Protective Effect of Kefir Against Hepatorenal Toxicities of Malathion in Male Rats

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Abstract:
Background: Malathion (MAL) is the most organophosphates which is capable to produce free radicals and induce disturbance in body antioxidant. This study was undertaken to assess the effects of Kefir (KF), a probiotic fermented milk, on oxidative stress, functional and metabolic, as well as histological damage induced by malathion to the kidneys and liver of rats. **Materials and Methods:** Thirty‑two Wistar rats were divided randomly into four groups (n = 8): sham (saline/corn oil), KF group (1.8 ml/day); MAL control treated groups (50 mg/kg); and KF/MAL‑treated group. Treatments were administered for 30 days through oral gavage. Twenty-four hours after the last treatment, Parameters related to the function and the histology of both kidneys and liver were evaluated and statistically analyzed from kidney, liver and blood serum samples in respect of the groups. **Results:** Malathion increased the levels of various serum marker enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) and it increased bilirubin concentration. MAL also increased serum urea, uric acid and creatinine, whereas it decreased albumin level. As well as MAL induced increase in MDA in both liver and kidneys tissues while induced decreases in the activities of SOD and CAT and GSH activity. Co-administration of KF with MAL resulted in improvement of all tested parameter. In histological study of liver and kidney, MAL induced damage in liver and kidneys tissues. However, KF administration to MAL-intoxicated animals resulted in overall improvement in liver and kidneys tissue damage, emphasizing its antioxidant role. **Conclusion:** In light of the available data, it can deduce that MAL-induced lipid peroxidation, oxidative stress, liver and kidneys damage in rats, and conjoint supplementation of KF has resulted in pronounced ameliorating effect in all tested parameter and histological picture of liver and kidney tissues.

Keywords: Malathion, Kefir, Toxicity.

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Introduction

Malathion (MAL) (known as O, O'-dimethyl dithiophosphate of diethyl mercapto succinate) is the most widely used organophosphorus pesticide (OP) on agricultural crops for insects or other pests control in Egypt[1]. However, the uncontrolled use of insecticides in agriculture and public health operation importantly increased the scope of ecological imbalance and thus many non-target organisms become victims[2]. The widespread use of MAL makes it important to study its role in human disease. The primary toxicological effect of MAL was reported to be long term neurotoxicity with reports of poor mental health and deficits in memory and concentration mediated by inhibition of cholinesterase activity[3]. Clinically, MAL has also been shown in some cases to impair function of non-neuronal human organs such as kidney[4,5], lung[6],and lymphocytes[7], Moreover, the lipophilic nature of MAL facilitated its interaction with cell membrane and leads to perturbations of the phospholipids bilayer structure of most visceral organs[8]. In addition, MAL as one of the OPs affects mitochondrial membrane transport in liver, and so it disturbs cytochrome P450 system in human liver[9,10].

Several studies have demonstrated that MAL intake is accompanied by renal injury that manifested by the renal histological damage. It also reduced activity of antioxidant enzymes such as catalase and superoxide dismutase as well as glutathione peroxidase (GPx) and subsequently increased MDA production in both hepatic and renal tissues[11,12,13,14,15]. It is noteworthy that the adverse effects of MAL can be reduced by the natural compounds found in agricultural foods[16,17,18,19].

These functional foods including kefir (KF) which has health promoting properties in both experimental and clinical studies[20,21]. Kefir is gotten from the Turkish word "keif" which signifies "nice feeling" and the drink started in the Caucasian heaps of Russia[22]. KF is a food product made via fermentation of milk with kefir particles or culture[23]. KF's probiotic property comes from KF grains or cultures containing various species of lactobacilli, lactococci, Leuconostoc spp., acetic acid bacteria, and yeasts that demonstrate several

biological activity including antioxidant activity[24,25]. The antioxidant capacity of kefirs may be attributed to their proton- contribute capacity, their decreasing force and superoxide dismutase (SOD)-like action and anti-inflammatory properties[26,27,28].

Besides, Sphingomyelins found in kefir and kefir oil are reported to stimulate the immune system against infections[29]. Many lactic acid bacteria have systems that metabolize oxygen radicals. Stecchini et al. reported that superoxide dismutase and high magnesium content in KF are the most important antioxidant systems[30]. Regular consumption of 500 mL of daily milk was shown to positively affect hepatic, biliary, renal function, and blood circulation, in addition to having a metabolism-stabilizing effect[31]. Besides, KF has many applications in a variety of medical conditions such as allergy, immunological disorders, and coronary heart disease and improves the digestive health through organic acids, peptides (bacteriocins), carbon dioxide, hydrogen peroxide, ethanol and diacetyl production[32,33].

Although the kefir drink is recommended in many countries for consumption because of its **probiotic bacteria and yeast mixture (Simova)**[34], in Egypt the grains are not commercially available and are culturally donated from person to person. Hence, the main objective of this study is to explore the potential protective effect of kefir on MAL‑induced impairments in both kidney and liver of male rats. So, we investigated the putative effect of MAL on the functional and metabolic parameters in kidney and liver of rat as well as the implication of oxidative stress, and assess whether these effects can be ameliorated by co-treatment with kefir.

2. Materials and Methods

2.1. Drugs and chemicals:

a) Malathion: high technical grade (98% purity), was provided from the branch of the Ministry of Agriculture, Egypt.

b) Kefir grains were kindly provided by the Food Technology Research Institute, Agricultural Research Centre, Giza, Egypt.

c) Kits: MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GSH, glutathione; were purchased from Sigma Chemical Company (St. Luis, MO, USA). All the utilized solvents in this research were of high grade and were purchased from Sigma Chemical Company (St. Luis, MO, USA).

2.2. Methods:

Kefir preparation is done once a week, thus ensuring freshness of the product according to El- Kewawy, H.E[35]

2.3. Experimental Design

a) Animals

Thirty two adult male Wistar rats of 170–220 g weight were obtained from the Nile Co. for Pharmaceuticals and Chemical industries, Cairo, Egypt. Rats were 10–12 weeks old, which is equivalent to young adult age in humans[36] . The study was conducted in the Laboratory of Animals Research Center in the Faculty of Medicine, Ain Shams University. Before the start of experiments, animals were left to acclimatize for two weeks and were maintained under controlled temperature and 12 hours light/12 hours dark conditions. They were allowed ad libitum access to standard laboratory feed and tap water, then, rats were randomly subdivided into four different groups, 8 animals each: (1) control (n=8) received corn oil daily as vehicle for MAL, oral administration of distilled water (1.8 ml/animal/day), (2) KF-treated group (n=8): receiving single daily oral dose of 1.8 mL/day via gastric lavage[37] (3) MAL-treated group (n=8): receiving 50 mg/kg in corn oil 0.5 ml/kg via gastric lavage) daily[38]. This dose is equivalent to occupational exposure level[39]; (4) MAL/KF-treated group (n = 8) receiving both MAL and KF at previously indicated dosage and time. All doses were administered orally for 30 days. Throughout the experiment, all animals were observed at least once a day, which is in line with euthanasia guidelines for clinical signs of toxicity related to MAL exposure.

b) Sampling and Tissue Preparations

At 24 h after the last dose, blood samples were collected from retro-orbital venous plexus, left for 60 min to clot and then centrifuged for 10 min at 2430×g. The obtained clear sera were stored at−80 °C for biochemical analysis. Rats were sacrificed by cervical dislocation. Both kidneys and liver tissue were

rapidly excised. One kidney and part of liver tissue were washed in saline, and fixed in 10% formalin was used for histo-pathological examination[40]. For biochemical analysis, kidney and part of liver tissue samples were blotted over a piece of filter paper and perfused with ice-cold 50 mM/L sodium phosphate buffer saline (100 mM/L Na2HPO4/NaH2PO4), containing 0.1 mM/L EDTA to remove red blood cells and clots. Tissue samples were then homogenized in ice-cold buffer (5 to 10 mL per 1 g of tissue) and centrifuged for 30 min at 2000g (5000 r/min). The obtained supernatant was kept in 1.5-mL eppendorf tubes at − 80 °C for evaluation of biochemical parameters.

c) Biochemical parameter in serum

The activities of some biochemical parameters representing liver and kidney functions were determined in rats' blood plasma calorimetrically as follows: Alanine and aspartate aminotransferase (ALT and AST) activities were determined according to the method of Reitman and Frankel[41], ALP and bilirubin were determined according to Tietz et al.[42] and Walter respectively[43]. Renal products including creatinine, urea, and uric acid were determined according to Larsen[44], Coulombe and Favreau[45], and Whitehead et al[46], respectively. We used the methods of Savory et al. to assess serum level of albumin[47].

d) Antioxidant enzymes in liver and kidneys homogenates:

Assessment of liver and kidney antioxidant defense mechanisms was done in tissue homogenates, these organs were chosen because the liver is primarily involved in the metabolism of xenobiotics including MAL, while the kidney is the main organ of excretion. The tissue level of malondialdehyde (MDA) was determined according to the methods of Mihara and Uchiyama[48]. Moreover, activities of antioxidant enzymes as SOD and CAT and GSH content were evaluated according to methods of Nishikimi et al.,[49] Aebi[50], and Beutler et al[51] respectively.

3. Statistical analysis

Results are presented as mean± SD for comparison of different experimental animal groups and control ones. The results were statistically analyzed by a one way ANOVA. P-value >0.05 was considered significant.

4. Result

1) Biochemical parameter in serum

1-A: Effects of Kefir treatment on liver functions in MAL - intoxicated rat

Liver is often the primary target for toxic effect of xenobiotics. It is known that the detoxification of the toxic materials which enter the body occurs mainly in the liver. Therefore, liver can be used as an index for the toxicity of xenobiotics. Animals treated with MAL showed a significant increase ($p < 0.05$) in serum levels of ALT, AST and ALP. However, MAL caused a significant ($p < 0.05$) increase in the plasma level of bilirubin and decrease albumin level (Fig: 1) whereas, animals treated with KF alone did not show a significant change in all parameter tested. Co-treatment of MAL-intoxicated rats with KF significantly ($p < 0.05$) reduced the alterations in all previous parameters with restoration of bilirubin normal level.

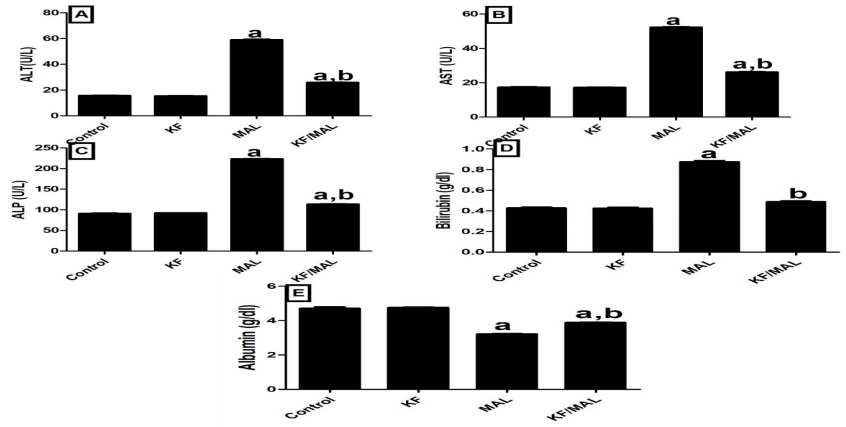

Figure 1: Effect of kefir (KF, 1.8 ml/day) on the plasma levels of ALT (A), AST (B), ALP (C), Bilirubin (D) and albumin (E) in rats exposed to Malathion (MAL 50 mg/kg) induced toxicity. Values are represented as means ± SD. (n=6).Significant difference is reported when $P < 0.05$.

[a] Significant difference compared to control, [b] significant difference compared to MAL gp. Data was analyzed by one-way ANOVA using Tuckey's post hoc test.

1-B: Effects of Kefir treatment on kidney functions in MAL - intoxicated rat:

Concerning kidney dysfunction, as we showed in Fig. 2 that MAL exposure induced an increase in urea, creatinine, uric acid levels and decrease albumin level. Whereas treatment of MAL-intoxicated rats with KF (1.8 mL/day) significantly ($p < 0.05$) reduced the alterations in all previous parameters. Moreover, Kefir restoring normal serum levels of creatinine

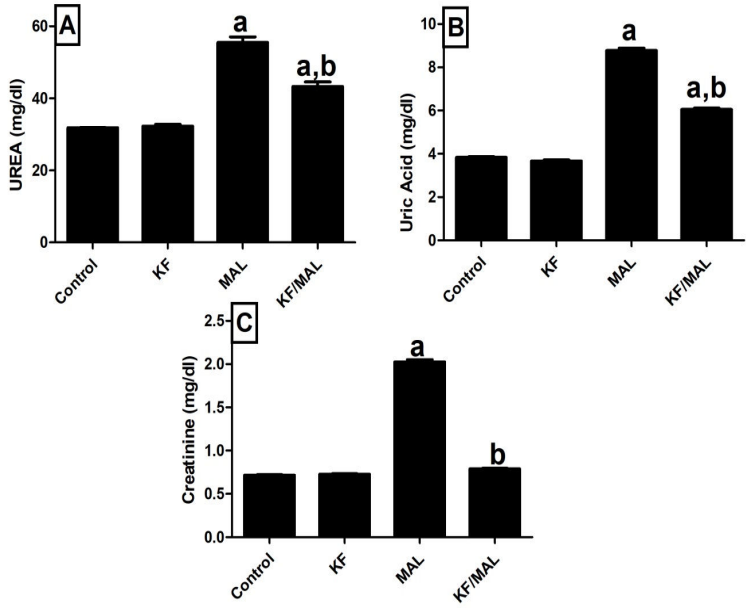

Figure 2: Effect of kefir (KF, 1.8 mL/day) on the plasma levels of Urea, Creatinine and Uric Acid in rats exposed to Malathion (MAL50 mg/kg) induced toxicity. Values are represented as means ± SD. (n=6).Significant difference is reported when $P < 0.05$.

[a] Significant difference compared to control, [b] significant difference compared to MAL.

Data was analyzed by one-way ANOVA using Tuckey's post hoc test.

2) Effects of Kefir treatment on Antioxidant activity in the hepatic and renal tissues in MAL - intoxicated rat:

Data from table 1 clearly revealed that MAL exposure significantly increased ($p < 0.05$) the MDA tissue content. In contrast, it significantly decreased GSH content, SOD and CAT activities in both liver and kidney tissues. Treatment with KF significantly reduced all these alterations with restoration of normal CAT enzyme activities in liver tissue.

Table 1: Effect of kefir on liver and kidney tissue oxidative stress markers and antioxidant parameters in MAL -intoxicated rats

Organ	Parameters	Control	KEFIR	MAL	KEFIR/MAL
liver	GSH (nmol/gm tissue)	23.85±0.13	23.20±0.65	14.63±0.19[a]	21.30±0.29[a,b]
	SOD (U/g protein)	16.90±0.29	17.58±0.46	5.100±0.41[a]	13.30±0.67[a,b]
	CAT (U/gm tissue)	2.200±0.08	2.250±0.13	0.740±0.01[a]	1.980±0.02[b]
	MDA (nmol/g tissue)	4.240±0.19	4.310±0.10	7.750±0.13[a]	4.900±0.17[a,b]
Kidney	GSH (nmol/gm tissue)	25.03±0.26	24.50±0.50	13.15±0.26[a]	16.58±0.36[a,b]
	SOD (U/g protein)	24.70±0.18	24.00±0.22	9.030±0.06[a]	22.30±0.51[a,b]
	CAT (U/gm tissue)	2.030±0.02	2.010±0.06	0.730±0.04[a]	1.730±0.04[a,b]
	MDA (nmol/g tissue)	3.620±0.17	4.060±0.13	7.070±0.10[a]	5.100±0.21[a,b]

Data are expressed as means ± SD (n = 6). [a] significant difference ($p < 0.05$) from the negative control (Kefir) group. [b] significant difference ($p < 0.05$) from the positive control (MAL) group. GSH reduced glutathione, SOD superoxide dismutase, CAT catalase, MDA Malondialdehyde

3) Effects of Kefir treatment on hepatic and renal histopathological examinations in MAL - intoxicated rat

3-A Histopathological Changes in the liver: Light microscopic observation of liver of control and KF treated rats showed regular and compact configuration with well-organized hepatic cell and central vain with preserved hepatic lobular architecture and patent sinusoids (Fig. 3 A and B). In contrast, the livers of the MAL-intoxicated rats showed severe hepatic lesion displayed as remarkable cell loss, focal necrosis, enlargement of sinusoids with inflammatory cells infiltration, dilation and hemorrhage associated and necrosis While the livers

of the malathion plus KF-treated animals exhibited less hepatic injury, unremarkable cell loss (minimal inflammatory reaction).

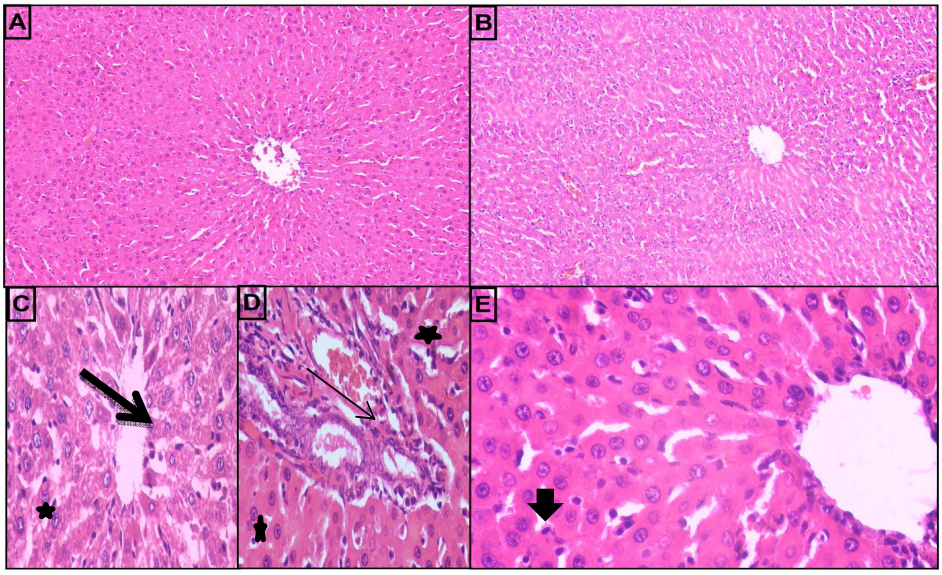

Figure 3: A photomicrograph of hematoxylin and eosin stained histological section in liver of A) control rats showed normal central vein and normal hepatocytes **(H & E X 100)**. B) The liver sections in the rats treated with *KF* alone showed normal portal tracts, central vein, and patent sinusoids parenchyma and normal focal aggregation of lymphocytes **(H & E X 100)**. C) A section of liver of MAL group showed hyperplasia of epithelial lining (arrow), vacuolar degeneration (star) **(H & E X 400).** D) A section of liver of MAL-intoxicated group showed focal hepatic necrosis associated with inflammatory cells infiltration(arrow), hemorrhage (star) as well as apoptosis of hepatocytes and Kupffer cell activation (arrow head) **(H & E X 400)**. E) A section of liver of rats treated with MAL plus *KF* showed much less damages in the hepatic parenchyma with very mild vacuolar degeneration with no evidence of necrosis, mild congestion and no inflammatory changes **(H & E X 400)**.

3-B Histopathological Changes in the Kidney: In light microscopic histopathological examinations on the kidney of control and KF groups rat showed regular structure with capillaries, tubules, glomerulus, and Bowman's capsule (Fig. 4 A & B). On the other hand, the areas of renal cortex containing renal corpuscles and associated tubules expressed more pronounced changes in MAL-treated animals compared with control. In the case of MAL-intoxicated group, there were pronounced changes in the structure of renal corpuscle including highly degeneration of glomeruli, Bowman's capsules and associated tubules structure, shrinkage of glomeruli, edema of renal tubules, raising of urinary space and inflammatory cell infiltration was also noticed (Fig. 4 C & D). KF treatment reversed abnormal histology of renal cortex areas induced by MAL intoxication.

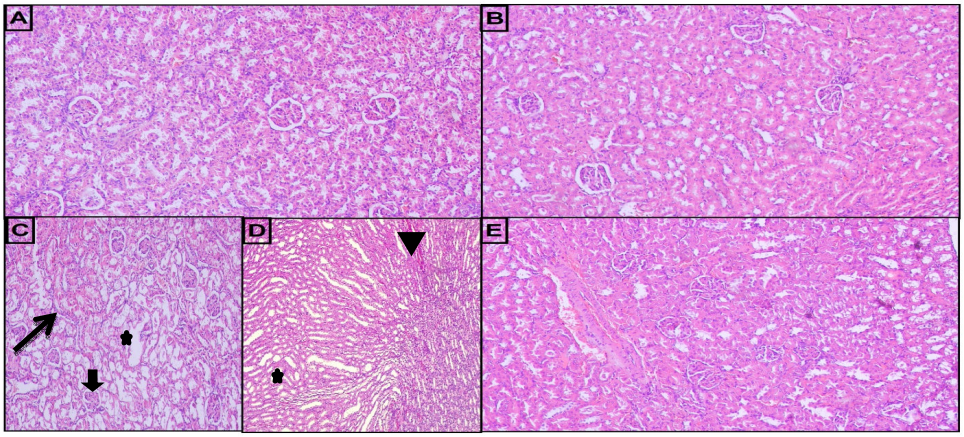

Figure 4: A photomicrograph of hematoxylin and eosin stained histological section in kidney of (A)&(B) Control and KF rats were having normal architecture of proximal tubules with intact brush border and vesicular nuclei of the epithelium lining, intact epithelium of the distal convoluted tubules. (C)& (D) showed swelling appearances, increasing of urinary spaces (star), highly degeneration of glomeruli (triangle) , Bowman's capsules (arrow head)and associated tubules structure with inflammatory cells infiltration (arrows).(E) *KF* treatment reversed abnormal histology of renal cortex areas induced by MAL intoxication. **(H & E X 200)**.

5. Discussion

Probiotics are microbial cell preparations or components of microbial cells with a beneficial effect on the health of the host. Recently, there is a strong focus on beneficial foods with probiotic microorganisms and functional organic substances especially kefir as it has a biological activity due to the presence of kefir's exopolysaccharides, known as kefiran[52,53] and considered mainly a probiotic resource because of its composition[54]. Many researchers proved that probiotic bacteria in kefir consumers' gut are increased and play an important role in health improvement[55,56].

There are many significant potential health hazards for all living organisms resulting from widely spread use of organophosphate pesticides as MAL[57]. MAL has been reported to be used extensively all over the world including Egypt in public health, agriculture and household purposes[58,59,60]. Several studies reported that MAL caused hepatotoxicity, testicular toxicity and genotoxicity besides biochemical and hematological parameters alteration in experimental animals[61,62,63,64,65]. Moreover, MAL caused changes in antioxidant enzyme activities in kidney, brain and liver[66,67,68]. The current study showed that elevated lipid peroxidation by MAL which evidenced by the increased MDA tissue levels which may be due to increased production of reactive oxygen. Moreover, significant decreases in the tissue levels of the non-enzymatic antioxidant (GSH), as well as SOD, and CAT enzymes in both liver and kidneys tissue in MAL-intoxicated rats, were observed, which reflect the exhaustion of the cellular antioxidant defense mechanisms. This may be associated with the ability of MAL to induce oxidative stress by changing the status of oxidant-antioxidant balance in many body organs as kidney, brain and liver[69,70,71,72].

In the present study, MAL significantly increased serum level of ALT, AST, and ALP than the control group which may be due to the decreased catabolism rate of these enzymes in plasma[73] . Moreover, MAL toxic effect on hepatocytes that associated with the alterations of their organelles, morphological change and damage of the cell membrane, result in secretion of several enzymes located in the hepatocyte cytosol including ALT, AST, and ALP into the blood[74,75,76].

Furthermore, ALP as an important critical enzyme in biological processes is responsible for detoxification, metabolism and biosynthesis of energetic macro-molecules for different essential functions. Any interference in this enzyme leads to biochemical alternation and impairment in the tissue and cellular function[77]. This is consistent with the damage to the hepatic tissues in the MAL-intoxicated rats seen by light microscopy. Results from histological investigation are in accord with diverse earlier studies which elucidate that the introduction to pesticides led to provoke intensive biochemical and physiological turbulence in experimental animals[78]. According to Tos-Luty et al., MAL intoxication led to injurious effects on the organization of the liver and kidney with the persistence of thin subcapsular infiltrations, diffused parenchymatous degeneration of single hepatocytes. Moreover, the increased bilirubin level that was reported in the present study may be due to pathological changes such as necrosis of hepatocytes, which cause increase in the permeability of cell membranes and hence of release of bilirubin in the blood stream[79,80].

Kidney is one of the targets organs that attacked by OP compounds[81,82] which revealed by alteration in plasma urea, uric acid , creatinine and albumin levels[83,84]. The present results showed a statistically significant increase in serum creatinine, urea and acid uric levels in MAL-intoxicated rats compared to control rats. Elevated blood urea level is correlated with an increased protein catabolism or the conversion of ammonia to urea as a result of elevated synthesis of arginase enzyme that involved in urea production or referred to glomerular damage with subsequent kidney dysfunction[85,86,87,88]. Moreover, this elevated urea level may result from increased breakdown of tissue or impaired excretion[89], so this elevation may be due to kidney damage caused by MAL. In context, the elevated uric acid level in this study may be related to either increased protein degradation, which is involved in uric acid formation, or the toxic effect of MAL on the kidneys as uric acid is the end product of purine catabolism and its elevated serum level in proportion to the decrease in creatinine clearance and correlate with the degree of renal tubulo-interstitial damage following oxidative damage of at least one constituent of the glomerular capillary wall[90,91,92,93]. Increased creatinine level is a risk marker

for chronic renal insufficiency that results from the glomerular and tubular damage in the kidneys[94,95,96]. In the present study, it was shown that degeneration of kidney tissues in MAL- intoxicated rats is correlated with the creatinine levels in plasma. So, the alterations in kidney functions were well correlated with the histological results in the present study. These results are in agreement with many researches[97,98,99].

In this study, a significantly lower albumin level was recorded in MAL-intoxicated animals than the control animals. Albumin, which is the most abundant blood plasma protein, is produced by the liver and it was proved by many researchers that albumin production can be decreased by OPs such as chlorpyrifos[100,101,102]. This reduction is generally suggestive of liver disease with subsequent alteration in protein and free amino acid metabolism and synthesis in the liver due to the harmful effect of MAL[103]. Hypoalbuminemia found in MAL- intoxicated animals in our study is thought to be a consequence of qualitative and quantitative disturbance of protein synthesis due to impaired hepatic function[104,105,106]. Moreover, it may be due to impairment of the glomerular function and tubular damage in the kidneys[107,108].

Kefir was chosen in our study as a potential protective agent because of its antioxidant and hepatorenal protective activity. Our results showed that treatment of MAL-intoxicated rats with KF minimized the biochemical and histopathological effects of MAL and kept the serum levels of hepatic and renal injury biomarkers nearly within normal ranges. Moreover, KF ameliorated the MAL-induced oxidative damage through reducing the generation of free radicals and increasing tissue activities of antioxidant enzymes. Previous studies suggested several underlying mechanisms for the antioxidant activity of KF which may be attributed to their proton-donating ability, their reducing power and SOD-like activity as evidenced through DPPH and superoxide radical-scavenging and lipid peroxidative inhibition[109,110]. Biochemical analyses showed higher superoxide dismutase, catalase, and glutathione levels in the MAL/KF group's renal and hepatic tissues compared with those in the MAL group these results are in line with Yadav et al and Kahraman et al who proved that KF increases the antioxidant capacity of the tissues.[111,112] In our

study, the reduction of malondialdehyde levels in both kidney and liver tissues is another biochemical effect of KF. MDA, an indicator of tissue damage, was significantly decreased in MAL/KF group compared to MAL group. These outcomes were in concurrence with Ozcan et al. and El- Kewawy as they reported that on supplementing diet by kefir, it reduced the oxidative damage induced by xenobiotic[113,114,115]. Accordingly, the oxidative damage induced by MAL and supported oxidative system may be indeed preventing by kefir.

Moreover, The improvement in histopathological characters of kidney and liver tissues in MAL/KF group compared to MAL group agree with (Amdekar and Singh, Momeni et al.and Karabacak et al), they found that the probiotics protect the tissue from damage caused by oxidation due to its effective role as a natural antioxidant by removing the free radicals that have been created[116,117,118]. besides, kefir was reported to stabilize metabolism, positively effect on liver, gallbladder, and renal function, and blood circulation[119] Thus, we considered kefir having alleviating effects on the renal tissue damage produced by different mechanisms.

CONCLUSIONS

In the light of the aforementioned results, it can be seen that biochemical parameters are in coincidence with histopathological observations which showed degenerative changes in liver and kidney of treated rats with MAL. The co-treatment of MAL-intoxicated groups with KF had attenuated all the biochemical, functional, and histopathological alterations induced by malathion especially restored the antioxidant enzymes status and the histoarchitecture of MAL-damaged hepato-renal tissue. The hepatorenal protective effect of kefir may be because of its antioxidant property and detoxification capacity. Furthermore, more attention is needed to limit the use of MAL, which is widely used in the developing countries.

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التأثير الوقائى للكفير ضد تسمم الكبد والكلى الذى يسببه الملاثيون فى ذكور الجرذان

مـروة مـدحت- منجود محمد منجود- نسرين عمر- محمد زكى حسين

يعد مبيد الملاثيون من أكثر المبيدات العضوية القادرة على إنتاج الشوارد الحرة وإحداث اضطراب فى مضادات الأكسدة فى الجسم. أجريت هذه الدراسة لتقييم تأثير الكفير، وهو حليب مخمر غنى بالبروبيوتيك، على الإجهاد التأكسدى والوظيفى، وكذلك الضرر النسيجى الناجم عن الملاثيون فى كل من كلى وكبد الجرذان. تم تقسيم اثنان وثلاثون جرذًا بالغًا إلى أربعة مجاميع بصورة عشوائية وخضعت للعلاج لمدة ٣٠ يومًا عن طريق الفم على النحو كالتالى: المجموعة الضابطة، ثم مجموعة الكفير؛ حقنت بـ١,٨ مل/ يوم، أما المجموعة الثالثة فحقنت بالملاثيون بجرعة٥٠ مجم/ كجم/ يوم، والمجموعة الرابعة حقنت بمادتى الملاثيون والكفير معًا بنفس الجرعات السابقة. تم إعطاء العلاجات لمدة ٣٠ يومًا عن طريق الفم، وبعد أربعة وعشرين ساعة من آخر جرعة، تم إجراء القياسات الخاصة بوظيفة وأنسجة كل من الكلى والكبد وتحليلها إحصائيًا. وقد أظهرت النتائج أن الملاثيون رفع من مستويات إنزيمات الكبد فى الدم مثل الأسبارتات أمينوترانسفيراز (AST)، آلانين أمينوترانسفيراز (ALT) والفوسفاتيز القلوى (ALP) كما أدى إلى زيادة تركيز البيليروبين وانخفاض مستوى الألبومين. أدى الملاثيون أيضًا إلى زيادة اليوريا وحمض البوليك والكرياتينين فى الدم، بالإضافة إلى زيادة مستوى مالوند الديهيد المسبب للأكسدة فى أنسجة الكبد والكلى مع حدوث انخفاض فى أنشطة مضادات الأكسدة. هذا وقد أدى استخدام الكفير مع مبيد الملاثيون إلى تحسن فى وظائف الكبد والكلى وكذلك تحسن عام فى أنسجة الكبد والكلى التالفة، مما يؤكد دوره كمضاد للأكسدة. واستنتج من هذه الدراسة أن للكفير تأثيرًا وقائيًا محتملًا لحماية الكلى والكبد من التأثيرات الضارة للملاثيون.