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Protective effect of ginger and zinc chloride mixture on the liver and kidney alterations induced by malathion toxicity

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Abstract

This study was carried out on four groups of male Wistar rats, 10 rats per group. Group I got open access to food intake and water with normal balanced diet. Group II was administered 400 mg ginger per kg body weight (BW) and zinc chloride ($ZnCl_2$) (300 mg/L) diluted in tap water for 4 months. Group III was administered malathion at a dose of 50 mg/kg BW/day in 0.2 mL corn oil via gavages for 4 months. This dose equal to 1/50 of the LD50. Group IV was given a mixture of 400 mg ginger per kg BW and $ZnCl_2$ (300 mg/L) diluted in tap water in addition to 100 mg malathion/kg BW for 4 months. The liver showed histopathological changes include congestion, edema, and leucocytic infiltrations which were ameliorated by the addition of ginger and $ZnCl_2$ mixture. The kidney showed cloudy swelling and hydropic degeneration of the renal tubules. These changes were ameliorated by the addition of ginger and $ZnCl_2$ mixture. Ki67 immunoreactivity was localized in the cytoplasm and nuclear membrane. Its expression was estimated as the percentage of cells positively stained by the antibody in the different groups. In conclusion, malathion was toxic to the liver and kidney and must be avoided and protected by the addition of ginger and zinc mixture.

Keywords

ginger, Ki67, kidney, liver, malathion, zinc chloride

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Malathion is one of the most widely used organophosphate insecticides throughout the world. It is used to control the pests of agricultural crops, ornamental plants, greenhouses, livestock, stored grains, forests, buildings, and gardens. DDT and other pesticides have been found to cause irreparable damage to human and environmental health, however, malathion may pose a greater risk than the product label would lead one to believe. The toxicity of malathion is compounded by its metabolites and contaminants. The metabolites produced by the oxidation of malathion in mammals, insects, and plants is the primary source of malathion's toxicity and it is 40 times more acutely toxic than malaoxon.[1]

Ginger is one of the most important exogenous antioxidants that is used for prophylaxis and treatment of various diseases caused by free radicals.[2] The oleoresin from rhizomes of ginger contains phenolic substances that have been found to have anti-inflammatory, analgesic, antipyretic, cardiotonic, antioxidant, and anti-hepatotoxic effects.[3,4]

Zinc supplementation is also postulated as an adjuvant in the therapy of mood disorders.[5] Zinc also protects against oxidative liver damage induced by chronic alcohol ingestion,[6] organophosphate treatment.[7] The aim of the current study was to evaluate the protective effect of ginger and zinc chloride ($ZnCl_2$) mixture on the histopathological

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alterations in the liver and kidney induced by malathion toxicity.

Materials and methods

Animals

Forty male Wistar rats were 7 weeks old and weighed 200–250 g. Wistar rats were housed at room temperature (28 ± 2°C) with a 12/12-h light/dark cycle. Wistar rats were handled daily for 10 days to recover the stress and injection factors. These rats were raised in the King Fahd Research Unit in King Abdulaziz University, Jeddah, Saudi Arabia.

Chemicals

Malathion solution was purchased from Taif pesticide control market. Ginger powder and zinc chloride powder were purchased from Sigma Aldrich Company, Germany.

Experimental design

Four groups of male Wistar rats, 10 rats per group, were used for this study. Group I got open access to food intake and water with normal balanced diet as control group. Group II was administered 400 mg ginger per kg body weight (BW) and $ZnCl_2$ (300 mg/L) diluted in tap water for 4 months. Group III was administered malathion at a dose of 50 mg/kg BW/day in 0.2 mL corn oil via gavages for 4 months. This dose equal to 1/50 of the LD50.[8] Group IV was given a mixture of 400 mg ginger per kg body weight and $ZnCl_2$ (300 mg/L) diluted in tap water in addition to 100 mg malathion/kg body weight for 4 months. At the end of the experiment, the livers and kidneys were collected for histopathological and immunohistochemical studies.

Histological techniques

At the end of the experiments, small pieces of the liver and kidney were collected directly in Susa fixative. The samples were processed for general and special stains.[9]

Immunohistochemical study of Ki67

Deparaffinized sections were dehydrated in a graded series of alcohol solutions. Sections were incubated in antigen retrieval buffer (boiling the sections at 98°C for 20 min in 10 mmol/L sodium citrate buffer), treated with 3% H2O2 to block endogenous peroxidase. Monoclonal antibody (Anti-ki67, DAKO Corp.) were applied on the slides and incubated in humid chamber overnight in refrigerator at 4°C. Secondary biotinylated antibody was then applied, followed by incubation with streptavidin peroxidase (DAKO Corp.). Sections were washed with phosphate buffer saline (PBS) three times after each step. Sections were stained with diaminobenzidine chromogen solution (DAB), and counterstained with hematoxylin.[10]

Results

The liver of the first and second group had the same histological structure. It consisted of hepatic lobules around the central vein. Each lobule consisted of hepatic cords. The hepatocytes represented the hepatic cords and consisted of polygonal cells with centrally basophilic nuclei and shiny acidophilic cytoplasm (Figures 1a and 1b). The liver tissue of the third group had circumscribed areas of focal leucocytic infiltrations and congestion of the central vein with hemolysis (Figure 1c). The liver of the fourth group was characterized by congestion of the central vein and increased interlobular CT (Figure 1d).

Ki67 immunoreactivity was localized in the cytoplasm and nuclear membrane of the hepatocytes. Its expression was estimated as the percentage of cells positively stained by the antibody. The liver of the first group characterized by single ki67 positive cell (Figure 2a), while the liver of the second group characterized by double or triple ki 67 positive cells (Figure 2b). Numerous ki67 positive cells in the liver of the third group (Figure 2c). The liver of the fourth group returned back to be like the first group (Figure 2d).

The kidneys from the first and second groups were characterized by renal corpuscles and renal tubules (proximal convoluted tubules, distal convoluted tubules, and loop of Henle) in the cortex and collecting tubules in the medulla (Figures 3a and b). The kidney from the third group was characterized by cloudy swelling of the renal tubules (Figure 3c), desquamation of the tubular epithelial cytoplasm (Figure 3d), and hydropic degeneration in the renal tubules and collecting ducts (Figure 3e). The kidney from the fourth group showed regeneration of the renal tissues with some cells still showed desquamations (Figure 3f).

Ki67 immunoreactivity was localized in the cytoplasm and in the nuclear membrane of some cells in the renal corpuscles. The kidney from the first and second groups were characterized by single ki67 positive cells (Figure 4a and b). There were numerous ki67 positive cells in the collecting tubules of the third group (Figure 4c). The kidney from the fourth group showed weak Ki67 immunoreactivity (Figure 4d).

Discussion

Organophosphorus insecticides form the largest and the most diverse group of insecticides. The wide application of organophosphorus insecticides in public health and agricultural programs was accompanied by a potentially hazardous impact on humans, animals, plants, and the environment (water, air, soil, and food) and causes severe acute and chronic poisoning.[11] In fact, the toxicity of organophosphorus insecticides results in negative effects on many organs

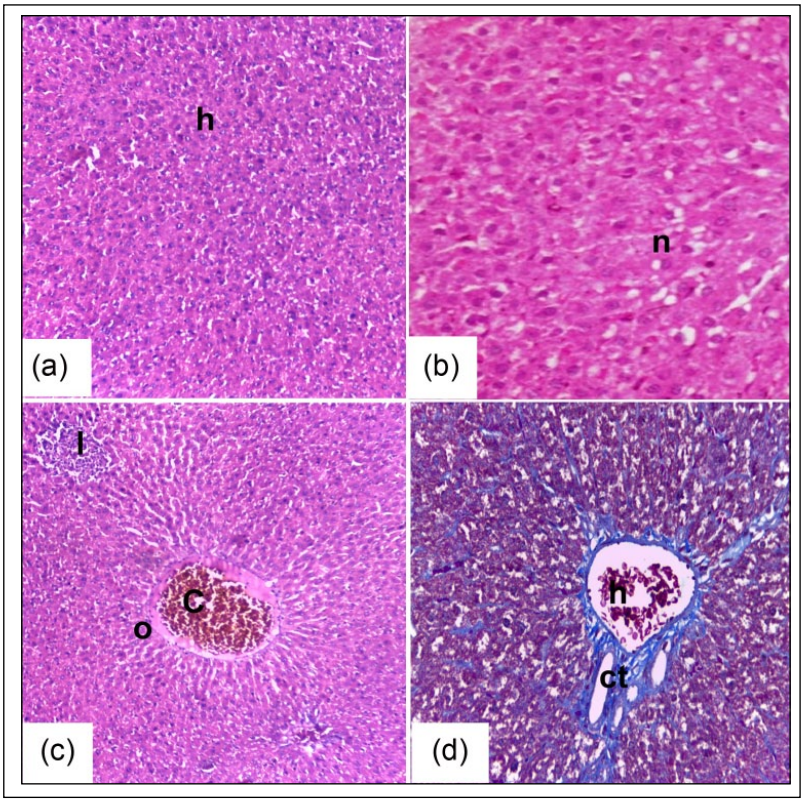

Figure 1. Photomicrograph of the liver. (a, b, H&E) Hepatic cords with polygonal cells and centrally located basophilic nuclei (n). (c, H&E) Congestion in the central vein (C) and hemolysis (O). Notice focal leucocytic infiltrations (l). (d, Masson trichrome) Congestion in the central vein (h) and increase interlobular CT (ct) (×20).

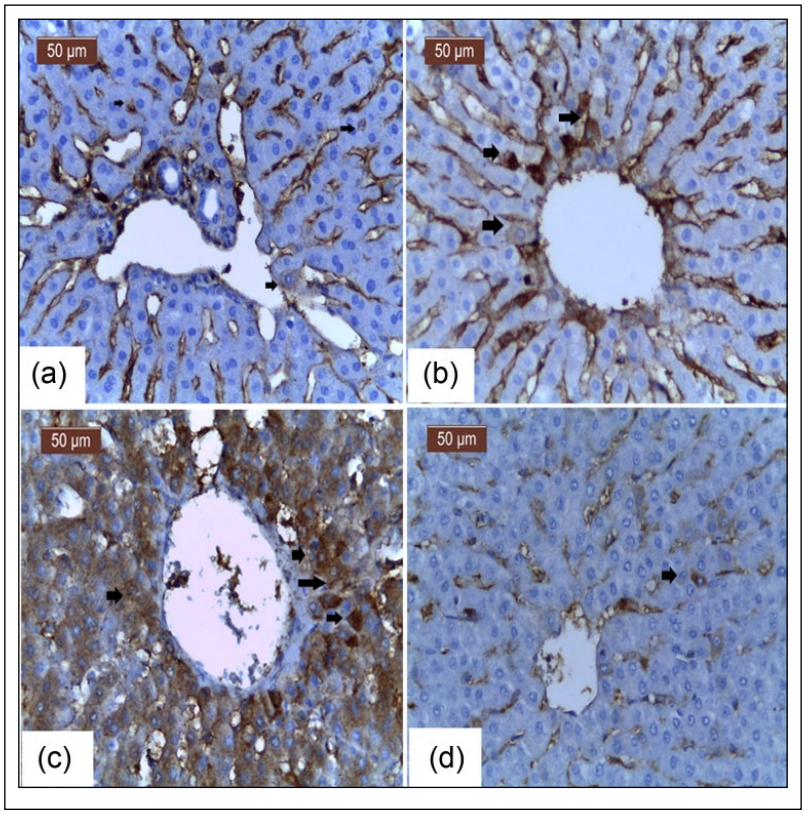

Figure 2. (a–d) Photomicrograph of the liver showing Ki67 positive cells with strong intensity of immunostaining in hepatocytes cytoplasm and nuclear membrane (arrows). Note the cells with cytoplasmic staining (arrows). IHC of Ki67 – bar = 50µm.

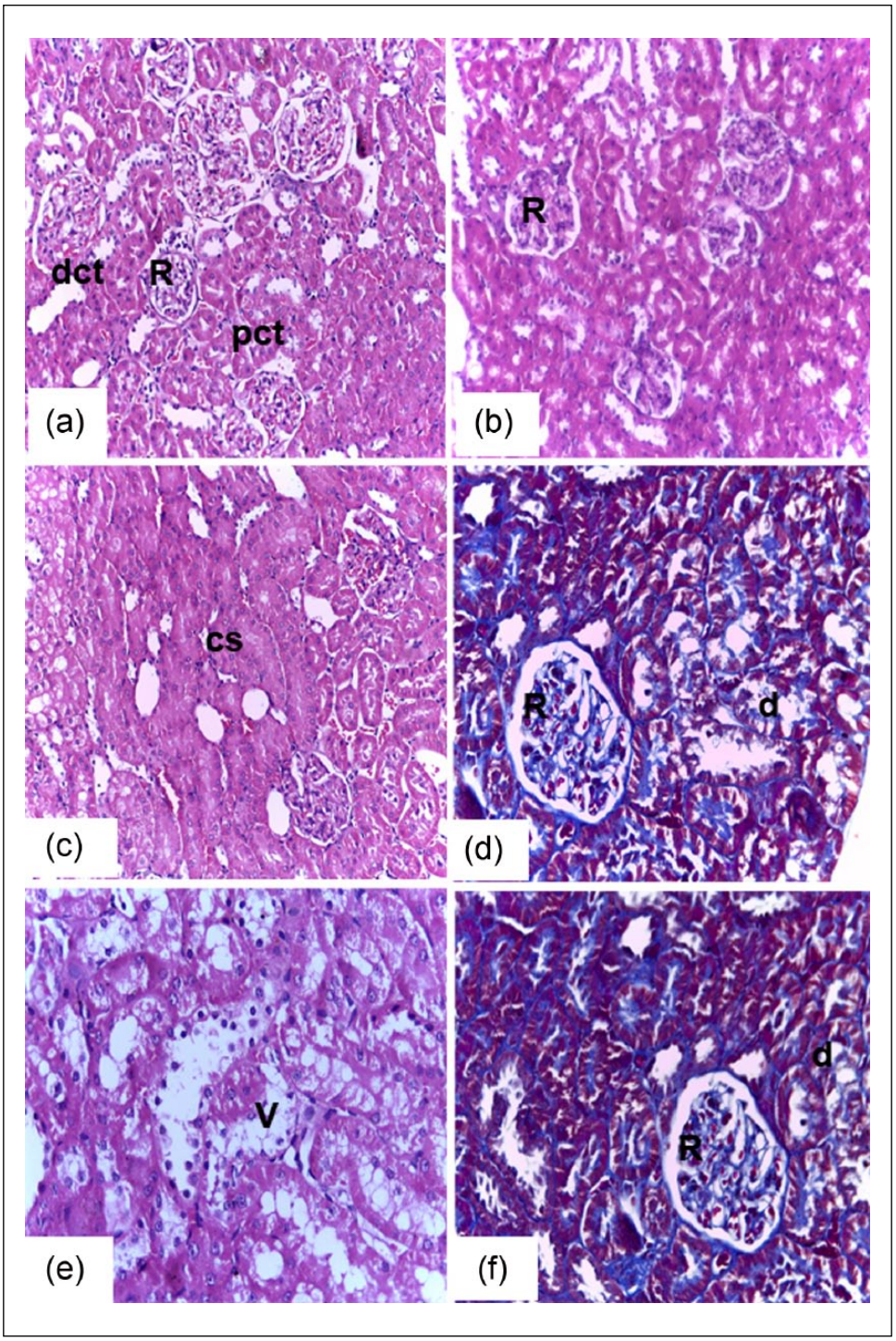

Figure 3. Photomicrograph of the kidney showing. (a, b, H&E) Renal corpuscles (R), proximal convoluted tubules (pct), and distal convoluted tubules (dct). (c, H&E) Cloudy swelling of the renal tubules (cs). (d, e, Masson trichrome and H&E) Hydropic degeneration of the renal tubules (V) and desquamation (d). (f, Masson trichrome) Renal corpuscle (R) and some cells of the tubules showed desquamation (d) (×20).

and systems such as the liver, kidney, nervous system, immune system, and reproductive system.[12,13]

The liver is one of the organs affected by malathion toxicity. It showed degenerative changes in the form of congestion of the central vein and focal areas of leucocytic infiltration in the hepatic architecture. These findings were supported by the findings in several studies.[14–17] The susceptibility of liver tissues to this stress due to exposure to

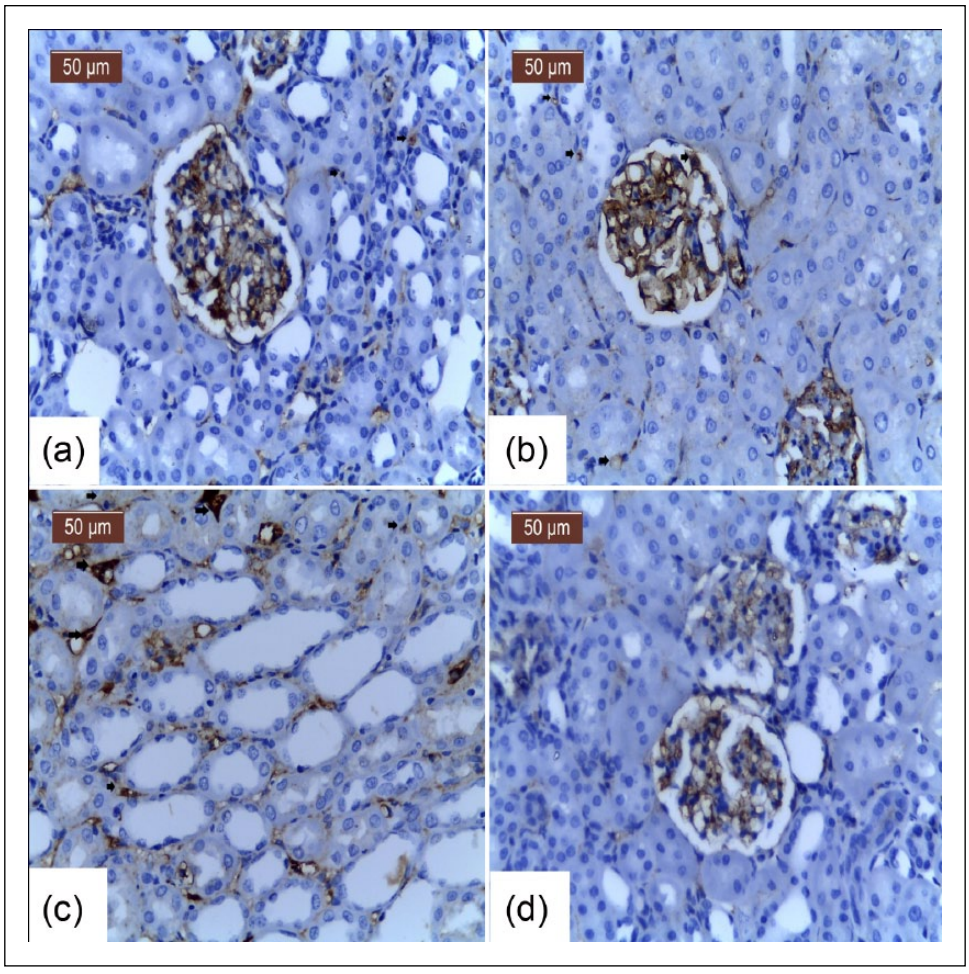

Figure 4. (a–d) Photomicrograph of the kidney showing Ki67 positive cytoplasm with faint intensity of immunostaining in renal cell nucleus (arrows). Note the cells with cytoplasmic staining (arrows). IHC of Ki67 – bar = 50µm.

pesticides is a function of overall balance between the degree of oxidative stress and the antioxidant capacity.[18]

Ginger extract possesses an antioxidative characteristic, since it can scavenge superoxide anion and hydroxyl radicals.[19] Ginger has a strong antioxidant and anti-hepatotoxic effects in treated rats.[20] The main constituents of ginger are gingerol, shagaols, zingerone, and paradol. It was reported that 6-gingerol and 6-shogaol are the major gingerol and shogaol present in the rhizome.[21] Therefore, the addition of ginger and zinc mixture leads to amelioration of the toxic effect of malathion due to its antioxidant effect.

The kidney is one of the target organs of experimental animals attacked by organophosphorous compounds.[13,22] In the present study, the renal tissues showed degenerative changes including cloudy swelling, hydropic degeneration, and cellular desquamations. These results were augmented by the findings of lesions in the kidney tissues of fish exposed to malathion. These were characterized by degeneration in the epithelial cells of renal tubule, pycnotic nuclei in the hematopoietic tissue, dilation of glomerular capillaries, degeneration of glomerulus, intracytoplasmatic vacuoles in epithelial cells of renal tubules with hypertrophied cells, and narrowing of the tubular lumen.[23] Administration of malathion to rats caused significant radioactivity to the liver, kidneys, and ileum.[24]

Ki67 is one of the many antigens protein that has been used as a proliferation marker for cancer cells.[25] The assessment of the presence of cell cycle-related proteins may yield important information about the biological behavior of a tumor.[26] Ki67 is a protein associated with active cell proliferation and expressed in all phases of the cell cycle, except G0, with the highest expression seen in G2/M.[27] The monoclonal antibody of Ki67 has been developed and used in evaluating cellular proliferation rates of malignant tumor.[28,29] Ki67 expression was associated with prognosis in prostate, breast, and lung cancer.[30–33] So the application of Ki67 immunohistochemistry in the liver and kidney toxicity can indicate the protective effect of ginger and $ZnCl_2$ mixture.

In conclusion, the toxicity induced by malathion can be ameliorated by the addition of ginger and $ZnCl_2$ mixture during the exposure to the insecticides.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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