



# Protective effects of kefir against deltamethrin-induced hepatotoxicity in rats

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## Abstract

Deltamethrine (DLM) is a synthetic pyrethroid with broad spectrum activities against acaricides and insects. Widely used for agricultural and veterinary purposes, its human and animal exposure occurs by ingestion of contaminated water and food and leads to serious health problems. Kefir is fermented milk with numerous health favors counting restorative properties of bacterial flora, immune system stimulation, cholesterol reduction, as well as anti-mutagenic and anti-tumor properties. The present study was undertaken to examine the hepatoprotective and antioxidant potential of kefir against DLM toxicity in male Wistar albino rats. DLM-treated animals revealed a significant increase in serum biochemical parameters as well as hepatic protein and lipid oxidations but caused an inhibition in antioxidant enzymes. Additionally, we have observed an increase in hepatocyte DNA damages. This toxic effect was confirmed by histological study. Kefir administration normalized the elevated serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T bilirubin), and cholesterol. It also reduced DLM-induced protein carbonyl (PC) and malondialdehyde (MDA) formations. Furthermore, Kefir treatment restored catalase (CAT) and superoxide dismutase (SOD) activities. The co-treatment as well as the pre-treatment by kefir showed an improvement of oxidative status as well as suppressed inflammation and DNA damages. However, the pre-treatment seems to be the most efficient. Therefore, it could be concluded that kefir is a natural product able to protect against the hepatotoxic effects of DLM by its free radical-scavenging and potent antioxidant activity.

**Keywords** Deltamethrine · Kefir · Oxidative stress · Hepatotoxicity

## Introduction

What and how pesticides are used represent the most researched topics in the agro fields. Knowing that pesticides can significantly increase crop productivity, they are widely used to protect vegetables and fruits against pests. They are also used as an ectoparasiticide in farm animals and even used in residential homes against garden insects. Due to their low

environmental persistence and toxicity, pyrethroids have replaced organophosphorus insecticides (Pérez et al. 2010). Pyrethroids are pyrethrin-synthetic derivatives, natural toxic substances obtained from *Chrysanthemum cinerariaefolium* flowers (Tewari and Gill 2014). Among pyrethroids, deltamethrin (DLM) is firstly thought to be the safest available insecticide (Shukla et al. 2002). Sadly, numerous recent reports considered DLM as an environmental and industrial pollutant that is toxic to non-target organisms living in the same ecosystem, leading to serious hazards (Yonar and Sakin 2011; Chandra et al. 2013). Contaminated water and food are considered as the main DLM population exposure sources because of its rapid oral absorption (Barlow et al. 2001).

The insecticidal effect of DLM resulted from its binding to a distinct receptor site on voltage gate sodium channels and blocking nerve impulse by modifying the open-state kinetics (Du et al. 2010; Mani et al. 2014). In addition to its neurotoxicity (Nieradko-Iwanicka and Borzecki 2015), DLM metabolites were found accumulated in the liver and the kidneys since

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DLM is mainly metabolized by liver microsomal enzymes and excreted by kidneys (El-Maghraby 2007; Abdelkhalik et al. 2015). Therefore, liver and kidneys are among the most chemically affected organs. Indeed, DLM through increasing oxidative stress status (Mazmanci et al. 2011; Abdel-Daim et al. 2014, 2015) can cause liver tissue alteration by inducing liver fibrosis and cirrhosis (Abdel-Daim et al. 2013, 2016). It also causes severe renal injury including inflammation, tubular cellular toxicity, and nephrotoxicity in rat models (Abdel-Daim and El-Ghoneimy 2015; Abdou and Abdel-Daim 2014).

Kefir is the result of fermenting milk with kefir grains. It is the beverage remaining after the removal of kefir grains (Bensmira et al. 2010). These grains resemble to small clumps of cauliflower forming a polysaccharide matrix of microbial symbiotic mixture. It contains yeasts and acetic acid and lactic acid bacteria (Jianzhong et al. 2009; Chen et al. 2015). During milk fermentation, many components are found in kefir such as lactic acid, acetic acid, ethanol, and aromatic compounds. Kefir holds equally essential amino acids, vitamins, minerals, and exopolysaccharides (Garrote et al. 2001; Ismaiel et al. 2011). However, kefir consumption has highly interesting healthy properties as the treatment of gastrointestinal disorders, hypertension, allergies, and ischemic heart disease (Farnworth 2005; Otles and Cagindi 2003; de Oliveira Leite et al. 2013). Additionally, immunomodulating (Vinderola et al. 2005), anti-microbial (Silva et al. 2009), anti-inflammatory (Prado et al. 2016), and anti-proliferative (Rizk et al. 2009) activities have been reported.

Therefore, the aim of the present study was to investigate the hepatoprotective effect of kefir against DLM-inducing hepatic alterations associated with oxidative stress in rats. This study is the first, to our knowledge, in which the protective effect of kefir was evaluated against DLM-induced hepatotoxicity.

## Materials and methods

### Chemicals

Deltamethrin ( $C_{22}H_{19}Br_2NO_3$ ) (99% pure) was dissolved in corn oil. The CAS chemical name is *a*-cyano-3-phenoxymethyl (1*R*, 3*R*)-3-(2,2-dibromovinyl)-2,2 dimethyl cyclopropane-carboxylate. It is manufactured by a Tunisian company of fertilizers, El Afrane-1009, Elouardia, Tunisia. All serum biochemical parameters were performed on the Synchrom CX7 Clinical System using the Beckman reagents (Beckman, Fullerton, CA). All products used in this study were of analytical grade.

### Production and preparation of kefir

Traditional cultured and original grains from Monastir, Tunisia, were used. Kefir culture was as follows: (10% w/v); 10 g kefir grains were inoculated in 100 ml of commercial liquid pasteurized full-fat cows' milk. The mixture was subsequently cultivated at 23 °C for 12 h, then was filtered. Grains were removed and kefir was stored at 4 °C. The beverage was freshly given to animals directly after the fermentation period.

### Kefir quality control

Knowing that, the most important parameter that can affect kefir quality is its geographical origin. However, variability in the microbial community composition has been reported in kefir grains from different geographical origins (Miguel et al. 2010; Garofalo et al. 2015). Thus, our Tunisian kefir, as reported by Ben Taheur et al. (2017), used equally a traditional culture containing lactic acid bacteria as the predominant microbial population ( $9.5 \pm 0.15 \times 10^{10}$  CFU/g) (Ben Taheur et al. 2017). Where, yeast were present at  $9.2 \pm 0.14 \times 10^6$  CFU/g. The milk nature (goat, cow, or ewe milk) can also significantly affect the microbial quality of kefir (Satir and Guzel-Seydimb 2016; Yilmaz-Ersan et al. 2018). Finally, the environmental production conditions as the proportion kefir grains (g)/milk (ml), the fermentation time, and the temperature can make differences in the kefir microflora. (Tamine 2006; Kim et al. 2018; Altuntas and Hapoglu 2019).

In all manipulations, we have kept our experimental conditions stable (kefir grains and milk origins, the proportion kefir grains (g)/milk (ml), the fermentation time and the temperature). Therefore, we estimated that we have maintained the invariability of microflora and a stable kefir quality.

### Animals and experimental design

A total of 25 male Wister Albino rats, weighing  $150 \pm 20$  g, purchased from the Central Pharmacy (SIPHAT, Tunis, Tunisia). Rats were housed in a ventilated room of normal 12:12 h light/dark cycle, relative humidity ( $55 \pm 5\%$ ) and temperature ( $25 \pm 2$  °C). Food and water were provided ad libitum. All experimental design and animal manipulations were approved according to the National Institute of Health Guidelines for Animal Care and approved by the local Ethics Committee. All prerequisites were taken to avoid animal stress.

After 2 weeks of acclimation, rats were randomly subdivided into five different groups, five animals each:

The first group (G1) administered corn oil and acted as a control group.

The second group (G2) received kefir.

The third group (G3) received DLM at a dose of 25 mg/kg bw (1/6 LD50) (Manna et al. 2006).

The fourth group (G4) received simultaneously kefir + DLM at a dose of 25 mg/kg bw.

The fifth group (G5) was given kefir 1 h before DLM administration at the same dose of groups 3 and 4.

All treatments were given orally, at a dose of 1 ml of vehicle/DLM/kefir/day, in the morning (between 0900 hours and 1000 hours) and continued for 3 days.

### Serum and liver collections

Animals were sacrificed at the end of experiments (24 h after the last DLM dose) by cervical dislocation. From the rats' brachial artery, blood serum was withdrawn in heparinized tubes. Then, centrifuged at 2200g for 15 min (4 °C). Livers were immediately removed and washed with ice-cold physiological saline solution for histological analysis, biochemical analysis, and comet assay.

### Liver extract preparations

Using Tris-HCl solution (10 mM, pH 7.4) and a potter (glass-Teflon), we have milled treated liver rats of each group. To obtain rich protein supernatants, a centrifugation (15,000g, 30 min, 4 °C) was performed. Protein extracts were stored at – 80 °C until used. Protein concentrations were calculated using commercial Bio-Rad protein assay, where bovine serum albumin was taken as a standard (Bradford 1979).

### Serum biochemical analysis

Serum liver biomarkers (aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, total bilirubin (T bilirubin), and cholesterol) were measured using an automatic biochemistry analyzer at the Biochemistry Department, Sahloul University Hospital, Sousse, Tunisia.

### Oxidative stress parameter assays

**Protein carbonyl measurement** Oxidative stress generation can attack proteins via ROS, leading to the formation of carbonyl groups, and the loss of their structure and activity. Our method measured the carbonyl groups reactivity by 2,4-dinitrophenylhydrazine (DNPH). Two hundred microliters of each liver extract was mixed with 800 µl of DNPH. After 1-h incubation and in order to precipitate proteins, we added 1 ml of trichloro-acetic acid. Then, to remove cell debris, we added 800 µl of ethanol-ethylacetate preparation. Finally, 500 µl of guanidine hydrochloride allowed solubilizing proteins complexed with the DNPH. The optical density was read at 340 nm (Mercier et al. 2004).

**Lipid peroxidation status** Malondialdehyde (MDA) is the ultimate fragment of the degradation of cell membrane

polyunsaturated fatty acids. This assay was founded on the formation of a fluorescent complex between thiobarbituric acid and MDA fragment. For that, 250 µl of each liver extract was mixed with 750 µl of both thiobarbituric acid and acetic acid. Then, the mixture was heated 2 h at 90 °C and chilled in ice for 10 min. Before optical density reading (540 nm), 2.5 ml of n-butanol pyridine (15:1 v/v) was added (Ohkawa et al. 1979).

**Catalase activity evaluation** Catalases (CAT) are enzymes that neutralize oxygen peroxide (H<sub>2</sub>O<sub>2</sub>). In a quartz cuvette of spectrophotometer, we put, respectively, 20 µl of each liver extract, 780 µl of phosphate buffer solution, and 200 µl of H<sub>2</sub>O<sub>2</sub> (the substrate of the enzyme). CAT activity was calculated after optical density measurement at 240 nm for 1 min of time interval (Clairbone 1985).

**Superoxide dismutase activity evaluation** Superoxide dismutases (SOD) are ubiquitous metallo-enzymes that catalyzes superoxide ion disproportionation into molecular oxygen and hydrogen peroxides. Indeed, the SOD method referring to the nitroblue tetrazolium (NBT) test is a photo-reduction assay. Therefore, the basis for SOD activity detection is the reduction of NBT by the anion O<sub>2</sub><sup>-</sup>. However, white light was triggered for 30 min on a reaction mixture containing 50 µl of liver homogenate, 950 µl of phosphate buffer, 1 ml of EDTA-methionine, 85.2 µl of NBT, and 22.6 µl of riboflavin. The riboflavin/methionine mixture undergoes photo-reduction-forming free radicals that will be oxidized by NBT resulting in a blue substrate. Thus, to calculate the enzyme activity, the optical density was measured at 540 nm (Beauchamp and Fridovich 1971).

### Histopathological analysis

Pieces of livers were firstly incubated for 24 h in 10% formaldehyde at room temperature. Then, they were dehydrated using graded ethanol and were embedded in paraffin. Sections were stained with eosin and hematoxylin. For liver tissue histopathological analysis, we examined 30 slides per group (6 slides per rat). Light microscopy was used to distinguish organ lesions and was classified as follows:

- Mild (+) = slight lymphocytic inflammatory infiltrate.
- Moderate (++) = moderate lymphocytic inflammatory infiltrate.
- Severe (+++) = marked lymphocytic inflammatory infiltrate.

### DNA damage assessed by comet assay

DNA damage was determined using the alkaline comet assay according to Tice et al. (2000). Each liver piece was placed in

0.5 ml of cold phosphate-buffered saline and thoroughly chopped to obtain a cellular suspension. Hepatocyte suspensions (5  $\mu$ l) were embedded in 60  $\mu$ l of 1% low-melting-point agarose and spread on a microscope slide previously covered with a 1% normal-melting agarose. For cellular and nuclear membranes lysis of the embedded cells and for DNA unwinding in alkaline conditions, slides were immersed overnight in freshly prepared ice-cold lysis solution (Banath et al. 2002). Then, slides were placed in an electrophoresis alkaline buffer for 30 min to allow for DNA unwinding. Using the same alkaline buffer, electrophoresis was performed for 20 min at an electric field of 25 V. Slides were then neutralized with 0.4 M of Tris (pH 7.5) and DNA was marked with 50  $\mu$ l of ethidium bromide (20  $\mu$ g/ml). The comets were detected and scored using a fluorescence microscope. The previous steps have been done in darkness to eliminate supplementary DNA damage. Images of 100 randomly selected cells (50 cells from each of two replicate slides) were analyzed from each rat. Based on the tail size, cells were visually scored into five classes. From undamaged (0) to maximally damaged (4), a single DNA damage score was resulted to each animal and consequently, to each group. Thus, the total score was calculated according to the following equation: (percentage of cells in class 0  $\times$  0) + (percentage of cells in class 1  $\times$  1) + (percentage of cells in class 2  $\times$  2) + (percentage of cells in class 3  $\times$  3) + (percentage of cells in class 4  $\times$  4).

### Statistical analysis

Data were expressed as the mean  $\pm$  standard deviation of the means. The data were statistically analyzed by one-way ANOVA, using Tukey's multiple comparisons test. In all cases,  $p < 0.05$  was considered statistically significant.

## Results

### Serum biochemical analysis

The effects of DLM-induced toxicity and kefir preventive effects on serum biochemical parameters are summarized in Table 1. Serum levels of liver function markers, AST, ALT, T bilirubin, and cholesterol, significantly increased in DLM-administrated rats (G3) as compared to control ones (G1/G2). Co-administration of kefir + DLM (G4) and 1-h pre-administration of kefir followed by DLM intoxication (G5) reduced the elevation in serum liver biomarkers. However, this amelioration in measured serum parameters depends on the treatment mode. Thus, the kefir pre-treatment seems to be the efficient one and kept the serum levels of analyzed markers within the normal levels.

### Oxidative stress parameters

**PC and MDA productions** Proteins and membrane lipid oxidations by DLM treatment as well as the preventive effects of kefir on liver tissue homogenate are represented in Table 2. We observed a significant increase ( $p \leq 0.05$ ) in protein carbonyl (PC) and MDA contents (229.7% and 181.55%, respectively) in DLM-treated group (G3) as compared to the control group (G1). Kefir itself does not induce stress oxidative markers as shown with the G2 group. Concerning co-administration of kefir + DLM (G4) and 1-h pre-administration of kefir followed by DLM intoxication (G5), liver PC amounts were decreased (168.94% and 119.72% respectively). It was the same for MDA amounts, which decreased to 134.5% and 107.69%, respectively, for the co-treatment and the pre-treatment.

**CAT and SOD activities analysis** Table 2 represented equally the effects of DLM intoxication and the preventive effects of kefir on liver antioxidant enzymes. Animals treated with DLM alone (G3) showed a significant decrease ( $p \leq 0.05$ ) in CAT and SOD activities (40.43% and 38.08%, respectively) as compared to the control group (G1). In contrast, those co-treated by kefir + DLM (G4) showed an increase in CAT and SOD activities to 70.61% and 60.39%, respectively. One more time, the pre-treatment 1 h by kefir followed by DLM intoxication (G5) seems to be the most efficient; it restores antioxidant enzymes activities to 91.4% and 79.95%, respectively, for CAT and SOD.

### Histopathology study

The histological changes in liver were evaluated as described in the "Materials and methods" section. Results are presented in Fig. 1. Histopathology of liver demonstrated that DLM administration caused an extensive histological damage (+++) (Fig. 1c) as compared to the control groups (normal hepatocytes shape) (Fig. 1a, b). Thus, we observed a lymphocytic inflammatory infiltrate in peri-portal and intra-lobular zones. Kefir administration decreased liver failure caused by DLM intoxication as shown in Fig. 1d (++) and Fig. 1e (+), respectively, for the co- and the pre-treatment.

### DNA damage

Comet assay is estimated as a well-sensitive technique for the measuring of DNA damages and apoptosis in individual cells. Figure 2 showed that DLM-treated rats (G3) represented a significant increase ( $p \leq 0.01$ ) in hepatocytes DNA damage score, which reached 190.23% when compared to the control group (G1). The co-treatment (G4) as well as the pre-treatment (G5) by kefir reduced significantly DNA fragmentation to 132.96% and 108.25%, respectively.



**Table 1** Effect of deltamethrin and/or kefir treatments on serum biochemical hepatic parameters

	G1	G2	G3	G4	G5
AST (IU/l)	107.15 ± 3.45	103.83 ± 2.02	177.12 ± 1.41*	132.75 ± 5.62* <sup>#</sup>	113.24 ± 0.87* <sup>#</sup>
ALT (IU/l)	41.98 ± 0.71	40.15 ± 1.81	61.23 ± 1.14*	53.07 ± 0.69* <sup>#</sup>	46.66 ± 3.51* <sup>#</sup>
T bilirubin (μmol/l)	2.75 ± 0.05	2.66 ± 0.02	4.12 ± 0.03**	3.48 ± 0.15* <sup>#</sup>	2.93 ± 0.03* <sup>#</sup>
Cholesterol (mmol/l)	1.61 ± 0.02	1.56 ± 0.05	2.13 ± 0.03**	1.92 ± 0.04* <sup>#</sup>	1.74 ± 0.11* <sup>#</sup>

Control animals (G1), animals receiving kefir alone (G2), animals receiving DLM alone (G3), animals receiving DLM + kefir (G4), and animals receiving kefir 1 h prior to DLM (G5)

Data are expressed as means ± SD for five rats per group

AST aspartate aminotransferase, ALT alanine aminotransferase, T bilirubin total bilirubin

Treated (G3–G5) vs control G1 \* $p \leq 0.05$ ; \*\* $p \leq 0.01$ . Treated (G4 and G5) vs treated G3: <sup>#</sup> $p \leq 0.05$

## Discussion

Several pathological processes including cardiovascular and neurodegenerative diseases and even cancer have been closely related to the cellular imbalance between free radical production and antioxidant defense systems (Pizzino et al. 2017). It is commonly called the oxidative stress and can attack macromolecules via increasing phospholipids, proteins, and DNA oxidations (Lopez-Pedraza et al. 2016). Therefore, free radicals are among the major etiological factors implicated in several pesticides toxicity (Satpute et al. 2017). However, through pyrethroids toxic mechanisms, oxidative invasion may play critical roles. Among pyrethroids, DLM was shown to have some toxic effects. Otherwise, DLM is mainly metabolized by liver microsomal enzymes and DLM toxic metabolites were found accumulated in the liver (El-Maghraby 2007; Abdelkhalek et al. 2015). So, in rat liver, Tuzmen et al. (2008) detected that hepatic lipid peroxidation and the hepatic antioxidant defense system were significantly altered after 16 weeks of exposure to low and high DLM doses and ultimately caused the formation of oxygen free radicals and hepatic toxicity (Tuzmen et al. 2008). Likewise, Swiss mice treated with DLM 5 mg/kg bw and 25 mg/kg bw exhibited degenerative changes in the liver and kidneys (Tos-Luty et al. 2001). Rats' exposure to DLM (0, 2, 5, 10, 20, or 40 mg/kg bw) for 7 days

caused dose-dependent neurotoxicity and liver dysfunction that was accompanied by elevated ROS levels (Ding et al. 2017). A similar study reported that administration of DLM (5.6 and 18 mg/kg bw) significantly increased mean lipid peroxidation values in mouse liver and kidney (Rehman et al. 2006). Insofar as, the wide usage of DLM as an insecticide (Mehlhorn et al. 2011), investigations that would provide prevention by natural products with antioxidant effects in response to such pyrethroid toxicity are still needed. In this line, we have been interested in probiotics as living organisms, which upon ingestion exert health benefits. Especially kefir, a fermented beverage product produced by a symbiotic microbial with powerful antioxidant compounds. However, there is a strong connection between the composition of the gut microbiota and liver disease (Gkolfakis et al. 2015). Therefore, the modulation of the gut microbiota using probiotics has been suggested as a novel therapeutic strategy (Aron-Wisniewsky et al. 2013; Seo et al. 2015). Thereby, the aim of the present study was to investigate the protective potential of kefir against DLM deleterious oxidative effects leading to hepatotoxicity in rats.

Our selected dose of DLM, 25 mg/kg bw (1/6 LD50), for 3 days was based on anterior reports where highest dose, 30 mg/kg bw (1/5 LD50) for 5 days, induced biochemical alterations in rats without causing morbidity (Abdel-Daim et al.

**Table 2** Effect of deltamethrin and/or kefir treatments on oxidative stress parameters

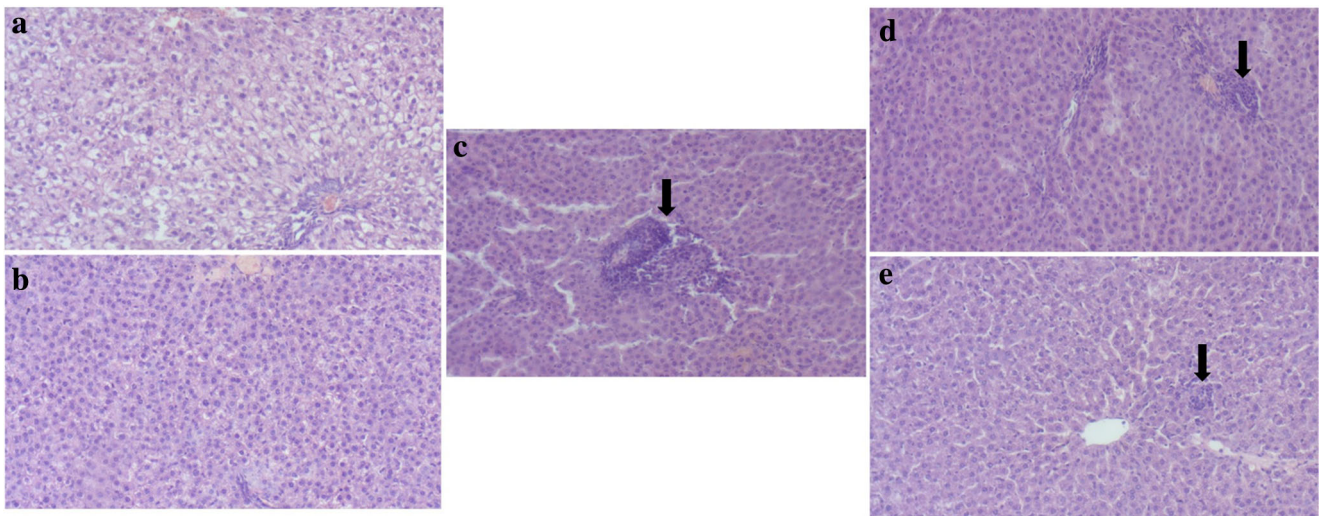
	G1	G2	G3	G4	G5
PC (nmol/mg of proteins)	14.01 ± 0.48	12.37 ± 0.54	32.18 ± 0.62*	23.67 ± 0.78* <sup>#</sup>	16.77 ± 0.47* <sup>#</sup>
MDA (nmol/mg of proteins)	1.45 ± 0.05	1.29 ± 0.08	2.64 ± 0.06**	1.95 ± 0.04* <sup>##</sup>	1.56 ± 0.07* <sup>#</sup>
CAT (μmol/min/mg of proteins)	519.24 ± 7.19	522.4 ± 3.97	209.96 ± 8.45*	365.64 ± 12.8* <sup>#</sup>	474.61 ± 5.3* <sup>#</sup>
SOD (IU/mg of proteins)	23.4 ± 0.5	24.89 ± 0.54	8.91 ± 0.68*	14.13 ± 0.33* <sup>#</sup>	18.71 ± 0.35* <sup>#</sup>

Control animals (G1), animals receiving kefir alone (G2), animals receiving DLM alone (G3), animals received DLM + kefir (G4), and animals receiving kefir 1 h prior to DLM (G5)

Data are expressed as means ± SD for five rats per group

PC protein carbonyl, MDA malondialdehyde, CAT catalase, SOD superoxide dismutase activities

Treated (G3–G5) vs control G1: \* $p \leq 0.05$ ; \*\* $p \leq 0.01$ . Treated (G4 and G5) vs treated G3: <sup>#</sup> $p \leq 0.05$ ; <sup>##</sup> $p \leq 0.01$



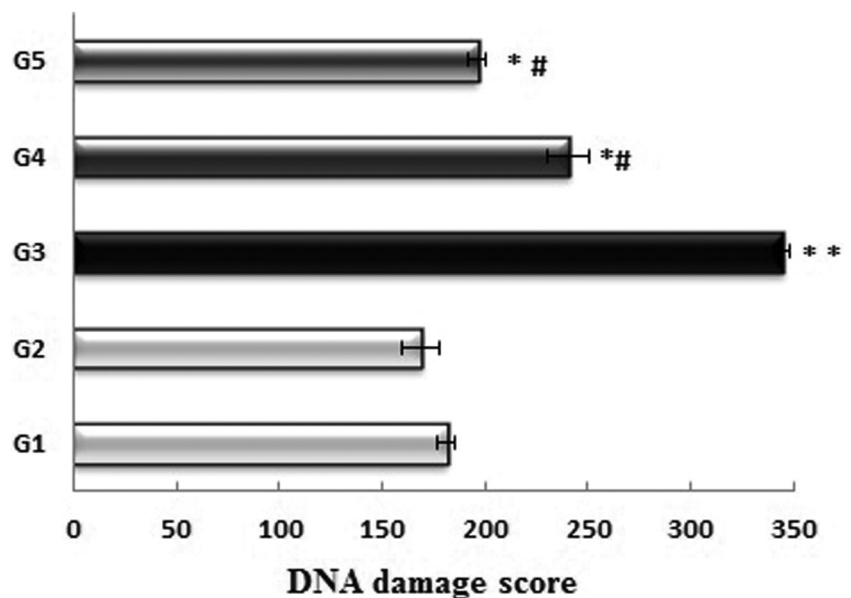
**Fig. 1** Hepatic sections of control animals (a), animals receiving kefir alone (b), animals receiving DLM alone (25 mg/kg bw) (c), animals receiving DLM (25 mg/kg bw) + kefir (d), and animals receiving kefir 1 h prior to DLM (25 mg/kg bw) (e)

2013). In these conditions, abnormally elevated serum ALT and AST levels is a sensitive marker of impaired liver function. Moreover, serum T bilirubin and cholesterol increased significantly. These perturbations in hepatic biochemical parameters were probably the consequences of DLM-induced liver pathological modifications. Our results are consistent with other studies which showed that oral administration of DLM to male rats for 5 or 30 days increased significantly liver biochemical parameters and particularly, ALT and AST enzymes (Abdel-Daim et al. 2013; Yousef et al. 2006; Maalej et al. 2017; Abdel-Daim et al. 2014).

PC and MDA, products of protein oxidation and lipid peroxidation, were significantly increased in response to DLM administration. Antioxidant enzymes such CAT and SOD that normally act to reduce and prevent tissue damages caused by

free radicals were decreased in our conditions. All these alterations are implicated in DLM-mediated hepatic toxicity; such toxicity is the consequence of oxidative injuries induced by oxidative stress occurrence causing protein dysfunctions and lipid peroxidation. Moreover, the DLM-induced hepatic failure is supported by the impairment of antioxidant enzymes' activities. Our results are in line with Abdel-Daim et al. (2013) that showed an increased in lipid peroxidation across high hepatic MDA level and inactivation of hepatic antioxidant enzymes (CAT, SOD) in response to orally DLM administration (30 mg/kg bw for 5 days). Saoudi et al. (2017) also reported that lipid peroxidation significantly increased while CAT and SOD decreased in the kidney and brain of rats due to DLM intraperitoneal administration at 7.2 mg/kg for 15 days. Our findings are equally concordant with those of

**Fig. 2** Total DNA damage induced in rat hepatocytes treated with DLM alone (25 mg/kg bw) (G3). Preventive effect of kefir on DNA fragmentation; performed in co-administration with DLM (25 mg/kg bw) (G4) or in pre-administration of 1 h followed by DLM treatment (25 mg/kg bw) (G5). Data are expressed as means ± SD for five rats per group. Control animals (G1), animals received kefir alone (G2). Treated (G3–G5) vs control G1: \* $p \leq 0.05$ ; \*\* $p \leq 0.01$ . Treated (G4 and G5) vs treated G3: # $p \leq 0.05$



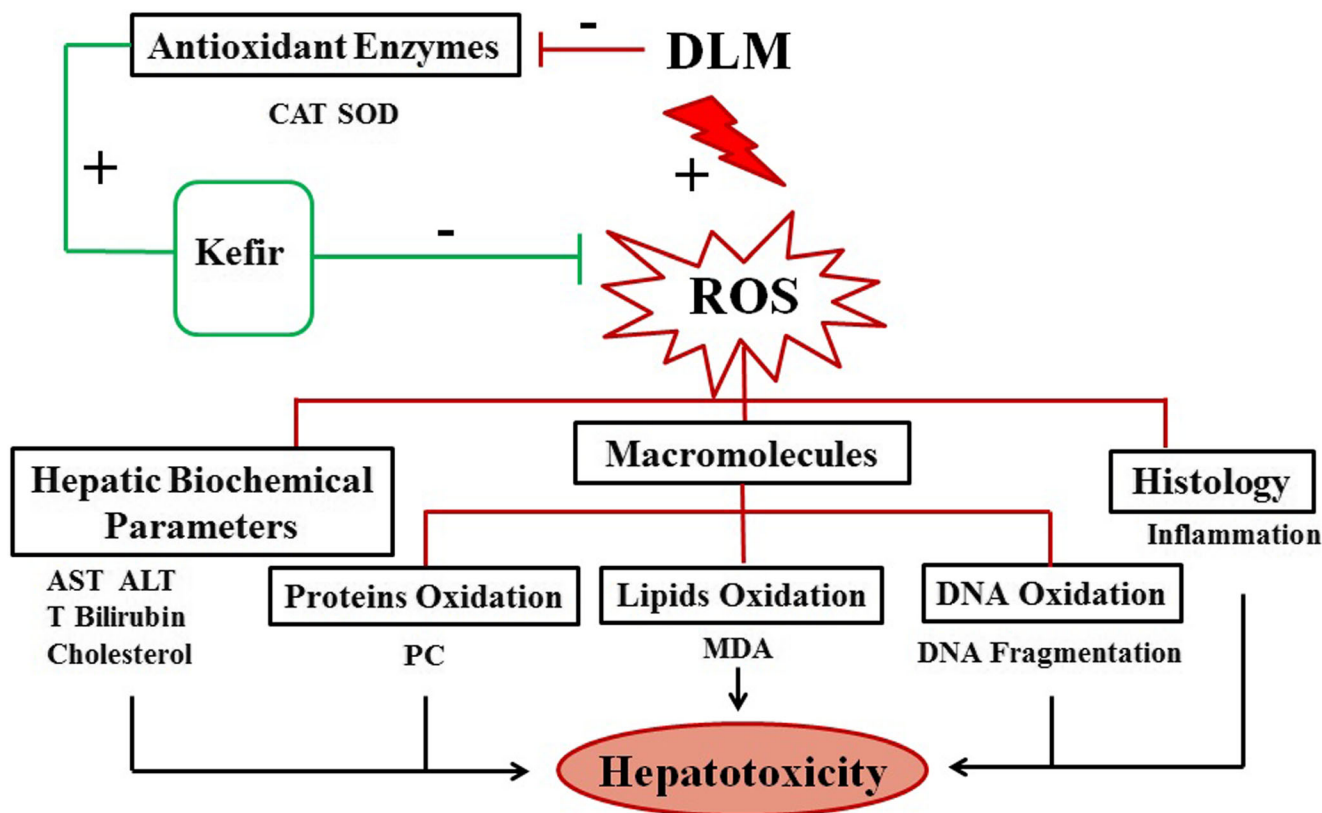


Fig. 3 Interaction of kefir with the signaling pathway of DLM-induced hepatotoxicity

Abdou and Abdel-Daim (2014) demonstrating increased serum MDA, but decreased CAT and SOD levels in rats treated intraperitoneally with DLM (30 mg/kg) for 5 days. Abdel-Daim et al. (2014) showed the same results in hepatic and renal tissues of rats administrated orally with DLM (15 mg/kg) for 30 days.

Excepting proteins and lipids, excessive ROS production can attack and disrupt the stability of DNA. Following our findings, DLM oral administration significantly advanced the DNA fragmentation score as assessed by comet test; a powerful technique in genotoxicity purview (Tice et al. 2002). DNA damage results are consistent with data reported by Ogaly et al. (2015). These authors showed that DLM significantly induced DNA damage expressed by the elevated tail moment and the extensive bright comet tail. Therefore, the ability of DLM to provoke DNA fragmentation may be due to its potential to outset a series of cell death signaling processes, which finally lead to apoptosis (Hossain and Richardson 2011).

All above results were supported by histopathological findings. However, the administration of DLM to rats showed disrupted hepatic architecture. Thus, histopathological changes covered inflammatory disorder, which might be due to the generation of ROS that provoked damages to different membrane constituents in hepatocytes (Rjeibi et al. 2016). Other reports also showed that DLM-induced hepatic lesions

indicating acute and subacute hepatic damages in rats treated with 30 mg/kg bw of DLM for 5 days (Abdel-Daim et al. 2013) and with 15 mg/kg bw for 30 days (Maalej et al. 2017). However, all alterations attributed to DLM administration might be attenuated or pronounced from one study to another depending on the dose and the exposure time.

Because oxidative stress is considered a key mechanism of DLM toxicity, the use of antioxidants to counteract ROS production and to prevent liver damages could be investigated. To this end, we have used kefir as a highly antioxidant beverage against DLM-induced hepatotoxicity in rats. However, some kefir probiotic strains can alleviate oxidative status. So, *Lactobacillus fermentum ME-3* has a manganese superoxide dismutase capable to decrease redox damage (Kullisaar et al. 2002; Songisepp et al. 2004). It was the same for lactobacilli and bifidobacteria strains that reduced especially lipid peroxidation and scavenged free radicals (Lin and Chang 2000; Wang et al. 2006; Savini et al. 2010). Moreover, the kefir polysaccharide, the kefiran, has many antioxidant activities (Mahapatra and Banerjee 2013). Additionally, this drink contained vitamins such as vitamins E and C and beta carotene with synergic antioxidant effects (Satir and Guzel-Seydim 2016; Yilmaz-Ersan et al. 2018). Our results indicate that Kefir has a supreme antioxidant power and it prevents liver ROS invasion. Consequently, kefir protects against protein, lipid, and DNA oxidations by the prevention of PC, MDA,



and DNA fragmentation. This prevention reached with the pre-treatment mode about 84%, 90%, and 91%, respectively. We have also demonstrated that the co-treatment as well as the pre-treatment by kefir reduced the serum hepatic injury biomarkers (AST, ALT, T bilirubin, and cholesterol). In addition, there was restoration of liver antioxidant enzyme (CAT and SOD) activities and prevention of histopathological changes in liver tissue to maintain the ultrastructure almost similar to the control one. Nevertheless, the pre-treatment remains the most efficient; it markedly improved or even totally reversed all toxicities observed with DLM administration. Recently, the hepatoprotective effects of natural antioxidant compounds of olive extracts and their critical roles in the improvement of liver functions have been reported (Mahmoudi et al. 2015; Maalej et al. 2017).

It is important to mention that kefir by itself reduces the intrinsic rats stress (G2); it ameliorates all biological parameters investigated within our study as compared to animals receiving the vehicle only (G1).

## Conclusion

Accordingly, our data indicated that kefir effectively abolished DLM-induced hepatotoxicity mediated by oxidative stress (Fig. 3). Thus, this fermented milk could be given as a dietary supplement to individuals who are constantly exposed to such insecticides.

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## Compliance with ethical standards

**Conflict of interest statement** The authors declare that they have no competing interests.

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