Toxicon:X 14 (2022) 100121

Contents lists available at ScienceDirect

Toxicon: X

journal homepage: www.journals.elsevier.com/toxicon-x

Does probiotic Kefir reduce dyslipidemia, hematological disorders and oxidative stress induced by zearalenone toxicity in wistar rats?

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ARTICLE INFO

Handling Editor: Dr. Ray Norton

Keywords:
Kefir
Zearalenone (ZEA)
Hepatotoxicity
Antioxidant potential
Rat

ABSTRACT

Zearalenone (ZEA) is a toxic metabolite of the genus *Fusarium*, which causes hepatotoxicity and induces oxidative stress. Kefir is an important probiotic dairy-product showing important *in vitro* antioxidant potential. In this study, the effect of Kefir supplementation to mitigate ZEA toxicity in rats was investigated. Animals were divided into four groups of five rats each, which received sterile milk (200 μL/day) during the first week. Then, they were switched to Kefir (200 μL/day), ZEA (40 mg/kg b. w./day) and Kefir + ZEA for the second week. Hematological and biochemical parameters, as well as liver histological analysis were determined. Kefir administration prevented the changes occurred in the count of all blood cells, and improved the antioxidant enzymes in the liver, such as catalase, glutathione peroxidase and superoxide dismutase activities that increased by 6, 4.5 and 1.3 folds, respectively, compared to ZEA group. Interestingly, the concurrent regimen Kefir + ZEA removed ZEA residues in the serum and liver. Furthermore, the Kefir + ZEA group showed a reduction in the levels of bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and hepatic malonaldehyde by ~82, 54, 66, 50 and 36%, respectively, compared to the ZEA group. The histopathological analysis showed a normal liver histological architecture in Kefir + ZEA group, while degenerative changes were observed in ZEA group. These results suggest that Kefir as probiotic consortium may have a hepatoprotective effect against ZEA poisoning.

1. Introduction

The zearalenone (ZEA) is a non-steroidal estrogenic mycotoxin produced by *Fusarium* species, which grown in humid and warm environment, and contaminated diverse crops, such as oat, wheat, corn, malt, rice, maize, and cereals (Llorent-Martínez et al., 2019). ZEA was observed in various animal feed and raw material samples around the world (Streit et al., 2013). Accordingly, ingesting food contaminated with mycotoxins could have serious adverse effects on human and animals health, leading to significant economic losses (Ben Taheur et al., 2019). It was reported that ZEA could have many disagreeable effects on animals, that may distress the reproductive performance of animals and even lead to death (Sirot et al., 2013). In fact, ZEA was shown to be toxic to many tissues in animals causing hepatotoxicity in piglets (Sirot et al., 2013), and oxidative stress, immunotoxicity and hematotoxicity in rats (Ben Salah-Abbès et al., 2016). Moreover, ZEA has high cytotoxicity, since its metabolites could alter the cell membrane structure and inhibit DNA replication, RNA transcription and protein synthesis inducing programmed cell death (Yu et al., 2011). On other hand, the ability of ZEA to bind to the estrogen receptor could lead to reactive oxygen species (ROS) production and lipid peroxidation (Salem et al., 2017).

Recently, many studies have developed new biological approaches aiming to avoid or at the least minimize the ZEA toxicity (Ben Taheur et al., 2017, 2020a; Wang et al., 2018). In this regard, attention has been focused on the use of microorganisms, especially those having advantages of high efficiency, high specificity and non-toxic metabolites.

For particular interest, Kefir was a traditional fermented milk originating from the North Caucasus Mountains (Amorim et al., 2019). Kefir was made by the fermentation of semi-skimmed or skimmed pasteurized milk (cow, sheep, camel, goat or buffalo) with little irregular masses of

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https://doi.org/10.1016/j.toxcx.2022.100121
Received 25 January 2022; Received in revised form 24 March 2022; Accepted 25 March 2022
Available online 29 March 2022

traditional or commercial Kefir grains, having yellowish color and gelatinous texture (Sharifi et al., 2017). Kefir grains were a complex ecosystem composed by a symbiotic association of yeasts, and lactic and acetic acid bacteria coexisting mutually in a gelatinous polysaccharide matrix, referred to Kefiran (Ben Taheur et al., 2017). Interestingly, Kefir was frequently recognized by its probiotic properties that could have beneficial effects on health, associated to its high composition in bioactive compounds (Fiorda et al., 2017; Amorim et al., 2019). *In vivo* and *in vitro* studies reported antibacterial, anti-fungal, antitumor, anti-apoptotic, anti-allergic, antioxidant, anti-mutagenic and anti-inflammatory effects of Kefir (Ben Taheur et al., 2019, 2020b).

To the best of our knowledge, no reports were available in the literature on the effect of Kefir administration to mitigate ZEA-induced oxidative stress in rats. Thus, hematological and biochemical parameters, antioxidant enzymes and lipid peroxidation in the liver, and histopathological analysis were studied in rats subjected to ZEA toxicity.

2. Materials and methods

2.1. Chemicals

All chemicals reagents used for the HPLC analysis were of analytical grade and obtained from Sigma Aldrich (St. Quentin Fallavier, France). Standard ZEA with high purity (>98%) was purchased from Sigma (St Louis, Mo, USA) and a stock solution was prepared in methanol. A 40 mg of ZEA was dissolved in phosphate-buffered saline (PBS, pH 6.5) for the *in vivo* experiments.

2.2. Kefir production

The Kefir grains used in this study were a traditional culture, and the original grain was from the Laboratory of Analysis, Treatment and Valorization of Environmental Pollutants and Products (Faculty of Pharmacy of Monastir, Monastir, Tunisia). For the Kefir production, commercial Ultra-high temperature cows' milk was inoculated by grains (10%, w/v) and incubated at optimized conditions (25 °C for 24 h). The Kefir maintenance and grain activation were previously described by Ben Taheur et al. (2017).

2.3. Animals and treatments

2.3.1. Animals

Femele Wistar rats (8 weeks of age; 130 g) were purchased from the Pasteur Institute of Tunis (Tunisia). Just after their arrival, animals were housed in an environmentally controlled breeding room maintained at 25 ± 2 °C, with a relative humidity of 60 ± 5% and a 12 h dark/light cycle in the animal house of the Faculty of Pharmacy of Monastir, Tunisia. Rats were allowed *ad libitum* access to filtered water and standard pellet diet (SICO, Sfax, Tunisia) throughout the experimental period. Alimentation was analyzed and confirmed to be free from mycotoxins. Animals were handled in laboratory under the Tunisian code of practice for the Care and Use of Animals for Scientific Purposes. Experimental procedures were realized using the guidelines of the Ethical Committee of Medicine Faculty of Monastir (Tunisia). All experiments were done in compliance with the rules of the European Communities Council Directive of November 24, 1986 (86/609/EEC).

2.3.2. Treatments

Before treatments, rats were fed as previously mentioned for a period of one week of acclimatization. Subsequently, the rats were divided into four groups, of five animals each ($n = 5$), and were subjected during 2 weeks to the following treatments:

(i) Group 1, received sterile milk (200 μL/day) by gastric gavage during the experimental period and referred to as "Control";
(ii) Group 2 received sterile milk (200 μL/day) by gastric gavage during the first week, and then the Kefir (200 μL/day) for the second week and referred to as "Kefir";
(iii) Group 3 received sterile milk (200 μL/day) by gastric gavage during the first week, and then the ZEA (40 mg/kg b. w./day) for the second week and referred to as "ZEA";
(iv) Group 4 received sterile milk (200 μL/day) by gastric gavage during the first week, and then the Kefir (200 μL/day) + ZEA (40 mg/kg b. w./day) for the second week as concurrent exposure regimen, and referred to as "Kefir + ZEA".

The administrated ZEA dose was chosen based on the literature, which represented 8% of the LD50 (Long et al., 2016). The Kefir was constituted with 8.4×10^7 CFU/mL lactic acid bacteria and 1.5×10^9 CFU/mL yeasts (Ben Taheur et al., 2017).

Twelve h after the end of the experimental period, rats were weighed. Blood samples were collected from the retro-orbital venous plexus and collected into EDTA tubes for hematological analysis. Another part of blood was distributed into heparinized tubes and was centrifuged (1100×*g* for 15 min at 4 °C) to obtain serum that was frozen, and stored at −80 °C for biochemical analyses and ZEA quantification. The liver was removed, weighed, washed with water and blotted on filter paper. The relative liver weight was calculated by dividing the organ weight by the total animal weight. Then, a piece of liver was fixed in 10% formaldehyde solution for histopathological analysis. A slice of liver was flash frozen in liquid N_2 and conserved at −80 °C for ZEA quantification. The remaining samples from liver were homogenized in TBS buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.4) and centrifuged (9000 rpm for 15 min at 4 °C). The resulting supernatants were collected and used for different biochemical analyses.

2.4. Hematological measurements

White blood cell: WBC; lymphocytes: Lym; monocytes: MON; granulocytes: GRAN; red blood cell: RBC; hemoglobin: Hb; hematocrit: Ht and platelets: PLT in plasma were measured using a H18 LIGHT automated hematology analyzer (SFRI, SaintJean d'Illac, France).

2.5. Biochemical assays

The levels of aspartate aminotransferase (ASAT) activity, alanine aminotransferase (ALAT) activity, alkaline phosphatase (AP) activity, total protein (TP), bilirubin (Bil), cholesterol (Chol) and triglycerides (TG) in serum were measured using an automatic biochemistry analyzer (Cobas Integra 400 plus system, Roche, Mannheim, Germany).

2.6. Protein determination

The total protein (TP) concentration in liver homogenate was measured colorimetrically at 595 nm using the method of Bradford. Bovine serum albumin ($E^{1\%}_{1\ cm} = 6.7$) was used as a standard (Bradford, 1976).

2.7. Antioxidant enzymes activities

Supernatants from livers, obtained as claimed in the sub-title 2.3.2, were used for the determination of catalase (CAT), dismutase (SOD), and glutathione peroxidase (GPx) activities.

The CAT activity was measured in liver using the method of Aebi (1984). The reaction was initiated by adding 500 mM H_2O_2 to 20 μL of the supernatant. The decomposition rate of H_2O_2 was spectrophotometrically followed at 240 nm. The CAT activity was expressed as U/mg protein in liver.

The SOD activity in liver was measured as previously described (Sun et al., 1988) and the absorbance was measured at 580 nm. The enzyme activity was based on the inhibition of nitroblue tetrazolium (NBT)

oxidation in the presence of the superoxide anion $O_2^{-}\bullet$. One unit of SOD activity was defined as the quantity of enzyme necessary to inhibit NBT reduction by 50% and the activity was expressed as U/mg protein in liver.

The GPx activity was measured as described formerly (Flohé and Günzler, 1984). The glutathione (GSH) oxidation by GPx was monitored spectrophotometrically following the transformation of 5,5′-dithio-bis-(2- nitrobenzoic acid into 2-nitro-5-thiobenzoate that absorb at 412 nm. The GPx activity was expressed as U/mg protein in liver. All experiments were conducted in triplicate.

2.8. Lipid peroxidation assay

The lipid peroxidation was estimated indirectly in liver tissue homogenates by measuring the malondialdehyde (MDA) level using the thiobarbituric acid reactive species (TBARS) assay as previously described (Yagi, 1976). Absorbance was measured at 530 nm and results were expressed as nmol MDA/mg protein in liver.

2.9. Mycotoxin determination in plasma and liver

Extraction of ZEA from plasma and liver was realized using the procedure previously described (Corcuera et al., 2011). The residue of ZEA in plasma and organs was quantified by HPLC as reported by Ben Taheur et al. (2017).

2.10. Histological evaluation

The liver tissue samples from different groups of rats were fixed in 10% formaldehyde solution. The organs were dehydrated in graded alcohol series (alcohol 96% and alcohol 100% for 16h30 min and overnight, respectively). Then, the livers were immersed in toluene for 1h30 min and were embedded in paraffin. Thin sections (3 μm) were cut, mounted onto glass slides and subjected to hematoxylin and eosin (H&E) staining for histopathological examination at 40× and 100× magnification using a Leica Orthoplan microscope (Leica, Solms, Germany).

2.11. Statistical analyses

All results were expressed as mean ± standard error, the data were analyzed using a one-way analysis of variance (ANOVA) to ascertain differences between experimental groups. Significant differences were determined at the $p < 0.05$ level using Tukey's test. Principal component analysis (PCA) was used to find correlations between the different biomarkers. Statistical analyses were done using the software STATISTICA (Statsoft STATISTICA version 6.1.478.0).

3. Results

3.1. Effect of ZEA and Kefir on weight gain and relative liver weight

All through the treatment period, no mortality and no clinical symptoms of disease were observed in any group. The rats were healthy with normal behavior, diet and water consumption, lively posture, clean bright coat and normal feces color through the whole experiment. The effects of Kefir, ZEA or both in combination on body weight and relative liver weight were measured (Fig. 1). Rats treated with ZEA failed to gain weight; while the combination Kefir + ZEA was successful in restoring body weight gain to be close to the control group (Fig. 1A). Indeed, at the end of the treatment, the weight gain of ZEA-treated group was reduced by 56% as compared to control rats, whereas it was decreased by 6% and 12.5% in Kefir and Kefir + ZEA groups, respectively (Fig. 1A). Furthermore, a significant reduction ($p < 0.05$) of 31% in relative liver weight in ZEA-treated rats was measured as compared to the other groups (Fig. 1B). Interestingly, these ZEA-induced changes were significantly alleviated ($p < 0.05$) by Kefir supplementation, which restored body weight gain and relative liver weight to be close to the normal rats.

3.2. Effect of ZEA and Kefir on hematological parameters

Hematological parameters were good indicators of rat physiology, health, and adaptation to gavage, as well as environmental conditions. Table 1 shows the hematological parameters in rats treated with ZEA and/or Kefir. The number of monocytes and granulocytes increased significantly ($p < 0.05$) in the rats treated with ZEA compared to control group (Table 1). Besides, ZEA significantly decreased ($p < 0.05$) the number of lymphocytes, platelets and red blood cells as compared to the control group. Interestingly, the Kefir administration prevented changes in the number of all blood cells (Table 1).

3.3. Protective effects of Kefir against ZEA-induced hepatotoxicity

3.3.1. Hepatic toxicity biomarkers

Hepatocellular markers were monitored by measuring the serum

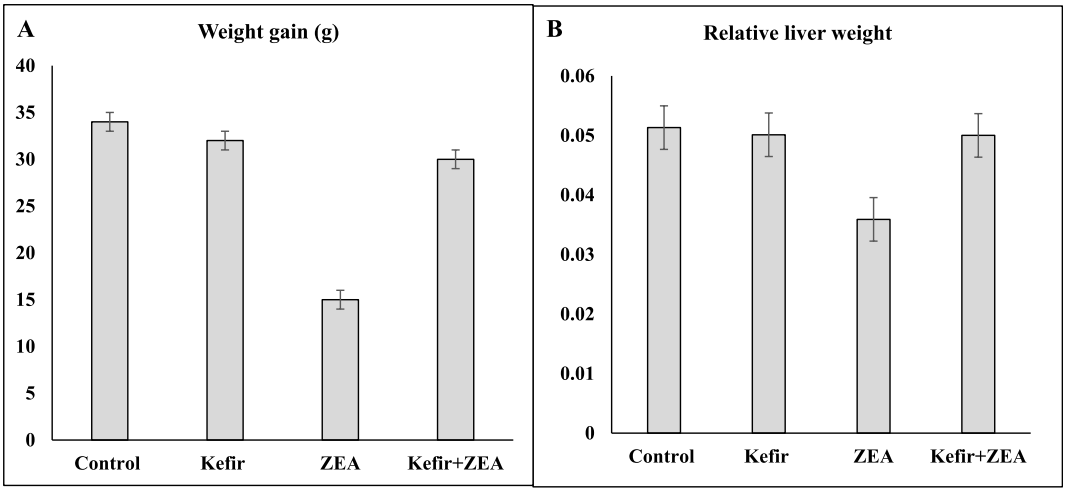

Fig. 1. Changes in (A) weight gain and (B) relative liver weight measured in rats exposed to different treatments. All groups received sterile milk (200 μL/day) during the first week. Control, Kefir, ZEA and Kefir + ZEA groups received sterile milk (200 μL/day), Kefir (200 μL/day), ZEA (40 mg/kg b. w./day) and Kefir (200 μL/day) + ZEA (40 mg/kg b. w./day) for the second week, respectively. Data represent the mean ± S.E. Asterisks indicate significant differences with the control group ($p < 0.05$).

Table 1
Effects of Kefir and ZEA on the blood count (NFS) parameters.

	Control	Kefir	ZEA	Kefir + ZEA
WBC (10^3/µL)	8.7 ± 1.25[a]	8.6 ± 1.05[a]	5.4 ± 1.12[b]	8.7 ± 1.04[a]
LYM (%)	83.2 ± 3.03[a]	83 ± 2.25[a]	61.9 ± 3.25[b]	83.9 ± 2.78[a]
MON (%)	5.6 ± 0.92[a]	5.6 ± 0.89[a]	8.9 ± 1.23[b]	5.3 ± 0.89[a]
GRAN (%)	8.2 ± 1.11[a]	8.4 ± 0.13[a]	10.2 ± 0.93[b]	8.1 ± 0.125[a]
RBC (10^6/µL)	7.35 ± 0.26[a]	7.67 ± 0.34[a]	3.93 ± 0.15[b]	7.41 ± 0.56[a]
Hb (g/dL)	15.2 ± 0.34[a]	15.6 ± 0.56[a]	15.9 ± 0.35[a]	15 ± 0.26[a]
Ht (%)	30.9 ± 0.37[a]	30.6 ± 0.67[a]	30.8 ± 0.78[a]	30.2 ± 0.23[a]
PLT (10^3/µL)	968 ± 68[a]	983 ± 71[a]	249 ± 28[b]	992 ± 45[a]

WBC: white blood cell; Lym: lymphocytes; MON: monocytes; GRAN: granulocytes; RBC: red blood cell; Hb: Hemoglobin; Ht: Hematocrit; PLT: platelets. The results are shown as mean ± ES of 5 rats per group. [a,b] Lower case letters denote significant differences between experimental groups ($p < 0.05$) using Tukey's test.

activities of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and alkaline phosphatase (AP), as well as the contents of total protein (TP), bilirubin (Bil), cholesterol (Chol) and triglyceride (TG) in different groups. Results relative to liver toxicity biomarkers for all experimental groups were shown in Table 2. Significant differences ($p < 0.05$) were obtained for all biomarkers following the exposure to ZEA, compared to the control group. Indeed, the ZEA-treated rats showed significant increase ($p < 0.05$) in the levels of Bil, ALAT, ASAT and AP, compared to the control group (Table 2). However, a decrease in TP, Chol and TG levels were observed in the ZEA-treated rats. Table 2 shows that Kefir-treated rats had similar values of liver toxicity biomarkers, compared to the control group. The Kefir administration was effective in increasing the serum TG, TP and Chol levels in Kefir + ZEA group, compared to the ZEA group. On the other hand, the Kefir + ZEA group showed a reduction in the levels of Bil, ALAT, ASAT and AP by ~82, 54, 66 and 50%, respectively, compared to the ZEA group.

3.3.2. Antioxidant enzymes and lipid peroxidation

The effect of Kefir administration, as a probiotic consortium with antioxidant potential, was studied against ZEA-induced hepatotoxicity in Wistar rats. Table 3 shows the effect of different treatments on the antioxidant enzymes activities and malondialdehyde (MDA) content in the liver. The superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) significantly decreased ($p < 0.05$) in the ZEA-treated rats, compared to the control group, suggesting therefore an acute oxidative stress. Besides, the MDA level was 1.8 fold higher in ZEA-treated rats than in the control group. Interestingly, in ZEA + Kefir group the CAT, GPx and SOD activities increased by 6, 4.5 and 1.3 folds, respectively; while the liver MDA content was reduced by ~36%, compared to the ZEA treatment.

Table 2
Effects of Kefir and ZEA on hepatic toxicity biomarkers.

	Control	Kefir	ZEA	Kefir + ZEA
ASAT (U/L)	213.1 ± 1.41[a]	221.1 ± 1.56[a]	931.65 ± 0.92[b]	315.2 ± 2.12[c]
ALAT (U/L)	63.57 ± 1.73[a]	67.98 ± 1.86[a]	161.4 ± 1.98[b]	73.85 ± 1.77[a]
AP (U/L)	108.95 ± 1.34[a]	116.3 ± 0.42[a]	247.65 ± 2.05[b]	124.8 ± 1.13[ac]
TP (g/L)	61.39 ± 0.72[a]	59.6 ± 0.85[a]	48.65 ± 1.06[b]	55.3 ± 1.13[ab]
Bil (µmol/L)	0.17 ± 0.04[a]	0.18 ± 0.03[ad]	1.37 ± 0.04[b]	0.25 ± 0.07[cd]
Chol (mmol/L)	2.04 ± 0.06[a]	1.95 ± 0.11[ad]	1.44 ± 0.01[b]	1.83 ± 0.07[cd]
TG (mmol/L)	0.97 ± 0.10[a]	0.82 ± 0.12[ac]	0.40 ± 0.11[b]	0.81 ± 0.10[a]

ASAT: aspartate aminotransferase; ALAT: alanine aminotransferase; AP: alkaline phosphatase; TP: total protein; bilirubin (Bil); cholesterol (Chol); triglyceride (TG). The results are shown as mean ± ES of 5 rats per group. [a,b,c] Lower case letters denote significant differences between experimental groups ($p < 0.05$) using the Tukey's test.

Table 3
Enzymes (CAT, SOD, GPx) activities and MDA level in liver tissues of different rats.

	Control	Kefir	ZEA	Kefir + ZEA
MDA (nmol/mg protein)	72.23 ± 2.32[a]	65.49 ± 2.10[a]	131.52 ± 4.22[b]	83.86 ± 3.66[a]
CAT (µmol/min/mg protein)	8.35 ± 0.58[a]	8.76 ± 0.60[a]	1.26 ± 0.09[b]	7.66 ± 0.46[a]
SOD (U/mg protein)	228.05 ± 1.67[a]	242.33 ± 1.36[a]	160.47 ± 2.60[b]	212.95 ± 1.96[a]
GPx (U/mg protein)	5.83 ± 0.18[a]	6.39 ± 1.25[a]	1.28 ± 0.13[b]	5.74 ± 0.16[a]

MDA: malondialdehyde; CAT: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase. The results are shown as mean ± ES of 5 rats per group. [a,b] Lower case letters denote significant differences between experimental groups ($p < 0.05$) using the Tukey's test.

3.4. Principal component analysis

Principal component analysis (PCA) was proposed as a method for obtaining an overall view of the results based on biochemical parameters data obtained from all treatments. In the current study, PCA done on biomarkers data extracted two main factors, which explained 96.85% of the total variance (Fig. 3). Factor 1 explained 94.91% of the total variance. However, factor 2 explained 1.94% of the total variance, which confirmed that ZEA treatment was the most toxic on the liver. The ZEA toxicity was mitigated by the Kefir administration, since the ZEA + Kefir treatment was less toxic than the ZEA treatment. The high correlation in the first component indicate that all biomarkers responses were correlated with the ZEA treatment.

3.5. Residues of ZEA in serum and liver

ZEA residues in the serum and liver of different treated rats were measured. ZEA was not observed in the control and Kefir groups. Nevertheless, the highest ZEA levels were observed in the ZEA-treated group. The ZEA concentration in the liver and plasma were 16.94 ng/kg b. w. and 9.38 ng/kg b. w, respectively. Interestingly, Kefir administration was effective to remove ZEA (0 ng/kg b. w) in the Kefir + ZEA group.

3.6. Histological study

Fig. 2 shows that macroscopic examination revealed a lesion in the liver of rat receiving ZEA alone (Group 3), compared to normal liver. The histological sections of the liver of the control group (data not shown) and the group receiving Kefir alone showed a normal liver structure (Fig. 4A). However, the microscopic examination showed a lesion and degenerative changes in the liver cells of rat receiving ZEA alone (Fig. 4B). Indeed, intense diffuse necrosis of hepatocytes, characterized by pycnosis and destruction of nuclei with narrowed

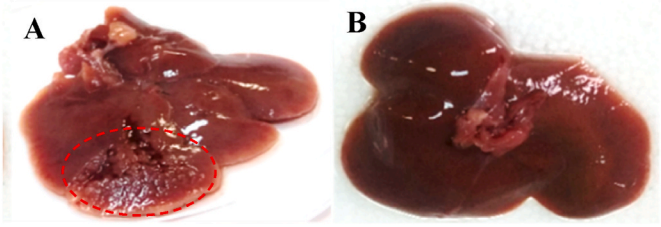

Fig. 2. Experimentally ZEA intoxicated rat showing liver lesions (A) compared to normal morphology of liver rat treated with Kefir (B).

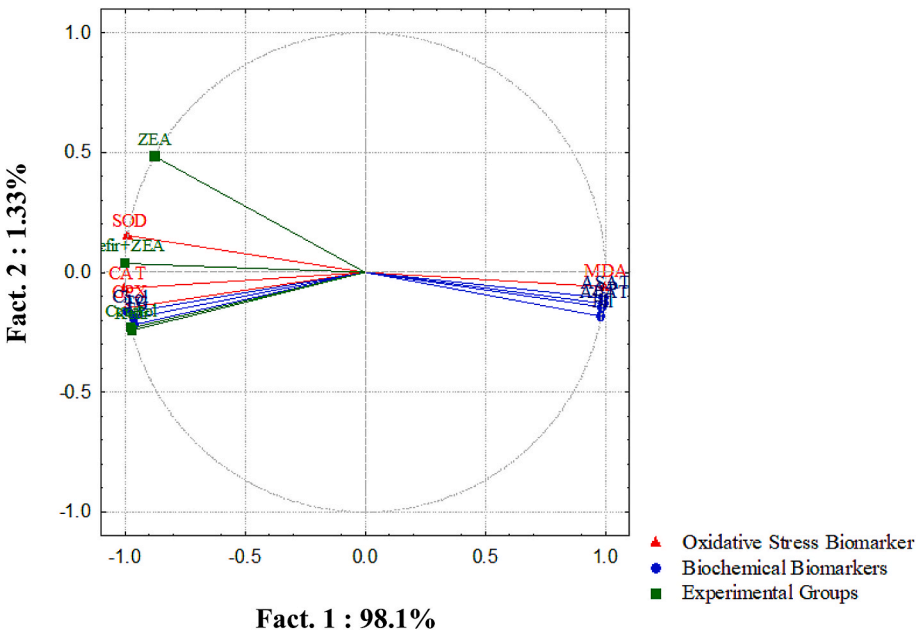

Fig. 3. Principal component analysis (PCA) of the two main Factors produced by liver toxicity (ASAT, ALAT, AP, TP, Bil, Chol and TG), oxidative stress (CAT, SOD and GPx) and perpxidation (MDA) biomarkers measured in liver tissues of different rat groups. ASAT: aspartate aminotransferase; ALAT: alanine aminotransferase; AP: alkaline phosphatase; TP: total protein; Bil: bilirubin; Chol: cholesterol; TG: triglycerides; CAT: Catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase; MDA: malonaldehyde.

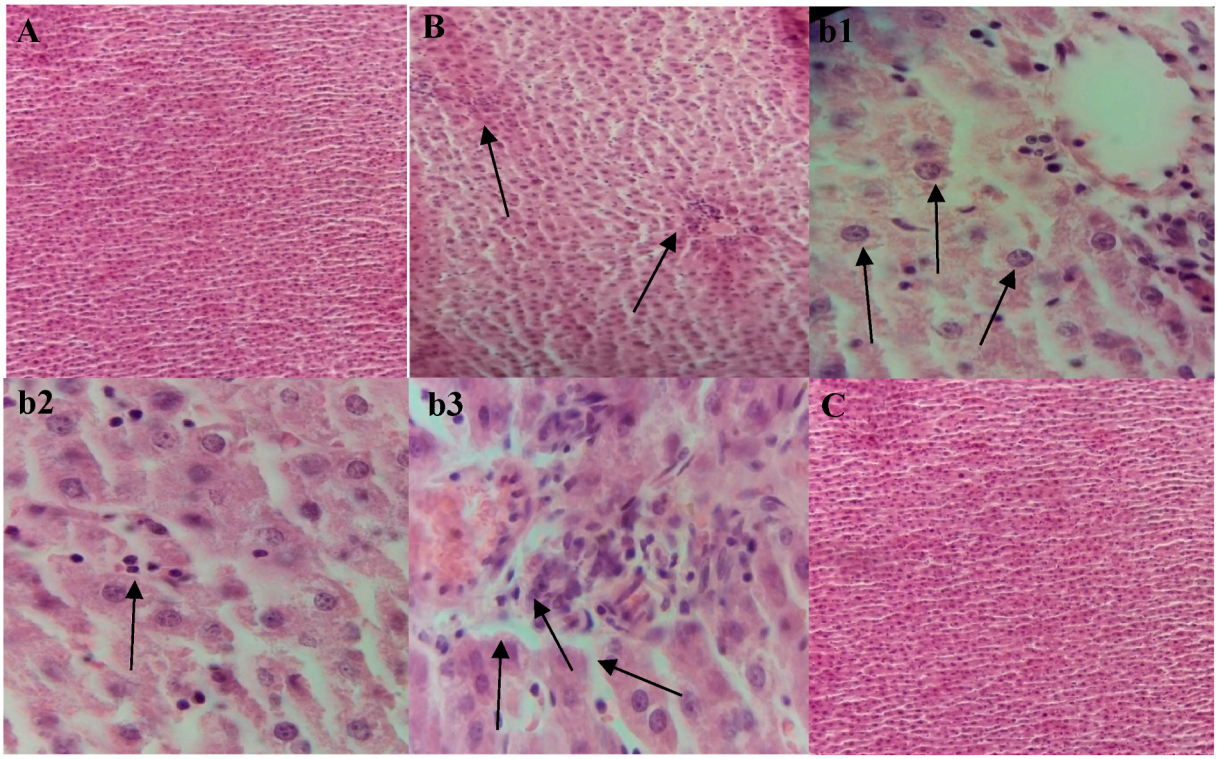

Fig. 4. Histopathological sections of H&E liver; capital letter (× 40) and lowercase letter (× 100): (A): rats treated with Kefir alone; (B, b1, b2, b3): rats treated with ZEA alone; (C): rats treated with Kefir + ZEA.

cytoplasm, accompanied by inflammatory infiltrates were observed (Fig. 4 b1, b2, b3).

4. Discussion

In the present study, a significant reduction ($p < 0.05$) in the weight gain and relative liver weight was observed in the ZEA-treated rats (Fig. 1). Similar results reported an 18% decrease in body weight of rats exposed to ZEA (20 mg/kg b. w.) for 6 weeks, which was prevented by silymarin supplementation at 100 mg/kg b. w. (Gao et al., 2018). As shown beforehand, oral administration of Kefir for 2 weeks significantly increased the body weight (Bakir et al., 2015). This may be due to the role of Kefir in improving the gastrointestinal microbiota, which implicated in nutrients decomposition and provided additional vitamins, enzymes, and amino acids to the rat.

The ZEA-treatment significantly decreased ($p < 0.05$) the hematological parameters (Table 1). Likewise, ZEA-treatment was reported to show a deficiency in platelet production, migration and aggregation, as

well as a reduction in red blood cells, which were indicators of anemia (Sabetghadam et al., 2013). In the present study, Kefir administration prevented these changes, which was consistent with the results reported by Boeira et al. (2015), who showed that lycopene mitigated the ZEA-hematotoxic effect.

The ZEA is mainly metabolized in the liver to α-zearalenol (ZOL) or β-ZOL (Olsen et al., 1986). It was reported that α-ZOL has stronger estrogenic activity than ZEA (Rajapaksa et al., 2007). Oğuz et al. (2000) suggested that chronic mycotoxin intoxication could be diagnosed by measuring changes in serum biochemical parameters prior to the occurrence of apparent clinical symptoms. The present study showed that ZEA-treatment resulted in significant differences ($p < 0.05$) for all hepatocellular markers (Table 2). Likewise, Maaroufi et al. (1996) reported that treatment of female rats with ZEA (1.5 mg/kg b. w.) for 48 h led to a change in the ALAT, ASAT, AP and Bil levels. Ben Salah-Abbès et al. (2008a) also reported a significant increase in ALAT in the ZEA-treated rat group (40 mg/kg b. w.). The relatively high serum ALAT and ASAT levels suggest that ZEA could cause liver damage (Ohgo et al., 2009). On the other hand, Abbès et al. (2006) showed a reduction in TP level and an impairment of immune function through the ZEA-exposure. Moreover, animals consuming a diet contaminated with ZEA at 1.3 mg/kg b. w. for 24 days showed a decrease in TG and lipoprotein HDL levels, compared to the control group (Jiang et al., 2010). The ZEA could be considered as an inhibitor of lipid secretion, since the liver was the main organ that led to triglycerides (Jia et al., 2014). Interestingly, the serum levels of the discussed above biomarkers significantly decreased ($p < 0.05$) in ZEA + Kefir group (Table 2), suggesting the Kefir hepatoprotective effect.

Oxidative stress occurs when the reactive oxygen species (ROS) were produced and oxidation exceeds the antioxidant capacity. Hence, the use of naturals antioxidants to avoid ROS production and to prevent liver damage could be an interesting alternative. The MDA is the end product of lipid peroxidation obtained by free radical attacks on unsaturated fatty acids of cell membranes, which was commonly used as a biomarker to monitor the lipid oxidation (Jia et al., 2014). In the present study, ZEA-treatment resulted in significant increase in the MDA level (Table 3), which was in accordance with earlier studies carried out on rats exposed to ZEA. In fact, a significant increase ($p < 0.05$) in MDA level was measured in rats after exposure to 40 mg ZEA/kg b. w. for 10 days (Ben Salah-Abbès et al., 2008b) or 20 mg ZEA/kg b. w. for 6 weeks (Gao et al., 2018). On the other hand, results showed that ZEA-treatment resulted in significant decrease ($p < 0.05$) in antioxidant enzymes activities (Table 3). Similar results were reported by Ben Salah-Abbès et al. (2009), who showed a reduction in GPx and CAT activities, which was explained by their conjugation with ZEA or its metabolites. Likewise, Ben Salah-Abbès et al. (2008b) showed that SOD activity decreased in the liver of ZEA-treated rats.

Excessive ROS production in organs of ZEA-exposed rats was reported to lead to genomic DNA damage and cell membranes disruption. Furthermore, the increase in lipid peroxidation could alter the cell membrane structure and consequently block cellular metabolism (Abid-Essefi et al., 2004; Ben Salah-Abbès et al., 2009). Consequently, the oxidative stress was a pathway of ZEA-induced toxicity. Overall, the obtained results showed that ZEA disturbed the pro-oxidant and antioxidant equilibrium resulting in oxidative damage, as previously described (Kouadio et al., 2007; Ben Salah-Abbès et al., 2009). Interestingly, Kefir administration to ZEA-exposed rats improved antioxidant status. Kefir significantly reduced ($p < 0.05$) the MDA level and increased the endogenous antioxidant enzyme activities.

It was important to note that the ability of Kefir to regulate antioxidant responses could be explained by its antioxidant potential. Indeed, Kefir was able to inhibit the peroxidation of linoleic acid, and to scavenge the superoxide and DPPH• radicals (Ozcan et al., 2009). Moreover, Kefir microflora (such as *Lactobacilli*, lactic *Streptococci* and yeasts) was able to attenuate oxidative stress either *in vitro* or *in vivo* (Güven et al., 2003). In fact, *Lactobacillus fermentum* ME-3 isolated from Kefir was able to decrease the redox status by a manganese superoxide dismutase (Khan et al., 2017). Additionally, probiotic *Enterococcus faecium* CRL 183 improved the lipid profile in rabbits with induced hypercholesterolemia (Cavallini et al., 2009). In addition, *Lactobacilli* and *Bifidobacteria* could protect against *in vitro* lipid peroxidation and decreased free radicals (Cavallini et al., 2009).

On the other hand, a polysaccharide isolated from Kefir referred to "kefiran" showed antioxidant activities (Chen et al., 2015). Furthermore, milk and Kefir compounds such as lipids, peptides, amino acids, enzymes, superoxide dismutase, catalase, glutathione peroxidase, milk oligosaccharides, phenolic compounds, vitamins (C and E) and beta carotene were known for their synergistic antioxidant activities (Yilmaz-Ersan et al., 2018; Khan et al., 2019). More particularly, milk fat richness in mono-unsaturated fatty acids allowed healthful properties and better oxidative stability (Khan et al., 2017). Moreover, it was reported the efficacy of Kefir in adsorbing ZEA *in vitro*, which suggest that Kefir consumption could help to decrease the gastrointestinal absorption of these mycotoxins and consequently to decrease the biomarkers of oxidative stress (Ben Taheur et al., 2017). For these reasons, Kefir has received attention as an efficient agent in oxidative stress prevention and treatment.

The ZEA residues in animal products increased serious concerns among consumers. Thus, in order to respect the public health and safety, it is necessary to control the quality of animal products and to analyze the ZEA residues in different animal tissues mainly in the liver because it is the detoxifying organ of xenobiotics using metabolic conversion and biliary excretion (Owumi et al., 2021). The obtained results showed that the Kefir administration was effective to remove the ZEA residues. It could be suggested that the use of Kefir had protective effects using the adhesion of ZEA, thus alleviated their bio-availability in the blood and the liver.

The histological study showed a lesion and degenerative changes in the liver cells of rat receiving ZEA (Fig. 4B). Similar histopathological finding were reported by other studies (Gao et al., 2018; Owumi et al., 2021). Remarkably, Kefir administration in Kefir + ZEA group reduced and prevented the hepatocytes damage (Fig. 4C), which support the above described biochemical results. The significant decrease in hepatocellular lesions was consistent with the reduction of serum ASAT and ALAT activities in the groups of rats treated with Kefir (Table 2).

5. Conclusions

The ZEA exposure resulted in a decreased of growth performance and relative liver weight. Furthermore, a severe toxicity was observed as shown by the disruption of hematological and biochemical parameters, histological changes, oxidative stress, and ZEA residues accumulation in serum and liver. The present study was the first report describing the Kefir potential to mitigate the ZEA-toxic effects. Interestingly, Kefir could be a promise candidate as an additive in food and feed to counteract the hazardous effects of ZEA.

Credit author statement

Fadia Ben Taheur: Conceptualization, Investigation, Methodology and Writing. **Chalbia Mansour:** Experiment and Formal analysis. **Sondes Mechri:** Experiment**Sihem Safta Skhiri:** Experiment. **Bassem Jaouadi:** Searching literature. **Ridha Mzoughi:** Searching literature. **Kamel Chaieb:** Searching literature. **Nacim Zouari:** Supervision, Writing-Reviewing and Editing.

Ethical statement

The study protocol was approved by the Ethical Committee of Medicine Faculty of Monastir (Tunisia).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are very grateful to the Laboratory of Analysis, Treatment and Valorization of Environmental Pollutants and Products, Faculty of Pharmacy Monastir and to Monastir University for their financial support.

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