

## Effect of *Laurocerasus officinalis* Roem. (Cherry Laurel) Fruit on Dimethoate Induced Hepatotoxicity in Rats <sup>[1]</sup>

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### Abstract

Dimethoate is one of the most important organophosphate insecticides and may cause oxidative stress leading to production of free radicals and alterations in antioxidants. The aim of this study was to investigate the protective effects of *Laurocerasus officinalis* Roem. fruit extract containing antioxidant compounds on dimethoate-induced hepatotoxicity in rats. The rats were divided into six groups as follows: Control group; dimethoate-treated group; *L. officinalis*-treated group; the group of pre-treatment with *L. officinalis* prior to dimethoate; the group of pre-treatment with vitamin C before dimethoate; the group of post-treatment with *L. officinalis* after dimethoate. Aspartate transaminase (AST), alanine transaminase (ALT), and total bilirubin (TBil) as liver function tests and the oxidative stress parameters such as total oxidant status (TOS), total antioxidant status (TAS), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) were measured. The DNA damage was determined with comet assay. The results indicated that dimethoate caused a significant increase in AST, ALT, TOS, MDA, DNA damage and an important decrease in TAS, SOD, CAT, GPx as compared to control group. However, administration of *L. officinalis* or vitamin C to dimethoate-exposed rats restored these biochemical and oxidative stress parameters to nearly normal levels. In conclusion, toxic effects of dimethoate on rat liver are mainly attributed to hepatic function enzymes, oxidative stress and DNA damage, while these effects were largely ameliorated by *L. officinalis* fruit extract.

**Keywords:** *Laurocerasus officinalis* Roem, Cherry Laurel, Dimethoate, Hepatotoxicity, Rat

## Sıçanlarda Dimetoatla Oluşturulan Hepatotoksisite Üzerine Taflan (*Laurocerasus officinalis* Roem.) Meyvesinin Koruyucu Etkisi

### Özet

Dimetoat en önemli organofosfatlı insektisitlerden biridir, serbest radikallerin üretimine ve antioksidanlarda değişimlere yol açarak oksidatif strese neden olur. Çalışmanın amacı, ratlarda antioksidan bileşikler içeren *Laurocerasus officinalis* Roem. meyve ekstresinin dimetoat kaynaklı karaciğer toksisitesindeki koruyucu etkilerini incelemektir. Ratlar aşağıdaki gibi altı gruba ayrıldı: Kontrol grubu; dimetoate ile muamele edilen grup; *L. officinalis* ile muamele edilen grup; dimetoat öncesi *L. officinalis* verilen grup; dimetoat öncesi vitamin C verilen grup; dimetoat sonrası *L. officinalis* ile muamele edilen grup. Karaciğer fonksiyon testleri olarak aspartat transaminaz (AST), alanin transaminaz (ALT) ve toplam bilirubin (TBil) ile toplam oksidan durum (TOS), toplam antioksidan durum (TAS), süperoksit dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz (GPx) ve malondialdehit (MDA) gibi oksidatif stres parametreleri ölçüldü. DNA hasarı komet testi ile belirlendi. Sonuçlar, dimetoat'ın kontrol grubuna kıyasla AST, ALT, TOS, MDA, DNA hasarında belirgin bir artışa ve TAS, SOD, CAT, GPx'de önemli bir azalmaya neden olduğunu gösterdi. Bununla birlikte, dimetoat'a maruz bırakılan ratlara *L. officinalis* veya vitamin C'nin uygulanması, bu biyokimyasal ve oksidatif stres parametrelerini neredeyse normal seviyelere getirdi. Sonuç olarak, dimetoat'ın rat karaciğeri üzerindeki toksik etkileri esas olarak hepatik fonksiyon enzimleri, oksidatif stres ve DNA hasarına atfedilirken, bu etkiler *L. officinalis* meyve ekstresi tarafından büyük ölçüde iyileştirilmiştir.

**Anahtar sözcükler:** Karayemiş, Taflan, Dimetoat, Hepatotoksisite, Sıçan



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## INTRODUCTION

Dimethoate (*O,O*-dimethyl *S-N*-methyl carbamoyl methyl phosphorodithioate) is one of the most significant and broad-use organophosphate insecticides on a great number of crops against various pests [1,2]. Producers, pesticide workers, and farm owners are the basic risk groups of dimethoate exposure [3]. Previous studies indicate that dimethoate leads to oxidative stress via production of free radicals and induction of lipid peroxidation [4-6]. Some studies have shown that dimethoate causes significant increase in lipid peroxidation by interacting with membrane lipids due to its lipophilic feature [7,8]. It has been noticed the dimethoate toxicity results in deleterious effects on various organs such as liver, brain, testes, pancreas of rats [9]. The liver is the target organ for chemicals and play a main role in xenobiotic metabolism. Therefore, evaluation of hepatotoxicity is an important process for the detection of toxic action of xenobiotics [10].

There is a growing interest in the importance of natural dietary antioxidant compounds as therapy to prevent damage in many health concerns related to oxidative stress [6,11]. Therefore, high fruit consumption may reduce the risk of some diseases such as hepatotoxicity, cancer, cardiovascular and coronary heart diseases, diabetes and atherosclerosis [12]. *Laurocerasus officinalis* Roem. (Cherry laurel) is grown as a native fruit in the coasts of the Black Sea region of Turkey and locally called "Taflan" or "Karayemiş" [13]. It was found that *L. officinalis* fruit is a rich source of antioxidant substances such as phenolics (chlorogenic acid, phenolic acids, anthocyanins, vanillic acid) and ascorbic acid [14-20]. Some studies indicated that *L. officinalis* fruit has radical scavenging action against superoxide and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals [15,19,21].

In previous studies, some pesticides have been shown to cause oxidative stress, DNA damage and cytotoxicity *in vitro* and *in vivo* test systems. Thus, the purpose of the research was to evaluate the hepatotoxic actions of dimethoate and the preventive efficacy of *L. officinalis* fruit on liver function markers, parameters of oxidative stress, and DNA damage in rats.

## MATERIAL and METHODS

### Chemicals

An emulsion form of dimethoate (Korumagor 40 EC, 40%, Koruma Agriculture, Turkey) was diluted in saline (0.9% NaCl) to get an effective dose (7 mg/kg body weight-bw) for rats. The percentage of the active content was used for the calculation of concentration of dimethoate. All other reagents were analytical grade and provided from Sigma Chemical Co. (St. Louis, MO, USA).

### Plant Material and Preparation of Extract

*L. officinalis* fruits were collected from Akçaabat, Trabzon

in Turkey and voucher specimen was deposited in the Herbarium of Pharmacy Faculty of Ankara University (AEF 26257). The fruits were washed with distilled water and dried at 40°C for 5 days. The fruits were macerated with methanol (MeOH) with magnetic stirrer for 8 h at room temperature and the extracts were filtered by Whatman No. 1 filter paper. This process was repeated twice with MeOH. All filtrates were dried at 40°C with a rotary evaporator and then lyophilized. The lyophilisate was kept in a freezer until usage.

### Determination of Total Phenolic Content

Total phenolic content of fruit extract was identified by the method of Ahmed et al. [22]. 0.5 mL extract and 0.25 mL Folin-ciocalteu reagent and 0.5 mL sodium carbonate (20% w/v) were mixed and incubated for colour development at room temperature for 60 min. The absorbance was recorded at 765 nm with spectrophotometer (UV-1800, Shimadzu Co., Kyoto, Japan). Total phenolic substance was defined as mg gallic acid equivalent (GAE)/g extract from the calibration curve. This experiment was repeated 3 times and the results were shown as mean±SD values.

### DPPH Radical Scavenging Activity

Determination of radical scavenging effect of the fruit extract was assayed according to the method of Pourmorad et al. [23]. 50 µL of the extract solution at various concentrations were allowed to react with the 950 µL methanolic solution of DPPH (100 µM) in the dark for 10 min. Thereafter, the absorbance change was followed at 515 nm with a spectrophotometer. Measurements were repeated in triplicate. The known antioxidants, gallic acid, chlorogenic acid, and quercetin were applied as standards. The value of inhibitory concentration (IC<sub>50</sub>) means the amount of sample, which is necessary to clearance 50% of DPPH free radicals.

### Animals and Experimental Procedure

The experimental procedure approved by the Ethical Committee for Animal Research at Erciyes University (approval date:15.08.2012; no:12/82) was in accordance with the European Union Directive 2010/63/EU for care and use of laboratory animals. Sixty male Wistar albino rats (weighing 200-250 g) were housed under standard laboratory conditions (in polycarbonate cages, 12 h/12 h light/dark cycle, 22-24°C temperature and 55-60% relative humidity).

The rats were divided randomly into six groups consisting of ten animals each and were applied with oral gavage during sixty days as follow: Group I (controls) received only saline; group II (D) was exposed to dimethoate; group III (LOFE) was applied with only *L. officinalis* fruit extract; group IV (LOFE + D) received *L. officinalis* fruit extract 30 min prior to dimethoate; group V (Vit C + D) was given vitamin C 30 min before dimethoate; group VI (D + LOFE)

received dimethoate during the first month, the rats were exposed to dimethoate 30 min prior to *L. officinalis* fruit extract throughout the second month. The doses for dimethoate (7 mg/kg/day)<sup>[2]</sup>, vitamin C (100 mg/kg/day)<sup>[5]</sup>, and *L. officinalis* fruit extract (4 mg/kg/day)<sup>[24]</sup> were determined by using the results of other studies.

### Sample Preparation

At the end of the experiment, blood was collected from anesthetized (xylazine/ketamine) rats and then centrifuged at 4400 rpm for 10 min at 4°C. Samples of plasma were kept at -20°C until analysis for the evaluation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (TBil) levels. Livers were removed and washed in ice-cold physiologic saline solution, blotted and divided into three parts. One part of liver was preserved in 10% formalin for histopathologic evaluation. For comet assay, a piece of the liver cut into thin slices were placed in phosphate buffered saline. Tissues were allowed to stir for dissociation at 500 rpm for 10 min to get the cell suspension<sup>[25]</sup>. For oxidative stress parameters, a homogenate from about 0.5 g of the remaining liver was obtained with a homogenizer (IKA Ultra-Turrax T10 basic model, Germany) in cold buffer solution of 140 mM KCl and 50 mM Tris-HCl (pH 7.6) for 2 min at 13000 rpm. Malondialdehyde (MDA) level was measured in the liver homogenate by using spectrophotometer. The homogenates were then centrifuged at 15000 rpm for 30 min at 4°C and clear supernatant was used for the assessment of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), total oxidant status (TOS) and total antioxidant status (TAS) levels. Protein content in tissue homogenate and supernatant was determined according to method of Lowry et al.<sup>[26]</sup>.

### Liver Function Tests

The levels of AST, ALT, and TBil in plasma were assayed spectrophotometrically using by commercial diagnostic kits (Biolabo Company, France)

### Oxidative Stress Parameters

Activity of SOD was evaluated as defined by Eken et al.<sup>[27]</sup>. In brief, 840 µL of substrate solution (0.05 mmol/L xanthine sodium, 0.025 mmol/L 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride, 50 mmol/L 3-(cyclohexylaminol)-1-propanesulfonic acid, and 0.094 mmol/L ethylenediaminetetraacetic acid-EDTA) (pH 10.2) were mixed with 30 µL supernatant. After addition of 130 µL xanthine oxidase (120 U/L) to the mixture, the absorbance increase was recorded by spectrophotometer at 505 nm for 3 min. The activity of SOD was defined in U/mg protein.

Activity of GPx was identified by the method of Pleban et al.<sup>[28]</sup>. 30 µL supernatant and 970 µL of the reaction mixture (50 mmol/L tris(hydroxymethyl)aminomethane, 1 mmol/L

EDTA-disodium salt, 2 mmol/L reduced glutathione, 4 mmol/L sodium azide, 0.2 mmol/L nicotinamide adenine dinucleotide, and 1000 U glutathione reductase) (pH 7.6) were mixed and incubated for 5 min at 37°C. When 8.8 mmol/L hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added, the decrement of absorbance was followed for 3 min by spectrophotometer at 340 nm. Activity of GPx was given as U/mg protein.

CAT activity was evaluated according to Aebi<sup>[29]</sup>. The rate of H<sub>2</sub>O<sub>2</sub> decomposition in supernatant was recorded at 240 nm for 30 sec at 25°C. Activity of CAT was given as U/mg protein.

MDA level was detected for marker of lipid peroxidation by the method of Ohkawa et al.<sup>[30]</sup>. The MDA in the sample was reacted with thiobarbituric acid reactive substance and then the product was measured spectrophotometrically at 532 nm. Tetramethoxypropane was used as a standard. The result was expressed as nmol/mg protein.

Assays of TOS and TAS developed by Erel<sup>[31]</sup> were carried out by commercial kits (Rel Assay Diagnostic, Turkey) in supernatant. Oxidative stress index (OSI), which is a marker of the ratio of oxidative stress, was calculated from the percent ratio of TOS to TAS.

### Determination of DNA Damage with Comet Assay

DNA damage was identified by comet technique as defined by Singh et al.<sup>[32]</sup> with some changes<sup>[33]</sup>. Firstly, microscope slides were pre-coated by using agarose with normal melting point (0.5%) in distilled water, drying at room temperature. Secondly, 10 µL of the cell suspension was mixed with 100 µL of agarose with low melting point (0.8%) in phosphate buffer saline at 37°C and dropped onto the first layer. Slides waited to concretion at 4°C in a humid container for 5 min were immersed in cold buffer solution (25 g sodium dodecyl sulfate in Tris Borate-EDTA) for 7 min at 4°C. The slides removed from the buffer solution was placed in the electrophoresis unit containing neutral electrophoresis solution (27.5 g boric acid, 54 g Tris, and 20 mL EDTA, pH 8.4) and left to unwind the DNA for 20 min. Electrophoresis was applied at 64 V for 2 min and was set to 250 mA. The neutralized slides were stained with 50 µL ethidium bromide. A fluorescent microscope (Olympus, BX51, Japan) was used for observations with a magnification of x400. Comet assay software project (CASP-1.2.2, Windows 2010) was applied for evaluation the images of 50 randomly selected nuclei. A tail of fragmented DNA that migrated from the cell head, causing a 'comet' pattern indicated damage. However, without a comet, whole cell heads were not regarded to be detriment.

### Histopathologic Evaluation

The liver pieces fixed in formalin (10%) were embedded in paraffin. Thick paraffin sections about 5 µm were cut from each specimen and were stained with hematoxylin and

eosin dye. A microscope (Olympus BX-51, Japan) equipped with a high resolution of camera (Olympus DP-71, Japan) was used for the histologic assessment.

### Statistical Analysis

Statistical package for the social sciences (SPSS version 18.0 for Windows, Chicago, IL, USA) was performed for the analysis of the data. The comparison of the values among the groups was performed by one-way ANOVA and followed by Tukey multiple comparisons test. The results were defined as mean±standard deviation (SD).  $P < 0.05$  value was considered significant.

## RESULTS

### Antioxidant Properties of *L. officinalis* Fruit Extract

The phenolic content of fruit extract was detected to be high ( $340 \pm 3.18$  mg GAE/g extract) which may be responsible for antioxidant activity. As presented in Table 1, DPPH scavenging effect of the extract was referenced to known antioxidants (quercetin, gallic and chlorogenic acid). *L. officinalis* fruit extract was demonstrated to possess DPPH radical scavenging activity.

### Hepatic Function Markers

Data represented in Table 2 shows the liver function tests of control and experimental rats. Treatment with dimethoate alone caused a significant increase in AST (+43%) and ALT (+59%), and the level of TBil (+22%) compared to the control rats ( $P < 0.05$ ). On the other hand, pretreatment with *L. officinalis* fruit extract (-37%) or vitamin C (-37%) greatly reduced the activity of ALT in comparison to the group of dimethoate-applied alone ( $P < 0.05$ ). Pre- (-42%) and post-treatment (-27%) with *L. officinalis* fruit extract or pretreatment of vitamin C (-30%) significantly decreased the activity of AST compared with the group of dimethoate-treated alone ( $P < 0.05$ ). Pre- (-29%) and post-treatment (-28%) with *L. officinalis* fruit extract or vitamin C treatment (-33%) remarkably diminished the TBil level compared with the group of dimethoate-applied alone ( $P < 0.05$ ). *L. officinalis* fruit extract treatment without dimethoate did not show any important change in hepatic enzymes of AST and ALT and TBil level when compared to control rats ( $P > 0.05$ ).

### Oxidative Stress Parameters

The results of Table 3 indicate the MDA levels and the antioxidant enzyme activities of SOD, CAT, and GPx. Dimethoate treatment raised to a considerable increase in MDA levels, while an important decrease was seen in SOD, CAT, and GPx as compared to the control rats ( $P < 0.05$ ). In contrast, pre- and post-treatment with *L. officinalis* fruit extract or vitamin C greatly ( $P < 0.05$ ) diminished the elevated MDA level, whereas the SOD and GPx were prominently raised in comparison to the dimethoate-

treated group ( $P < 0.05$ ). Pre-treatment with *L. officinalis* fruit extract or vitamin C treatment remarkably elevated the activities of CAT as compared to the dimethoate-applied group ( $P < 0.05$ ). Administration of *L. officinalis* fruit extract did not show any significant change in SOD, CAT, GPx and MDA when compared to controls ( $P > 0.05$ ).

TOS and TAS levels and the mean value of OSI were shown in Fig. 1A, 1B, and 1C, respectively. An important rise in the values of TOS and OSI was observed in dimethoate administered rats, while a substantial decrement was detected in the TAS in comparison to the control rats ( $P < 0.05$ ). On the other hand, pre- and post-treatment with *L. officinalis* fruit extract or vitamin C treatment significantly ( $P < 0.05$ ) reduced the elevated TOS and OSI levels, whereas the TAS level was greatly increased in comparison to dimethoate-treated group ( $P < 0.05$ ). There was not seen any substantial change in the values of TOS, TAS, and OSI in the *L. officinalis* fruit extract-applied rats alone as compared to the controls ( $P > 0.05$ ).

### DNA Damage

In this study, the tail DNA% in total comet DNA in liver cells of control and experimental rats was measured via comet assay as an indicator of DNA damage. The results are illustrated in Fig. 2 and comet images of rat liver cells for each experimental group are shown in Fig. 3. An important increase by 94% in parameter of the tail DNA% was observed in rats receiving dimethoate alone compared with the control rats. Pre-treatment (-48%) and

**Table 1.** Comparison of DPPH radical scavenging activity of *L. officinalis* fruit extract and those of standards

Samples	Concentration (mg/mL)	Scavenging (%)	IC <sub>50</sub> (mg/mL)
<i>L. officinalis</i>	50	83±0.94	17±0.09
Gallic acid	0.125	85±1.2	0.05±0.02
Quercetin	0.125	74±1.5	0.08±0.06
Chlorogenic acid	0.226	85±0.98	0.13±0.08

Each value in the table was obtained by calculating the average of three experiments±standard deviation

**Table 2.** Toxic effect of dimethoate and the ameliorative potency of *L. officinalis* fruit extract on liver function tests

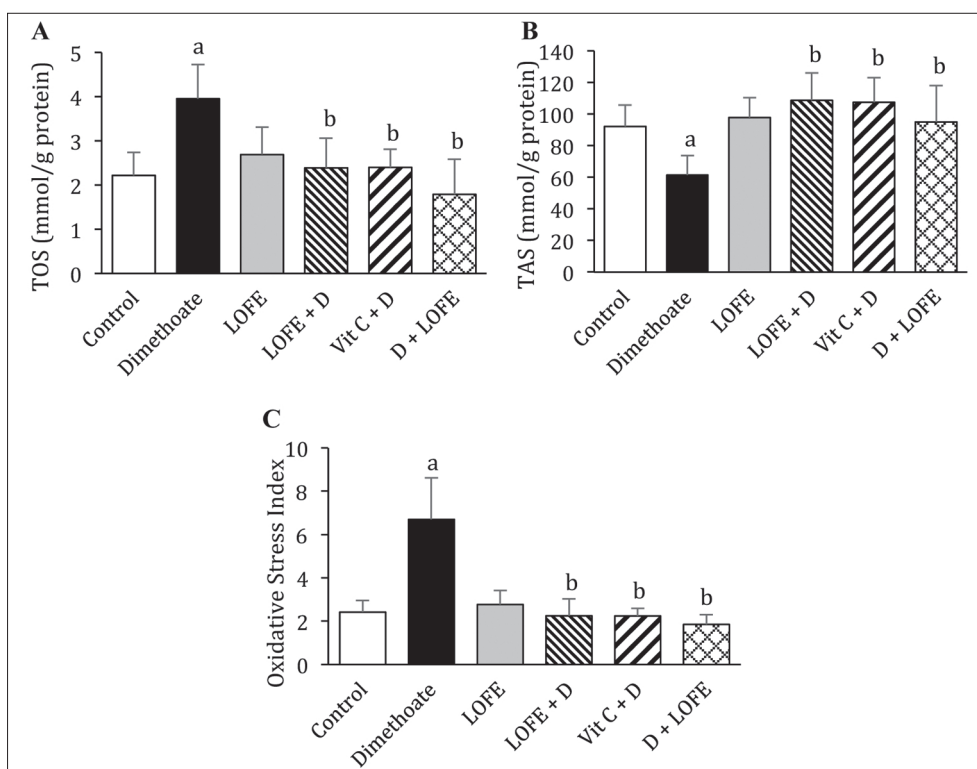
Groups	AST (μKat/L)	ALT (μKat/L)	TBil (mg/dL)
Control	0.63±0.17	0.37±0.07	1.00±0.14
Dimethoate	0.90±0.16 <sup>a</sup>	0.59±0.07 <sup>a</sup>	1.22±0.21 <sup>a</sup>
LOFE	0.55±0.20	0.43±0.11	0.93±0.12
LOFE + D	0.52±0.14 <sup>b</sup>	0.37±0.10 <sup>b</sup>	0.87±0.14 <sup>b</sup>
Vit C + D	0.63±0.15 <sup>b</sup>	0.37±0.14 <sup>b</sup>	0.82±0.10 <sup>b</sup>
D + LOFE	0.66±0.13 <sup>b</sup>	0.57±0.15	0.88±0.21 <sup>b</sup>

Values are expressed as mean±SD. LOFE: *L. officinalis* fruit extract; D: dimethoate; Vit C: vitamin C; AST: aspartate aminotransferase; ALT: alanine aminotransferase; TBil: total bilirubin. <sup>a</sup> $P < 0.05$  vs. control group; <sup>b</sup> $P < 0.05$  vs. dimethoate-treated group

**Table 3.** Toxic effect of dimethoate and the ameliorative potency of *L. officinalis* fruit extract on oxidative stress parameters

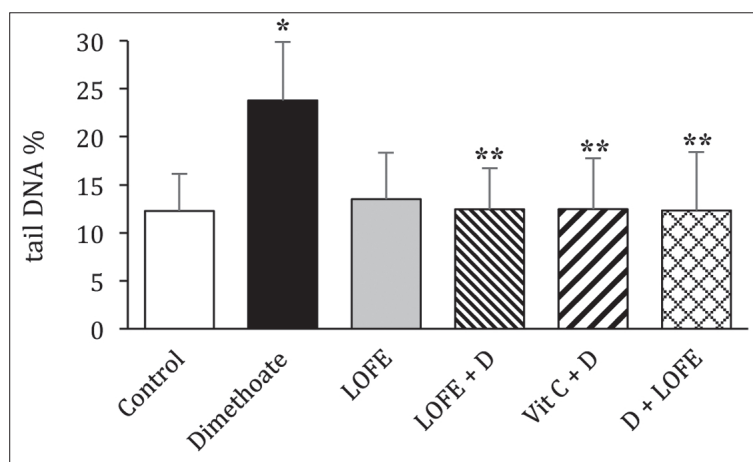
Groups	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)
Control	0.45±0.07	7.03±0.75	637.37±59.22	9.84±0.67
Dimethoate	0.97±0.06 <sup>a</sup>	5.88±0.76 <sup>a</sup>	496.24±54.99 <sup>a</sup>	7.64±0.73 <sup>a</sup>
LOFE	0.48±0.08	6.93±0.75	575.46±57.45	9.15±0.65
LOFE + D	0.57±0.08 <sup>b</sup>	6.94±0.58 <sup>b</sup>	579.36±31.09 <sup>b</sup>	9.38±0.49 <sup>b</sup>
Vit C + D	0.50±0.06 <sup>b</sup>	6.99±0.63 <sup>b</sup>	594.55±60.22 <sup>b</sup>	9.65±0.59 <sup>b</sup>
D + LOFE	0.55±0.09 <sup>b</sup>	6.96±0.49 <sup>b</sup>	514.67±50.29	9.42±0.65 <sup>b</sup>

Values are expressed as mean±SD. LOFE: *L. officinalis* fruit extract; D: Dimethoate; Vit C: vitamin C; MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase. <sup>a</sup>P<0.05 vs. control group; <sup>b</sup>P<0.05 vs. dimethoate-treated group



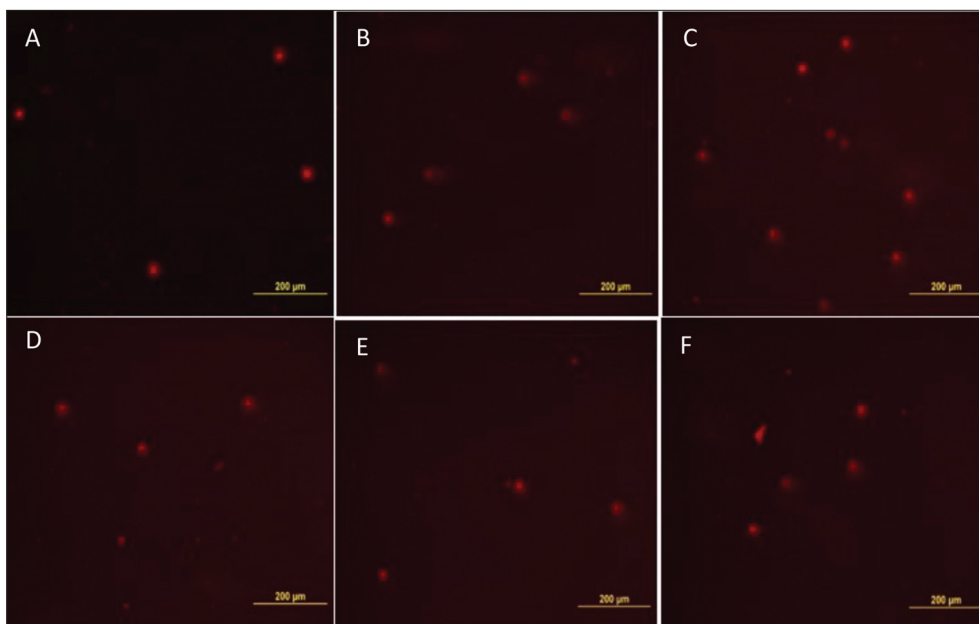
**Fig 1.** Toxic effect of dimethoate and the ameliorative potency of *L. officinalis* fruit extract on TOS (A), TAS (B), and OSI (C) parameters. LOFE: *L. officinalis* fruit extract; D: Dimethoate; Vit C: vitamin C; TOS: total oxidant status; TAS: total antioxidant status; OSI: oxidative stress index; Values are expressed as mean ± SD; <sup>a</sup>P<0.05 vs. control group; <sup>b</sup>P<0.05 vs. dimethoate-treated group

**Fig 2.** Toxic effect of dimethoate and the ameliorative potency of *L. officinalis* fruit extract on the tail DNA% with comet assay in hepatocytes of rats. LOFE: *L. officinalis* fruit extract; D: Dimethoate; Vit C: vitamin C; Values are expressed as mean ± SD. <sup>\*</sup>P<0.05 vs. control group; <sup>\*\*</sup>P<0.05 vs. dimethoate-treated group



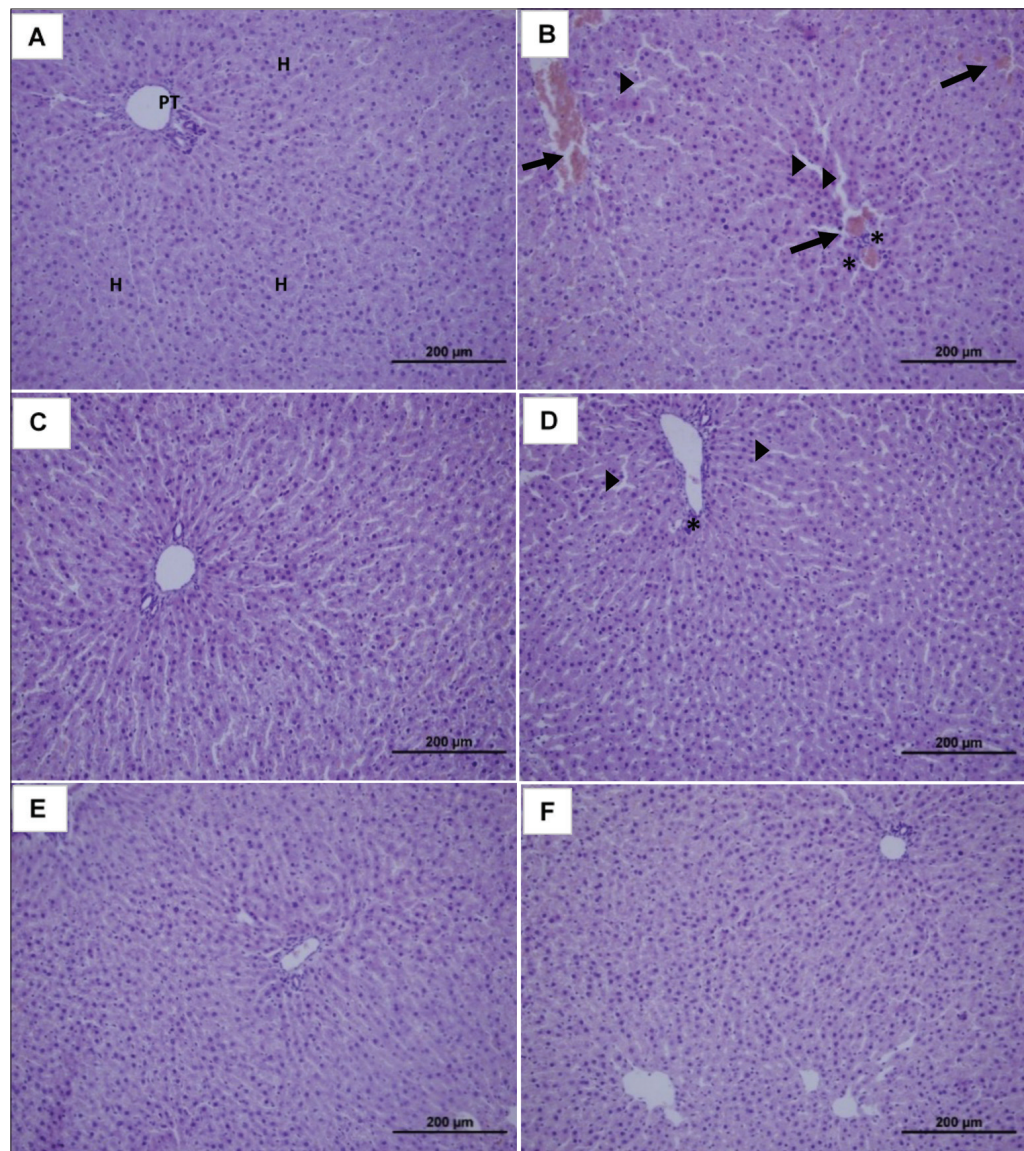
post-treatment (-48%) with *L. officinalis* fruit extract or vitamin C administration (-48%) reduced DNA damage in

rat hepatocytes as compared with dimethoate-applied alone group (P<0.05).



**Fig 3.** Comet images of rat liver cells for each experimental group (200× magnification). Control group (A), dimethoate-treated group (B), *L. officinalis* fruit extract-treated group (C), in group of pre-treatment with *L. officinalis* fruit extract before dimethoate (D), vitamin C-treated group (E), post-treatment with *L. officinalis* fruit extract after dimethoate (F)

**Fig 4.** Photographs of sections of the liver from rat (H&E, x20). The liver of control rats had normal histological structure of hepatocytes (H), portal triad (PT), and blood sinusoid (A); Dimethoate-treated group showed dilated sinusoids (*arrow head*), congested blood vessels (*arrow*), lymphocytes infiltration (*star*) (B); *L. officinalis* fruit extract-treated group indicated normal architecture (C); The most of the hepatocytes exhibited normal histological morphology, although there was dilated sinusoids (*arrow head*) and lymphocytes infiltration (*star*) in group of pre-treatment with *L. officinalis* fruit extract before dimethoate (D); an improvement of liver morphology was observed in groups of vitamin C-treated (E) and post-treatment with *L. officinalis* fruit extract after dimethoate (F)



### Histopathologic Evaluation

The liver section of control animals displayed a normal liver structure, including well-protected cytoplasm and nucleus of hepatocytes as shown in Fig. 4A. Dimethoate exposure exhibited severe histopathological changes in the liver sections compared with control rats as presented in Fig. 4B. The hepatocytes appeared large-sized cytoplasmic vacuolization. Moreover, dilated sinusoids, congested blood vessels, lymphocytes infiltration, and hepatocellular damage were observed. In addition, focal hepatocytes necrosis was also found. *L. officinalis* fruit extract-treated group did not indicate any pathological changes and the liver tissues appeared as the control group (Fig. 4C). Liver sections in group of pre-treatment with *L. officinalis* fruit extract showed a reduction in the injury with little pathological alterations such as dilated sinusoids and lymphocytes infiltration when compared with only dimethoate-treated group (Fig. 4D). In group of pre-treatment with vitamin C (Fig. 4E) and post-treatment with *L. officinalis* fruit extract (Fig. 4F) indicated an improvement of liver morphology.

## DISCUSSION

Findings in the current study showed that the subchronic exposure to dimethoate caused hepatotoxicity in rats. Only administration of dimethoate alone caused a significant increase in AST and ALT, and TBil level compared to the control group. Conversely, pretreatment with *L. officinalis* fruit extract or vitamin C severely alleviated the activity of ALT compared with the group of dimethoate-treated alone. Pre- and post-treatment with *L. officinalis* fruit extract or pretreatment with vitamin C greatly decreased the activity of AST and TBil level in comparison with the group of dimethoate-treated alone. These findings were compatible with those obtained by other research. Saafi et al.<sup>[5]</sup> reported that dimethoate exposure produced liver toxicity in rats, increasing AST and ALT in comparison to controls. However, pre- and post-treatment with fruit extract of date palm (*Phoenix dactylifera* L.) significantly ameliorated these parameters in comparison with dimethoate-applied rats.

Oxidative stress mainly occurs through production of free radicals that consequently react with biological molecules, causing damage to membranes and tissues leading to lipid peroxidation<sup>[34,35]</sup>. It has been notified that the exposure to dimethoate induces oxidative stress in rats, as evidenced by enhanced lipid peroxidation, accompanied by concomitant decrement in the SOD, CAT, and GPx activities in tissues<sup>[34,36]</sup>. MDA is an oxidation product of polyunsaturated fatty acids and serves as an important biomarker of lipid peroxidation<sup>[37]</sup>. Increased MDA level indicates an enhanced lipid peroxidation, leading to tissue injury and failure of the antioxidant defense mechanism to prevent the excess production of free radicals<sup>[35]</sup>. Vitamin C is a well-known

antioxidant that capable of scavenging free radicals such as superoxide, hydroxyl and singlet oxygen with its reversible properties of oxidation and reduction<sup>[4,24]</sup>. The enzymes of SOD, CAT, GPx act as the important antioxidant defense mechanism and provide protection against the deleterious effect of toxicants by means of their being free radical scavengers<sup>[38]</sup>. In the present research, MDA as a lipid peroxidation index, antioxidant enzymes of SOD, CAT, GPx were carried out to determine the oxidative damage in rat liver. Moreover, the values of TOS, TAS, and OSI were determined to more accurately assess oxidative stress. The current study results showed that subchronic subject to dimethoate caused a substantial increase in MDA and TOS levels and OSI value, while an important reduction was seen in SOD, CAT, and GPx enzymes and TAS level as compared with the control rats. Confirming the data in the current research, Sharma et al.<sup>[39]</sup> indicated that oral treatment with dimethoate at a dose of 30 mg/kg/day bw for 30 days markedly elevated the MDA level as compared with control rats. Sivapiriya et al.<sup>[36]</sup> observed that subacute exposure to dimethoate (18 mg/kg/day bw) for 14 days resulted in a significant rise in MDA, but a reduction in SOD, GPx, glutathione-S-transferase (GST), glutathione reductase (GR), and glutathione in liver of experimental mice.

In the current study, pre- and post-treatment with *L. officinalis* fruit extract or vitamin C significantly diminished the elevated the levels of MDA, TOS and OSI, while the activities of SOD and GPx and the level of TAS were increased in comparison with the dimethoate-treated group. Pre-treatment with *L. officinalis* fruit extract enhanced the activities of CAT as compared to the dimethoate-applied group. Consequently, *L. officinalis* fruit extract provided protective effects against oxidative stress induced by dimethoate exposure. This may be reason that the antioxidant properties of *L. officinalis* fruit<sup>[15,19,20]</sup> and its hepatoprotective effect on rat liver toxicity induced by carbon tetrachloride<sup>[40]</sup> have been reported by the previous studies. In recent times, there is an attention for the significance of antioxidant compounds such as phenolic compounds present in the plants, which prevent toxic effects of free radicals because of their radical scavenger efficacy and have beneficial on human health<sup>[41]</sup>. We detected that *L. officinalis* fruit extract has activity of DPPH radical scavenging and antioxidant effect due to their high phenolic content. Our results were compatible with those presented by some studies. Saafi et al.<sup>[5]</sup> found that pre- and post-treatment with extract of date palm fruit alleviated the oxidative stress caused by orally exposure to dimethoate (20 mg/kg/day for 2 months), including improvement of antioxidant enzyme activities and histopathological alterations and the prevention of lipid peroxidation in liver of rats. Salim et al.<sup>[6]</sup> also reported that dimethoate administration (20 ppm) for 6 weeks produced a highly significant increase in the lipid peroxidation and an important decrease in antioxidant defense system in comparison to control rats. However, administration of

pomegranate seed due to its antioxidant content such as polyphenols, total flavonoids and phenols provided protection against dimethoate-induced oxidative stress. Similarly, Abu El-Saad and Elgerbed [34] demonstrated that dimethoate (21 mg/kg bw) treatment alone for 7 weeks produced a highly major rise in MDA level and a significant reduction in SOD, CAT, and GST compared with control rats. In contrast, treatment with vitamin E greatly reduced the MDA level and substantially elevated the SOD, CAT, and GST activities in comparison with dimethoate exposed group. Al-Awthan et al. [9] observed that the orally subject to dimethoate (7 mg/kg bw) for 28 days resulted in a significant increase of MDA, while major reductions in CAT, GST enzyme activities. On the other hand, co-treatment with vitamin C and vitamin E to dimethoate-applied guinea pigs restored all those parameters to nearly normal levels.

In this study, the tail DNA% in total comet DNA in liver cells of control and experimental rats was measured via comet assay as an indicator of DNA damage. Because the tail DNA% covers a widest range of damage and is linearly related to break frequency over most of this range [42]. An important increase in parameter of the tail DNA% was observed in rats receiving dimethoate alone compared with the controls. However, pre-treatment and post-treatment with *L. officinalis* fruit extract or vitamin C administration reduced DNA damage in rat hepatocytes as compared with dimethoate-treated alone group. This results were confirmed by the other studies. Similarly, Ayed-Boussema et al. [43] found that treatment with dimethoate (at doses ranging from 1 to 30 mg/kg bw) for 30 successive days produced DNA damage in liver of mice. In another study carried out by Abu El-Saad and Elgerbed [34], the increased DNA damage was found in dimethoate-applied rats compared to control group. Conversely, the combined treatment with vitamin E and N-acetylcysteine considerably decreased DNA damage in comparison to dimethoate group. Histopathological findings of the present research demonstrated that exposure to dimethoate exhibited severe changes in the liver including dilated sinusoids, congested blood vessels, lymphocytes infiltration, large-sized cytoplasmic vacuolization in hepatocytes, focal hepatocytes necrosis, and hepatocellular damage compared with control group rats. Our results are compatible with similar findings reported in the other studies [2,36,44]. Sharma et al. [39] found that dimethoate (6 mg/kg) administered group has portal inflammation in rats, and dimethoate (30 mg/kg) treatment caused variable portal inflammation, centrilobular congestion, and foci of necrosis, suggesting hepatocellular damage. In the present study, liver sections in group of pre-treatment with *L. officinalis* fruit extract indicated a reduction in the damage with little pathological changes such as dilated sinusoids and lymphocytes infiltration compared to only dimethoate group. In group of vitamin C-treated and post-treatment with *L. officinalis* fruit extract displayed an improvement of liver histology. It was also

identified that treatment with fruit extract of the date palm showed improvement the hepatotoxicity induced by dimethoate in rats [5].

According to the findings of this study, dimethoate caused a significant oxidative damage in rat liver as evidenced by increase in lipid peroxidation and DNA damage, alteration in antioxidant status, depletion in the activities of CAT, SOD and GPx, and histopathological changes. *L. officinalis* fruit extract or vitamin C, on the other hand, was observed to ameliorate these disturbances induced by dimethoate. Thus, it can be suggested that the supplementation of a natural antioxidant such as vitamin C or *L. officinalis* fruit extract may act as a protective agent against dimethoate-induced hepatotoxicity. However, further studies are required to propose the potential therapeutic use of *L. officinalis* fruit extract in preventing the liver from xenobiotic-induced oxidative damage. In addition, further work should unravel the detailed role played by the individual components present in the *L. officinalis* fruit extract.

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