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HISTOLOGICAL INVESTIGATION OF HEPATIC TISSUES OF MALE MICE TREATED WITH *OLEA OLEASTER* LEAVES EXTRACTS AGAINST MALATHION TOXICITY

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ABSTRACT

People of all ages and professions can be exposed to chemicals through the soil, water, air, food, and the impacts of chronic and acute poisonings have been well established for the common and noxious toxins. Malathion (MAL) is an organophosphate insecticide that disrupts the body's antioxidant system; it is one of the earliest organophosphate insecticides extensively used as dust, emulsion, and vapor to control a wide variety of insect pests under different conditions. This study was designed to investigate the histological effect of *O. oleaster* leaves extracts on male mice hepatic tissues against malathion toxicity. Experimental mice were acclimatized for one week before experimental treatments. After the acclimatization period, the mice were divided into four groups. Mice of the first group were served as control. The experimental animals of the second group were exposed to malathion. Mice of the third group were treated with malathion at the same dose given to the second group and orally supplemented with *O. oleaster* leaves extracts. The animals of the fourth group were treated with *O. oleaster* leaves extracts. At the end of experimental period (8 weeks), mice of group 2 showed significant reduction of body weight and severe hematological and biochemical alterations including significant increases of ALT, AST, ALP and total bilirubin values. Severe histopathological alterations of liver tissues were observed in exposed MAL-intoxicated mice. Administration of the *O. oleaster* leaves extracts reduced the detected biochemical, histopathological modifications caused by MAL intoxication.

Keywords: Malathion; Olea oleaster; Mice; Liver; Toxicity

1- INTRODUCTION

Environmental pollution is the contamination of the ecosystem that causes harm or discomfort to the physical systems or living organisms. The increase in pollution is a global problem, due to the use of toxic chemical substances or by synthetic compounds such as pesticides. Humans whose position in the food chain is at the top were exposed to various types of environmental contaminants at different stages of life, majority of which are harmful. An increasing number of natural and man-made pollutants have pervaded the environment in the last few decades, which ultimately affect the health and well being living organisms. Health risks due to pesticides toxicity are one of the world's current problems. Environmental pollutants are believed to be factors adversely affecting animal and human organisms **[1, 2]**. Pesticides are widely utilized chemicals in agriculture, intended to preserve the productivity of crops and the quality of harvests. The universal use of assorted groups of pesticides causes global environmental pollution, as well as the accidental exposure of humans to these pesticides **[3]**. Exposure to pesticides may be a major cause of various disorders in humans and animals **[4]**. Malathion is one of the most widely used organophosphorus insecticides throughout the world. Malathion is used mostly in agriculture and in public health programs to control infestations of insects. It is also used as pest control for agricultural food and feed crops **[5]**. Malaoxon, the oxon generated from malathion, is more toxic than malathion and is formed by the oxidation of malathion and may also be present as an impurity in the parent compound. Structurally, malathion has similarities with naturally occurring compounds, and their primary target of action in insects is the nervous system **[6, 7]**. One of the main toxic effects of malathion on the central nervous system is related to the inhibition of acetylcholinesterase (AChE) activity, which produces acute hypercholinergic syndrome **[8]**. Moreover, previous experimental investigations showed that malathion induced various biochemical and histological changes in experimental animals **[9-12]**.

Nature has been a source of medicinal treatments for thousands of years and plant-derived products continue to play an essential role in the primary health care of about 80-85% of the world's population. Despite the trends of molecular biology and chemistry providing fast escalation of synthesized *de novo* drugs, plants still remain a traditional source of medicinal compounds; up to 40% of modern drugs may directly or indirectly be related to natural compounds **[13]**. Phytotherapy consists of the use of medicinal plants in

order to prevent, cure or threat illnesses **[14]**. Kingdom of Saudi Arabia has abundant and wide variety of medicinal plants whose therapeutic effects have not been adequately studied **[15]**.

The wild olive trees *O. oleaster*, family *Oleaceae,* and those olive trees originated in the southwest of Saudi Arabia and eastern Mediterranean. This plant contains secoiridoids such as oleuropein, ligostroside, dimethyl oleuropein and oleoside, flavonoids, phenolic compounds, such as caffeic acid and tyrosol **[16]**. Moreover, this plant until now in all studies showed that olives extracts are used to provide nutrients, and help fighting against a variety of illnesses also as control weight loss-fat diet **[17]**. Additionally, **[18]** reported that olive leaf extract has a natural antioxidant and it influences the gastric defense mechanism, gastroprotective,. Although extracts from olive leaves and olive fruits exhibited a protective role and induced DNA damage by themselves **[19]**. The efficacy of olive leaf extract on metabolic, hemodynamic and anthropometric measures was studied in a clinical trial in borderline hypertensive monozygotic twins. A significant reduction in both systolic and diastolic blood pressure was observed in borderline hypertensive monozygotic twins **[20]**. Experimental animal studies on different total olive leaf extract or their constituents have demonstrated, hypotensive, anti-atherosclerotic, antiarrhythmic and vasodilator effects **[21, 22, 23]**. Antimicrobial, antiviral, anti-tumor, anti-inflammatory activity and anti-diabetic effect were also reported **[24-27]**. Moreover, **[28]** concluded that the pretreatment of carbendazim (a fungicide)-exposed male rats with olive leaves extract showed marked improvement in both physiological and histopathological alterations. They suggested that the olive leaves extract is a promising chemotherapeutic agent for reducing the toxicity of carbendazim. **[29]** suggested that the extracts of tea and olive leaves and their combination can be considered as promising therapeutic agents against hepatotoxicity, cardiotoxicity, nephrotoxicity, and metabolic disorders induced by exposure to diazinon (an organophosphorus insecticide) in male mice. Additionally, **[30]**, showed that the olive leaves extracts possess hepatoprotective properties against hepatic cirrhosis induced by thioacetamide in rats and mice.

This is the first study designed to evaluate the effect of *Olea oleaster* leaves extracts on the toxicity induced by malathion in male mice. This study will add significant understanding to the body of knowledge of the activities of *Olea oleaster* leaves extracts against the toxicity of malathion and may be

eventually lead to a preventive treatment for the toxic effect of malathion. The possibility of using the results of the current study in the fields of medical treatments and pharmaceutical, and strengthen the areas of the use of natural products and herbal extracts for the treatment of the toxic effect of malathion and other pollutants instead of synthetic chemical drugs which have harmful side effect and more expensive. The anticipated positive findings from the proposed study will lead to further studies to better understand the mechanism of their protective action, and thus this study opens up new areas of scientific investigations in the fields of biomedical science.

2- MATERIALS AND METHODS

Experimental animals:

Fourty matured male albino mice weighing 23.0–27.0 g was taken for the present study. The principles of laboratory animal care were followed throughout the duration of experiment and instruction given by King Abdulaziz University ethics committee was followed regarding experimental treatments. The mice were distributed into four groups (ten mouse per group) and were housed in standard cages at an ambient temperature of 20 ±1 _C with 12-h light:12-h dark cycle and humidity of 65%. The mice were fed ad libitum on normal commercial chow and had free access to water.

Experimental design: The animals were divided into four groups, each of which contained 10 mouse and they randomized into the following groups:

Group1: The mice of the first group were untreated and served as control.

Group 2: The experimental animals of the second group were orally treated with malathion as dose of 150mg/kg body weight, BW, five times weekly for a period of eight weeks.

Group 3: The mice of the third group were orally supplemented with *O. oleaster* leaves extracts at a dose of 800 mg/kg BW and after three hours treated with malathion as same dose given to group 2.

Group 4: The animals of the fourth were orally supplemented with *O. oleaster* leaves extracts same dose given to group 3.

Extraction of *O. oleaster*

The fresh leaves of *O. oleaster* were directly collected from the outskirts of Albaha region of Saudi Arabia. The collected leaves were completely washed, air dried at room temperature and stored in a dry plastic container until use for extraction processes. The method of **[29]** was used to prepare the extracts. The dried leaves of *O. oleaster* (50 g) were powdered, added to 2 liters of cold water and mixed using an electric mixer for 20 min. Thereafter, the solutions of *O. oleaster* was gently filtered. Finally, the filtrates were evaporated in an oven at 40 °C to produce dried residues (active principles). With references to the powdered samples, the mean yield of *O. oleaster* was 19.3%.

Furthermore, these extracts were stored in a refrigerator for subsequent experiments.

Body weight changes

Body weight changes of all mice were measured at the start of the experimental duration and after eight weeks using a digital balance. The experimental mice were observed for signs of abnormalities throughout the period of study.

Biochemical Analyses:

At the end of experimental period, rats were fasted for 6 hours and anaesthetized with diethyl ether. Blood specimens were collected from orbital venous plexus in vacuum tubes containing EDTA (k3) as anticoagulants. Blood specimens were centrifuged at 200 ×g for 10 minutes, and the clear samples of blood plasma were separated. Plasma ALT, AST, ALP and total bilirubin were estimated using an automatic analyzer (Reflotron Plus System, Roche, Germany). The method of **[31]** was used to determine the levels of serum ALT, AST and ALP. Serum level of total bilirubin was estimated using the method of **[32]**.

Histopathological examination

Liver tissues excised from each mouse were fixed in 10% buffered formaldehyde immediately after removal from the animals. Fixed tissues were routinely processed, then embedded in paraffin, and cut into 4 lm thick sections; they were mounted on slides for hematoxylin and eosin staining. Qualitative examinations of prepared tissues and the obtaining of their photos were carried out using a light microscope (Olympus BX61-USA) connected to motorized controller unit (Olympus bx-ucb, USA) and photographed by a camera (Olympus DP72, USA).

Statistical analysis:

The data were expressed as mean± standard deviation (SD) and were analyzed using the Statistical Package for Social Sciences (SPSS for windows, version 22.0). Statistical comparisons were performed by a two-way analysis of variance (ANOVA). The results were considered statistically significant if the P-values were less than 0.05.

3- RESULT

Biochemical Findings:

The body weight after eight weeks of all experimental groups are gradual increase in the body weight gain of normal control mice (54.1%) and this supplemented with *O. oleaster* leaves extracts (53.6%) compared with their initial body weights. Moreover, significant increases of body weight gain were observed in rats treated with *O. leaster* leaves extracts plus malathion (48.3%). The minimum body weight gain was noted in mice treated with only malathion (34.1%).

The levels of serum ALT, AST, ALP and total bilirubin are shown in **Table 1**. The levels of serum ALT were significantly elevated in mice treated malathion ($P < 0.000$), *O. leaster* plus malathion ($P < 0.05$). The levels of serum AST were markedly

raised in mice exposed malathion (P < 0.000), *O. leaster* plus malathion (P < 0.05). Furthermore, statistically increases in the level of serum ALP were noted in mice treated with malathion (P < 0.000), *O. leaster* plus malathion (P < 0.05). Serum total bilirubin level was statistically enhanced in mice treated with malathion (P < 0.000), *O. leaster* plus malathion (P < 0.05). Insignificant alterations of serum ALT, AST, ALP and total bilirubin levels were noted in *O. leaster* (group 4) treated mice as compared with normal control mice of group 1.

Histopathological findings

Histopathological examination of the livers showed that the control and *O. oleaster* treated mice exhibited unremarkable pathological changes, preserved hepatic lobular architecture, arrangement of the hepatocytes in thin double cell thick plates, patent sinusoids, and regular hepatocytes cytomorphology in the form of central vesicular nuclei and abundant eosinophilic cytoplasm (**Figure** A). In contrast, the livers of the malathion-treated animals showed severe hepatic lesion displayed as remarkable cell loss, focal necrosis (severe inflammatory reaction), cytoplasmic clearing, focal nucleomegaly with hyperchromasia, focal nuclear pyknosis, and congested portal veins with areas of interstitial hemorrhage as well as focal obliterated sinuses (**Figure B-C**). While the livers of the malathion plus *O. oleaster* - treated animals exhibited less hepatic injury, unremarkable cell loss (minimal inflammatory reaction), and normal cytoplasmic staining with minimal clearing, the nuclei were relatively hyperchromatic, yet with regular nuclear-cytoplasmic ratio, the sinusoids were patent with no evident interstitial hemorrhage; however, the portal veins showed mild congestion and revealed mild expansion of portal areas **(Figure D-E)**.

Table 1: Serum ALT, AST, ALP and total bilirubin levels in control (group 1), malathion (group 2), *Olea oleaster* leaves extract plus malathion (group 3), *Olea oleaster* leaves extract (group 4) treated mice after eight weeks

	Parameters			
Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Total bilirubin (µmol/L)
Group1	53.19±7.11	94.20±7.40	170.10±13.30	2.11±0.50
Group2	98.29±11.61ab	153.42±12.60ab	265.87±12.12ab	7.3433±1.08a
Group3	66.69±7.81a	111.43±6.75a	202.66±10.88a	3.32±0.94a
Group4	77.64±7.64a	122.60±7.48a	231.51±8.78a	4.77±1.10a

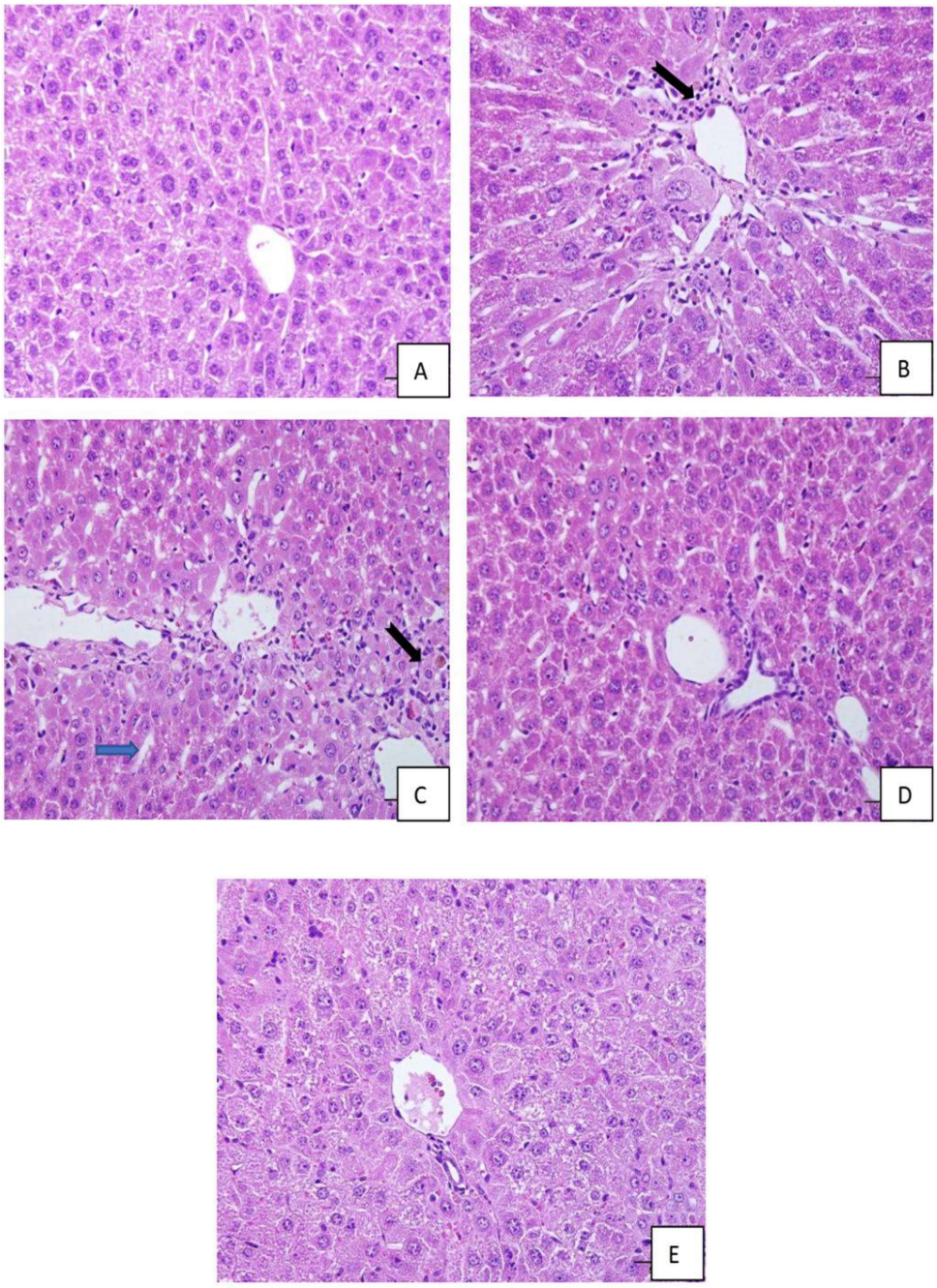

Figure 1: Effects of Olea oleaster leaves extract on histological evaluation in malathion -induced liver damage (H&E staining, magnification (X400). (A) NC group: visible central veins and thin sinusoids; (B-C) malathion Model group: severe liver damage, inflammatory infiltration and necrosis (black arrows) and hepatic sinusoid (blue arrows); (D-E) Olea oleaster leaves extract group : well-formed hepatocytes and minor histopathology change

4- DISCUSSION

Usage of herbal medicine has been on the rise due to ease of accessibility without prescription, reduced expenses, and minimal after effects **[33]**. Olive is the important nutritional material with several inclusions, including monounsaturated fatty acids (MUFA), which could carry nutritional advantages. Olive also contains phytochemicals such as phenolic compounds, which are beneficial for health **[10]**. Furthermore, the tree of *O. oleaster*. also holds a significant place in the history of medicines that have been in traditional use as a treatment for several diseases. The proof of this is the olive-rich diet of Mediterranean countries, which demonstrates the benefits of *O. oleaster* **[34]**.

The protective effects of *O. oleaster* L. against several chemical toxicants linked with the disruption of oxidative damage caused in investigated animals were reported by earlier research studies. Moreover, It was shown that total olive leaves extract had antioxidant activity higher than vitamin C and vitamin E, due to the synergy between flavonoids, oleuropeosides and substituted phenols **[35]**. As seen in the present study, the administration of malathion for eight weeks that malathion induced a notable decrease of body weight gain and significantly increase of serum ALT, AST, ALP and total bilirubin. Histopathologically, severe alterations of liver structure were noted **[36]**. The animals were administered malathion for eight weeks and the microscopic slide showed major histological changes in their liver tissues. Changes observed from light microscopes showed a varied scope of occurrence and different degree of intensity according to the dose of malathion **[37]**.

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 calves **[38]**, mice **[39]**, rabbits **[40]**, and rats **[41, 42]**. According to Tos-Luty *et al*. **[43]**. 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 present work showed that the treatment of mice with *O. oleaster* leaves extracts and their combination attenuated the physiological and histopathological changes induced by malathion administration. Moreover, the most improvements were observed in mice supplemented with O. oleaster leaves extract. This indicated the effectiveness of

these extracts in prevention of malathion toxicity. It may therefore be suggested from the evidence from the present study, that the supplementations of the studied extract may give some beneficial results for people with some hepatic diseases. Additionally, this study suggests that the supplementation of these extract may act as antioxidant agents and could be an excellent adjuvant support in the therapy of hepatic diseases induced by malathion and different pathogens.

CONCLUSION

This study indicated that use of malathion as pesticide is injurious to health and as an important histological marker for pesticide toxicity. Additional, MAL dosing causes apoptosis, inflammation, oxidative stress, and nephrotoxicity in mice. The test *O. oleaster* improved liver function, Liver tissue damage, and reduced inflammation and apoptosis, in MAL-induced mice.

Compliance with Ethical Standards:

Conflict of interests

The author declares that he has no conflict of interest.

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Ethical approval:

All applicable international, national, and institutional guidelines for the care and use of animals in Al Baha University, Saudi Arabia were followed and revised in March 2022 according to REF.7.1.4.2.

Consent for publication

Not applicable

Consent for Participation

Not applicable

Data Availability:

All data generated in this study is found in this manuscript

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