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Research Article
Short-time Effects of Malathion Pesticide on Functional and Histological Changes of Liver and Kidney in Female Mice

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Abstract

Background and Objective: The malathion is one of the most important organophosphorus pesticides used in Iraq. The present study was designed to investigate the short-time effects of the malathion on the biochemical parameters of AST, ALT, ALP, urea, creatinine, total cholesterol, triglycerides and total protein as well as histological changes of the liver and kidneys of female laboratory mice for an interval of 6 days. **Materials and Methods:** The animals were divided into 3 groups, each group included 8 mice. They were injected with the pesticide in the intraperitoneal region. The 1st group (the control group) was injected with 0.1 mL of distilled water, the 2nd group (the low dose group) was injected with 0.1 mL of the pesticide solution at 3 mg/body weight while the 3rd group (the high dose group) was injected with 0.1 mL of the pesticide solution at a concentration of 6 mg/body weight. **Results:** The biochemical tests of the liver and kidney showed significant elevation in serum AST, urea, creatinine and cholesterol concentrations in mice compared to control group ($p \leq 0.05$). In addition, the results showed a significant decrease in the ALP, triglycerides and the total protein in serum of the treated mice. Also, the results of histological sections of the liver and kidneys included congestion, necrosis, degeneration of cytoplasm, blood congestion, apoptosis, bleeding and sloughing of epithelial cells to the renal tubular lumen. **Conclusion:** Finally, the results indicated that malathion pesticide has the ability to induce hepatic and renal toxicity in mice within 6 days.

Key words: Malathion, liver, kidney, lipid profile, urea, histopathology, cholesterol, triglycerides

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Malathion pesticide is widely used all over the world in general and in Iraq in particular to eliminate pests of poultry and various agricultural crops, combate household insects and protect stored grains[1,2]. Organophosphorus pesticides cause harmful effects to humans and animals through inhalation, skin contact or contamination of water and food with pesticides[3-5]. The main mechanism of organophosphorus toxicity in the nervous system is the inhibition of the enzyme acetylcholinesterase[6].

The liver is an essential organ for metabolizing many chemicals by biotransformation, with the kidneys participating in the disposal of toxic products[7]. Therefore, these organs are a major target for malathion toxins[8]. Exposure to malathion for long intervals can damage the nervous system[9] as well as induce cancer, mutation and DNA mutilation[10].

Some of the problems of chronic exposure to pesticides are to have cancer, neural and immune toxicity and its effect on growth and reproduction[11]. Many previous studies have shown the effects of the chronic administration of the pesticides on laboratory animals as the chronic administration of diazinon in mice for an interval of 45 days resulted in inducing histological changes in spleen, thymus and lymph nodes[12]. Baconi *et al.*[13] reported that exposure to malathion and diazinon in laboratory mice for 35 days caused histological and biochemical changes in the liver and kidneys. Moreover, Hernandez *et al.*[14] examined the chronic exposure of farmers to organophosphorus pesticides. They noticed that there were significant changes in some biochemical indicators, such as AST, ALT, CK, LDH, urea, creatinine and lipid profile. Furthermore, the injection of laboratory mice with Dimethoate for 30 days caused negative changes in the function of the liver and kidneys accompanied by histopathological damages in the form of degeneration, necrosis, bleeding, congestion of blood vessels, cellular dissection and inflammatory cell infiltration[15]. Also, the injection of male laboratory mice with dichlorvos for 15 days led to changes in the levels of protein, total cholesterol and AST enzyme as well as a functional disorder in fertility[16,17].

Although there are many studies on the chronic administration of pesticides, there is a lack of research on the effect or short-time administration of malathion, therefore the present study was conducted to examine the effect of malathion on the biochemical and histological parameters of the liver and kidneys of female laboratory mice.

MATERIALS AND METHODS

Laboratory animals and chemical: Adult female mice *Mus musculus* L. weighting (23-25 g) and 12 weeks old were used. The animal were provided by the animal house of biology Department, College of Education for Pure Science, University of Basrah, Iraq. The mice were maintained under controlling conditions of temperature (25±2) and photoperiod 12 h light/12 h darkness. They were fed on standard laboratory pellet chow and water *ad libilum*. The malathion pesticide powder (1 kg) was supplied from local market, Vapco company Ltd. Jordan.

Experimental design and serum preparation: Twenty four female mice were divided randomly into 3 groups, each group contain 8 mice. Mice were daily injected with malathion intraperitoneally (i.p.) for 6 days. Malathion solutions were prepared by dissolving the powder in distal water. The control group were given 0.1 mL of distal water only. Whereas, the treating groups were received pesticide doses as following: low dose group were injected with 0.1 mL of malathion solution at 3 mg/body weight, while the animals in high dose group were injected with 0.1 mL of malathion solution at 6 mg/body weight. Later, the animals were anaesthetized with chloroform after the last day injection. Blood samples were withdrawn from heart by 1 mL plastic syringe. Then blood was transferred to tubes without anticoagulant. The serum was separated by centrifuge at (3500 rpm/15 min) and stored at -20°C to be used in subsequent biochemical tests. The study was carried out at Biology Department, Animal physiology Lab from January, 2018-October, 2019.

Biochemical assays: Serum concentration of alanine transaminase enzyme (ALT), aspartate transaminase enzyme (AST), alkaline phosphatase enzyme (ALP), urea, creatinine, total protein, triglyceride and cholesterol were measured by spectrophotometer kits which were provided by Biolabo and biomerieux companies, French. Procedure instruction of each kit was followed[18-22].

Statistical analysis: Statistical Package for the Social Sciences (SPSS ver.23) was used to analyze the results statistically and the significance was tested using analysis of variance (ANOVA) and the least significant difference LSD under the probability level ($p \leq 0.05$).

RESULTS

Liver functions tests: The effect of malathion treatment in liver function tests are shown in Table 1. The serum AST level was significantly elevated at ($p \leq 0.05$) in compare with the control group and there was a significant decrease in serum ALP. No significant changes were found in serum ALT level.

Kidney functions tests: Statistical analysis showed a significant increased in serum creatinine and urea levels with both doses (3.6 mg/day) of malathion pesticide at ($p \leq 0.05$) when compared with the control group (Table 2).

Lipids and total protein tests: The malathion pesticide caused a significant increase in cholesterol level of female mice treated with both doses at ($p \leq 0.05$), whereas the results revealed a significant decrease of serum triglyceride level in two doses compared with the control group. The results also showed a significant decrease of serum total protein level in high dose (6 mg/day) only (Table 3).

Histological effects: Histological examination of the female mice liver and kidney treated with malathion pesticide of both doses (3 and 6 mg/day) revealed many histopathological changes. Histological sections of liver showed congestion of sinusoids, infiltration of inflammatory cells, accumulation of hyalin materials in hepatocytes, necrosis, malformation of nucleus, degeneration of cytoplasm, aggregation of inflammatory cells, blood congestion and apoptosis were observed (Fig. 1b, 2f) compared with normal histology of liver (Fig. 1).

The kidney section was normal in control mice (Fig. 2g, h). Injection of malathion of both doses caused the aggregation of inflammatory cells in female mice kidney, bleeding, degeneration, sloughing of epithelial cells to renal tubular lumen, necrosis, malformation in nucleus and hyalin materials were observed as in (Fig. 3a-h).

DISCUSSION

The results of the current study showed a significant increase in the level of AST and decrease in the level of ALP in female mice treated with malathion. This finding might be related to the biological activity of organophosphorus pesticides which cause a superior generation of reactive oxygen species (ROS)[23]. Consequently, increasing the concentrations of ROS cause an obvious tissue damage and oxidation of lipids, proteins and DNA[24]. The increase of AST concentration in plasma is certainly an indicator of cellular damage. This increase was caused by the necrosis and damage of hepatic cell membranes that lead to the release of their enzymes toward circulation[25,26]. Malathion significantly restrains the reduction ability and inhibition of various antioxidants, such as superoxide dismutase, glutathione peroxidase and catalase[27], which negatively influence the level of malondialdehyde (MDA) and lipoperoxidation in liver and kidney tissues[28]. The malondialdehyde produced due to pesticide toxicity is toxic, mutagenic and inhibitory for enzymes. Subsequently the decrease in the effectiveness of antioxidants and an increase in the level of MDA cause biological disorders through the generation of free radicals[29], especially in liver where is an important site for metabolism of

Table 1: Effect of malathion on liver function tests in female mice (n = 8) (Mean±SD)

Treatments	AST (IU L^{-1})	ALT (IU L^{-1})	ALP (IU L^{-1})
Control (Distilled water)	76.84±6.60[a]	53.57±3.04[a]	118.42±11.95[a]
Low dose malathion (3 mg/day)	93.88±7.37[b]	57.49±4.57[a]	66.50±9.13[b]
High dose malathion (6 mg/day)	96.13±12.09[b]	49.63±4.31[a]	53.99±6.67[b]

[a,b]Significant difference ($p \leq 0.05$) compared with the control group

Table 2: Effect of malathion on kidney function tests in female mice (n = 8) (Mean±SD)

Treatments	Creatinine (mg dL^{-1})	Urea (mg dL^{-1})
Control (Distilled water)	0.60±0.11[a]	38.02±3.62[a]
Low dose malathion (3 mg/day)	1.91±0.33[b]	44.38±4.06[b]
High dose malathion (6 mg/day)	2.02±0.24 [b]	51.36±5.10[b]

[a,b]Significant difference ($p \leq 0.05$) compared with the control group

Table 3: Effect of malathion on lipids and total protein in female mice (n = 8) (Mean±SD)

Treatments	Cholesterol (mg dL^{-1})	Triglyceride (mg dL^{-1})	Total protein (g dL^{-1})
Control (Distilled water)	110.77±11.57[a]	149.63±12.95[a]	7.68±0.86[a]
Low dose malathion (3 mg/day)	133.08±10.91[b]	124.43±14.76[b]	7.52±0.55[a]
High dose malathion (6 mg/day)	154.35±13.25[b]	94.98±8.85[b]	6.44±0.35[b]

[a,b]Significant difference ($p \leq 0.05$) compared with the control group

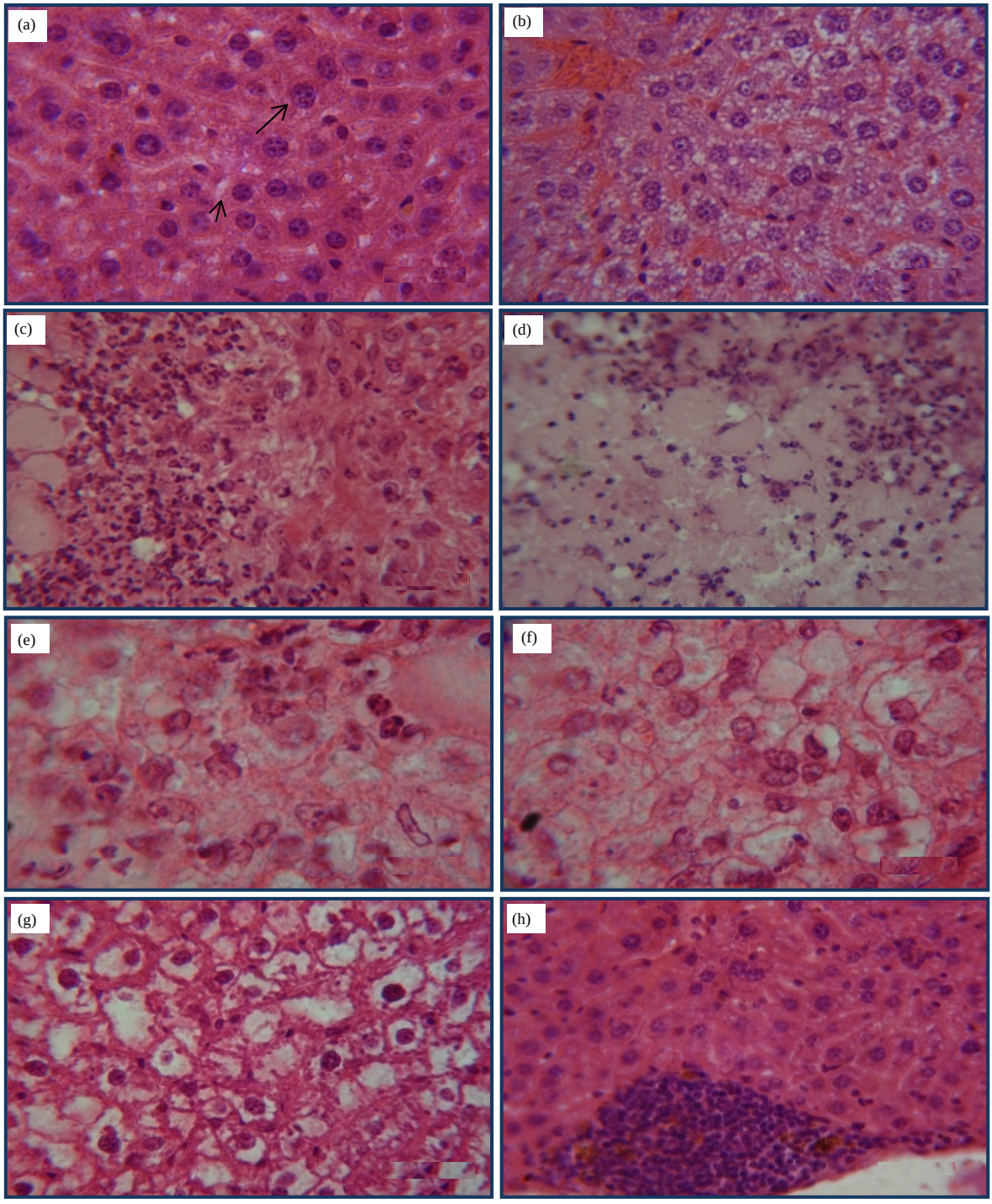

Fig. 1(a-h): (a) Liver section from a control female mice shows normal hepatocyte (arrow) and hepatic sinusoid (arrow heads), (b) Liver section from treated mice with malathion shows congestion of sinusoids, (c) Liver section from treated mice with malathion shows infiltration of inflammatory cells, (d) Showing accumulation of hyalin materials in hepatocytes, (e) Liver section from treated mice with malathion shows necrosis, (f) Shows malformation of nucleus, (g) Liver section from treated mice with malathion shows degeneration and (h) Liver section from treated mice with malathion shows aggregation of inflammatory cells

All Liver sections from treated mice with malathion (3 and 6 mg /for 6 day) were stained with Hematoxylin and Eosin (400X)

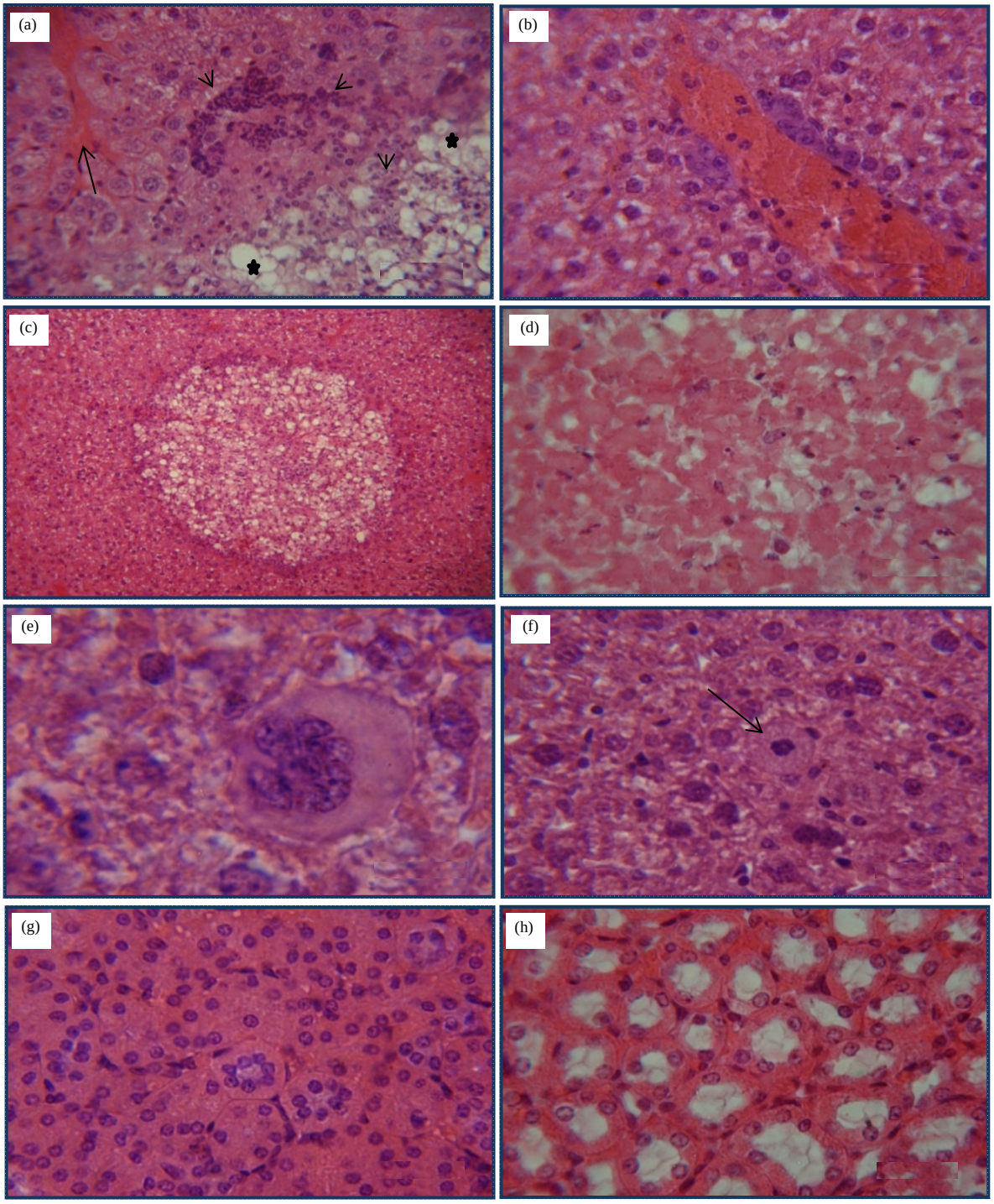

Fig. 2(a-h): (a) Liver section from treated mice with malathion shows congestion of sinusoids (arrow), inflammatory cells (arrow heads) and degeneration (stars), (b) Liver section from treated mice with malathion shows blood congestion, (c) Shows nodule of degeneration (200X), (d) Shows also accumulation of hyalin materials in hepatocytes and disappear of nucleus, (e) Liver section from treated with malathion shows giant cell (1000X), (f) showing apoptosis (arrow) and (g, h) Kidney section from control mice shows renal tubules

All Liver sections from treated mice with malathion (3 and 6 mg /day) and control kidney sections were stained with Hematoxylin and Eosin (400X)

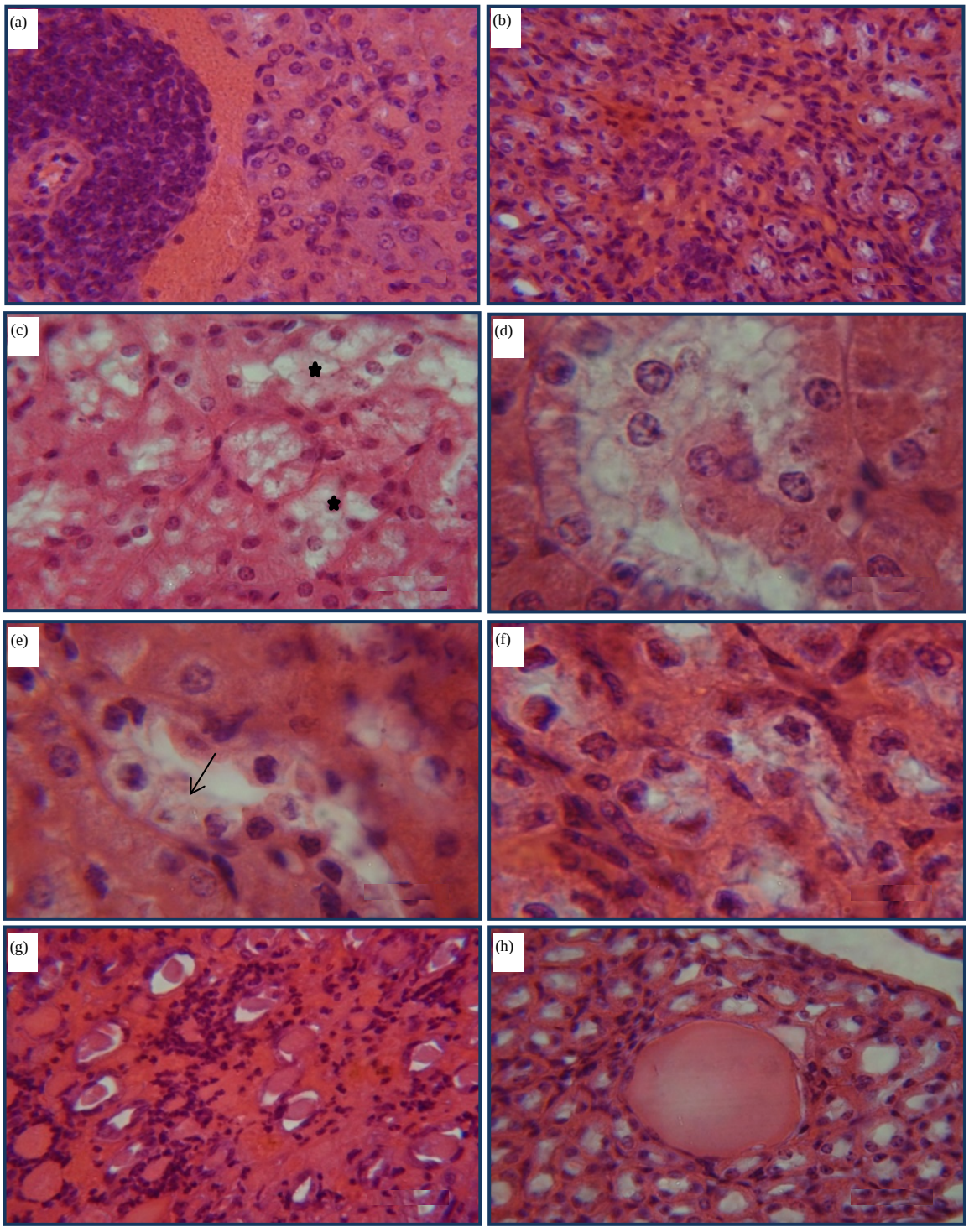

Fig. 3(a-h): (a, b) Kidney sections from treated mice with malathion of both doses show aggregation of inflammatory cells and bleeding, (c) Kidney section from treated mice with malathion shows degeneration (stars), (d) Shows sloughing of epithelial cells to renal tubular lumen, (e) Shows necrosis of renal cells (arrows), (f) Kidney section shows malformation in nucleus of renal cells and (g, h) Kidney sections from treated mice with malathion shows hyalin materials in renal tubules

All kidney sections from treated mice with malathion of both doses (3 and 6 mg/day) were stained with Hematoxylin and Eosin (400X)

different substances such as drugs and pesticides[30]. Thus, in current circumstance the hepatotoxicity was generated by free radicals and oxidative stress that act as oxidants to damage or disable enzymatic defense systems[31-34]. Many studies have shown that malathion works to break down fats of membranes through oxidative stress[35].

The ALP enzyme plays an important role in biological processes, including the toxic removal, metabolism and biosynthesis necessary in the different functions in cells. So that, any disturbance in this enzyme contributes to biochemical changes and damage of tissues and their cell functions[36]. The decrease in ALP may be attributed to damage in the hepatocytes caused by the toxicity of the pesticide, which caused damage and fracture in the DNA which reflected negatively in not creating the mRNA strand responsible for synthesis this enzyme[37,38].

In fact, the injection of female laboratory mice with malathion caused a significant increase in both urea and creatinine. These findings were consistent with Selmi *et al.*[39] and Yassin and Al-Shanti[40] results which confirmed that the treatment of mice with organophosphorus pesticides caused the changes in functional and histological tests of the kidneys. It is possible that the reason for this change in the level of urea is the decrease in the filtration rate as a result of the physiological impairments in kidney functions. Extensive exposure to pesticides induces toxic changes in biochemical parameters in the liver and kidneys[41]. Creatinine is a metabolic waste that is excreted with urine, its proportion increases with kidney malfunctions[42,43]. The main reason behind the increase in the urea concentrations and creatinine were renal tubular insufficiency, poor glomerular filtration and kidney damage due to exposure to pesticides[44].

Increasing of the total cholesterol and a decrease in the level of both triglycerides and total protein were detected in current findings. In general, organophosphorus pesticides increase the total cholesterol[45] and this increase can be explained by the effect of pesticides on the permeability of the cellular membranes of the liver[46] In addition, it may cause blockage of the bile duct and reduction in the secretion of cholesterol to the duodenum[47]. On the other hand, malathion may lead to induction of dyslipidemia as a result of hyperglycemia and insulin resistance, which increase lipids[45]. Lower level of the total protein could be caused by the qualitative and quantitative disruption in the production of proteins resulted from the impairments in liver function[48]. For instance, the organophosphorus pesticide Chlorpyrifos caused an acute shortage of the albumin protein produced in the liver and so that the depletion in the level of this protein can be associated with liver disease. Organophosphorus pesticides, including malathion, may alter the metabolism of proteins and free amino acids and their synthesis in the liver[49]. The results of the histological examination clarified in the images of the liver and kidney tissues of female mice treated with malathion showed histopathological changes. Organophosphorus pesticides are known to induce various histopathological changes in the liver and kidneys[50,51]. These include bleeding, infiltration of inflammatory cells and necrosis[52]. Histological damage may generally arise from the toxic effect of malathion in disrupting the mechanism of toxic removal in the liver[53]. There are a lot of research showing the harmful effects of chemicals and pesticides that are primarily responsible for the chemical and pathological changes in the liver and kidney tissues, which included necrosis, nuclear pyknosis and cytoplasmolysis, which lead to weakening and dissociation of tissues[51,54]. Moreover, it is more likely that the disruption effects of organophosphorus pesticides are in the histoarchitecture of the liver which is associated with metabolic capacity. In turn, these changes may interference with the common enzymatic pathways in the metabolism of fats, carbohydrates and proteins in the cytoplasm, mitochondria and peroxisomes[55]. The long-term treatment with malathion for four weeks caused hepatomegaly, necrotic damage, cytoplasmic vacuolation around the nuclei, sinusoid expansion and atrophy of the hepatocytes of rat[56]. Malathion and Diazinon also induced apoptosis of the cells[57]. In addition, a high dose of these two pesticides has led to swelling of hepatic cells, nuclei change, degeneration and cytoplasm vacuolization[58,59].

The inflammations in different tissues occur as a result of exposure to toxic substances, that to a serial of physiological and histological changes such as, congestion of blood vessels, lack of normal providing of cells with oxygen and nutrients, that accompanied with the beginning of necrosis in the regions surrounding the inflammatory areas[60]. Bleeding in the kidney may explain the effect of toxins on the cellular connections that bind the endothelial cells with the blood vessels[61]. It has been proven that the toxic compounds impede the phosphorylation of proteins. Subsequently it leads to an imbalance in the process of building and polymerization the components of the cellular structure and then leads to blood leaking from the blood vessels into the surrounding tissues[62].

CONCLUSION

In light of what has been obtained from the results of the current study, it can be noticed that the malathion pesticide has a great ability to induce changes and damage in the

biochemical and histopathological criteria of the liver and kidneys during short time of exposure to the 2 doses. The reason for these damages may be attached to the toxic potential of the pesticide to generate super free radicals and to the oxidative stress to the destruction of cellular membranes.

SIGNIFICANCE STATEMENT

This study discovers the adverse effects of malathion on liver and kidney that can be reflected the toxicity of pesticide. This study will help researchers to know the biological hazards of pesticide usage in controlling pests as well as environmental pollution. It is noticed that many farmers are unaware of the seriousness of pesticides. Thus, the new theory shows that this pesticide is caused toxicity in short time.

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