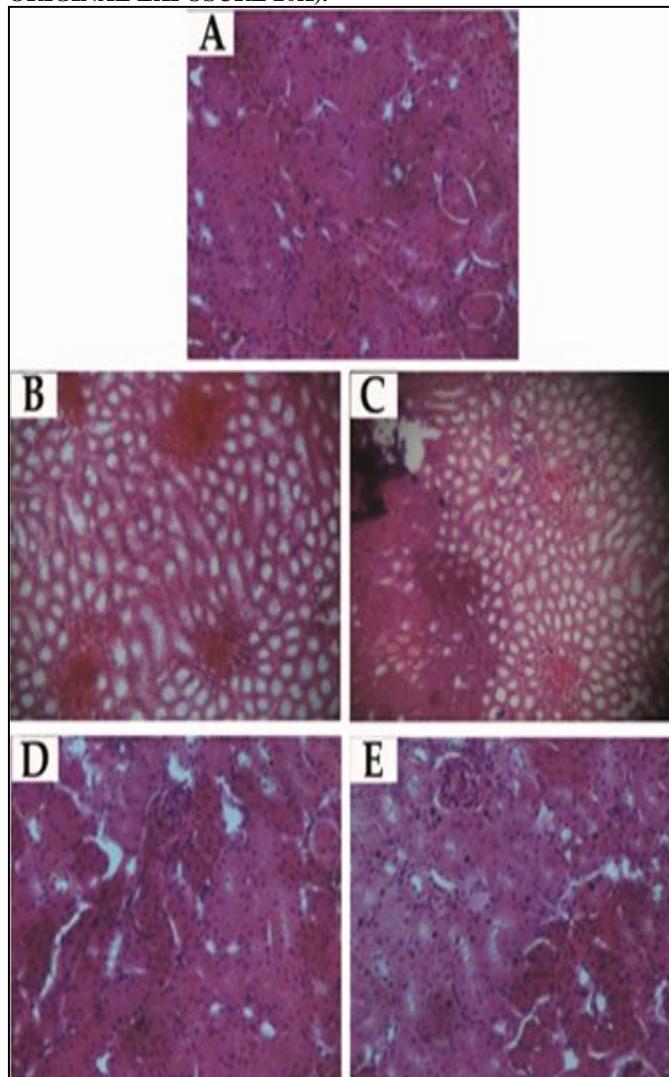


FIGURE 2: LIGHT MICROSCOPIC SLIDES OF KIDNEY SECTIONS OF MICE. A. KIDNEY SECTION OF CONTROL MICE SHOWS REGULAR STRUCTURE. B-E. KIDNEY SECTION OF TREATED MICE SHOWING VARIOUS HISTOLOGICAL ALTERATIONS. B REPRESENTS FOR TREATMENT GROUP 1. C STANDS FOR TREATMENT GROUP 2. D SYMBOLIZES TREATMENT GROUP 3 AND E REPRESENTS TREATMENT GROUP 4. (IMAGES ORIGINAL EXPOSURE 20X).

DISCUSSION: The animals were orally administered malathion with ordinary food for fifteen days and the microscopic slide of selected organs showed major histological changes in their liver and kidney tissues. Changes observed from light microscopes showed a varied scope of occurrence and different degree of intensity according to the dose of malathion. Mammals are liked to be harmfully affected by oral, dermal and inhalation administration to malathion^{23, 24}. Malathion not only toxic to mammals but also toxic to different kinds of non target organisms like as

fish, chicken and invertebrate. In the present study, the dose of group 1 (1300mg/kg) was the LD₅₀ of female mouse, dose of group 2 (260mg/kg) was five time dilution of LD₅₀, dose of group 3 (52mg/kg) was five times dilution of group 2 and the dose of group 4 (10.5mg/kg) was five time dilution of group 3.

Results from histological investigation are in accord with diverse earlier studies which indicated that the introduction to malathion and other pesticides led to provoke intensive physiological and biochemical turbulence in experimental animals, buffalo calves²⁵, goats²⁶, mice²⁷, cockerels²⁸, poultry²⁹, rabbits³⁰, and rats^{31, 32}. According to Tos-Luty et al.³³ malathion intoxication led to injurious effects on the organization of the liver and kidney with the persistence of thin subcapsular infiltrations, diffused parenchymatous degeneration of single hepatocytes, and the presence of tinny foci constructed of plasmatic cells, and histiocytes situated between hepatic plates.

The submicroscopic organization of hepatocytes showed lucid parts of cytoplasm carrying the residues of cell organelle and lipid vacuoles. Mitochondria were frequently distended, indicating a transference of the matrix and obliteration of crests. Furthermore, they indicated that the histopathological alteration in the kidneys occurred in all animals. These alterations demonstrated parenchymatous deterioration of the cells of renal tubules and hyperemia of the cortical area of the kidney, particularly of renal glomeruli, with infiltrations. A number of experiments indicated that malathion caused hepatotoxicity³⁴, testicular toxicity³⁵, hematotoxicity^{36, 37}, genotoxicity³⁸ and nephrotoxicity³⁷.

However, diverse works showed that malathion as well as other pesticides provoked histopathological alterations of liver and kidney in different rodent animals^{39, 40}. In conclusion, the results of present study indicated that, even oral LD50 value of malathion for male mice is 1300mg/kg, however, it can cause severe damage to hepatic and renal organization when exposure to such doses that much lower than LD50 value of malathion. The current experiment mainly focused on the fact that,

the duration (15 days) of exposure of malathion to experimental animal is less than many of other studies³⁷, but this short term exposure is enough to cause extent damage to hepatic and renal structure. Further investigations are needed to carry out for the discovery of probable therapeutic agent which completely detoxifies this toxic substance and searching out probable pesticide which is safe to use. Finally, the current work showed that the short term exposure and low doses besides high dose exposure of malathion is injurious to mice in approximately the same way to chronic treatment.

CONCLUSION: The present experiment indicated that the use of malathion as pesticide is injurious to health and as an important histological marker for pesticide toxicity. Therefore strict law need to be formulated to stop such oblivious use of malathion during applying in the field and it ought to be reflected in our health policy. In addition to the novel regulation, the law enforcement agencies of Bangladesh ought to be more watchful for appropriate implementation of the regulation. On the other side, Scientists have to explore and develop non pollutant and biological method to manage pest as an option of chemical insecticide. Eventually awareness should be spread among the general public about the continuous utilization of pesticide and their deleterious effect on public health.

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