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Investigation of protective roles of myricetin and quercetin against nephrotoxicity caused by malathion intoxication in rats

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Abstract

In this study, the protective roles of myricetin (MYC) and quercetin (QRS) against nephrotoxicity caused by malathion (MLT) intoxication were investigated. A total of 42 male rats were used and divided into 6 groups. The dose of MLT was fixed at 108 mg/kg/day and MYC or QRS were administered separately at doses of 100 and 200 mg/kg/day. All chemicals were orally administered for 28 days. At the end of the study, sera and kidney tissues were obtained. Serum urea, creatinine and uric acid levels, kidney malondialdehyde (MDA), nitric oxide (NOx), protein carbonyl (PCO), 8-hydroxy-2'-deoxyguanosine (8-OHdG), prostaglandin E2 (PGE2), reduced glutathione (GSH), catalase, superoxide dismutase (SOD), glutathione redustase (GSH-Rx), glutathione peroxidase (GSH-Px), paraoxonase (PON), and arylesterase (ARE) levels were measured as well as histological examinations in kidney tissue were performed. MLT treatment increased serum urea and creatinine with increased levels of kidney MDA, NOx, PCO, 8-OHdG, PGE2 and decreased levels of catalase, SOD, PON, ARE, GSH, and GSH-Px in the kidney ($p<0.05$). Also, MLT intoxication disrupted the histological structure of kidney tissue. MYC and high concentration of QRS were able to reverse this effect by reducing oxidative stress in the kidney and improving the histology of kidney tissue ($p<0.05$). In conclusion, MLT caused an increase in oxidative stress and inflammatory response with impaired kidney functions in rats, but MYC and QRS treatments can prevent MLT-induced nephrotoxicity in rats.

Keywords: Malathion, myricetin, quercetin, oxidative stress, inflammation, nephrotoxicity

Introduction

Pesticides are chemicals used to prevent or to control agricultural pests. The increase in the world population with increasing demand for food has led to gradually increasing use of pesticide on global scale. This increase becomes as a problem that required to be controlled due to both environmental pollution and negative effects on living things. The term pesticide covers a wide range of uses such as insecticide, herbicide, fungicide, rodent and nematicide killer. With the widespread use of pesticides in agriculture, the spread of these chemical residues to the environment destabilizes terrestrial and aquatic ecosystems, and consequently poses a serious threat to human health [1].

Numerous studies show that pesticides are associated with diseases such as cancer, obesity, asthma, and allergies. Even in the case of exposure to very low doses, it can cause birth defects and fetal death, especially in the early stages of development [2]. Around the world, approximately 355,000 people die every year due to the misuse of pesticides [1]. It has been stated that insecticides, as a subclass of pesticides, are extensively used in the fight against harmful insects, and they have an important

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role in both increasing the yield in agricultural production and protecting public health. According to their toxic effects and chemical properties, insecticides are classified as organochlorines, organophosphates, pyrethroids, carbamates and other insecticides. Organophosphate insecticides are the most widely used group of insecticides. The best known organophosphate insecticides are parathion, fenthion, dichlorvos, diazinon and malathion (MLT) [3]. MLT is a broad-spectrum organophosphate insecticide with low toxicity in mammals, used for protection against insects worldwide. MLT accumulates especially in adipose tissue, liver and kidneys and can affect the immune system, brain, liver, muscles, urinary system, reproductive system, pancreas and circulatory system in both humans and animals [4].

Acute and chronic doses of MLT cause oxidative stress in humans and animals [5]. One of the organs most affected by oxidative stress resulting from MLT intoxication is the kidney. Oxidative stress causes many renal disorders, from acute renal failure, obstructive nephropathy, hyperglycemia and glomerular damage to chronic renal failure, which results in hemodialysis and inflammation. In addition, due to the effect of reactive oxygen species (ROS) on mesenchial and epithelial cells, it can change the structure and function of the glomerulus. It is known that the glomerulus is more sensitive to oxidative damage than other nephron regions. Renal tubular epithelium may be exposed to damaging agents due to loss of permeability of the glomerulus or due to increased plasma levels of chemicals [6].

The use of plant extracts, and the flavonoids obtained for reducing the oxidative stress that may occur in the tissues for various reasons has attracted the attention of scientists for a long time. To date, more than 4000 naturally occurring flavonoids in foods have been identified. Flavonoids show anti-inflammatory, antihistaminic, antiviral, antibacterial and antitumoral activities as well as free radical scavenging and antioxidant activities. It is known that flavonoids show antioxidant properties by inhibiting lipid peroxidation, scavenging free radicals and chelation of metal ions [7]. Although the effects of some flavonoids against oxidative stress resulting from exposure to various insecticides have been demonstrated, to the best of our knowledge, there is no study for investigating the protective effects of myricetin (MYC) and quercetin (QRS). These compounds are known to have strong antioxidant properties against oxidative stress caused by MLT intoxication.

Various studies have shown that MYC protects cells from the damage caused by free radicals, which is reported to have anti-cancer, anti-diabetic and similar therapeutic properties as well as antioxidant properties [8]. QRS is a flavonoid found in red onions, apples, strawberries, citrus fruits, broccoli, tea and red grapes, which is reported to show antioxidant, antiatherogenic, antihypertensive, anti-inflammatory, anticancer and antihyperglycemic activities [9]. The aim of this study was to reveal to what extent MYC and QRS administrations could prevent oxidative stress and inflammatory response in kidney tissue in rats caused by experimental MLT intoxication.

Material and Methods

Materials

In the current study, 42 male rats (weighing 250-300 g) were used. The study was started after the approval of the local Ethics Committee of Animal Experiments of Van Yuzuncu Yil University with the decision dated 25.06.2020 and numbered 2020/06-06. MYC (purity ≥95 %) and QRS (purity ≥95 %) was purchased from Xi'an Natural Field Biological Technology Co., Ltd., Xi'an, China. MLT (Gold Malathion® 65 EC, 650 g/L) was purchased from Safa Tarım Inc. Co., Konya, Turkey. In the literature review, the lethal dose-50 (LD50) value of orally administered MLT was found to be 1080 mg/kg for rats. It was decided to use a dose of 108 mg/kg/day, which is 1/10 of the LD50 value of MLT. The experiments showed that MLT applications at this dose do not cause significant death in rats, but they can cause organ and tissue damage to the desired extent [10, 11]. MLT used in the study was prepared by dissolving in corn oil. MYC and QRS solutions were prepared by dissolving the substances in 1% (w/v) carboxymethyl cellulose (CMC) solution. MLT and all phytochemicals were administered orally for 28 days, and the phytochemical application was performed at least 2 hours after MLT administration.

Methods

Experimental groups

Rats were randomly selected and divided into 6 groups. Groups were formed with attention to provide homogeneous groups regarding the total weight of the rats in the groups is approximately the same. The obtained groups are provided below:

Control group (n=7): Rats in this group were administered only 1 mL of corn oil and 1 mL of % 1 CMC solution by gavage for 28 days without any treatment.

MLT group (n=7): Rats in this group were administered 1 mL MLT (108 mg/kg/day) and 1 mL % 1 CMC solution by gavage for 28 days.

MLT+MYC100 group (n=7): Rats in this group were administered orally 1 mL MLT (108 mg/kg/day) and 1 mL MYC solution (100 mg/kg) for 28 days.

MLT+MYC200 group (n=7): Rats in this group were administered orally 1 mL MLT (108 mg/kg/day) and 1 mL MYC solution (200 mg/kg) for 28 days.

MLT+QRS100 group (n=7): Rats in this group were administered orally 1 mL MLT (108 mg/kg/day) and 1 mL QRS solution (100 mg/kg) for 28 days.

MLT+QRS200 group (n=7): Rats in this group were administered orally 1 mL MLT (108 mg/kg/day) and 1 mL QRS solution (200 mg/kg) for 28 days.

Sacrification of animals and collection of blood and tissue samples

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On day 29 of the study, rats under xylazine (10 mg/kg/i.p.) (Rompun, Bayer, Turkey) and ketamine (50 mg/kg/i.p.) (Ketalar, Eczacıbaşı, Turkey) anesthesia were bled by cardiac puncture and kidney tissues were removed. The blood was taken into serum separator tubes containing micronized silica particles, centrifuged at 3500 rpm for 15 minutesi and serum was obtained. The obtained serum samples were stored at -20°C until the study day. The obtained serums were used directly or diluted on the study day. Kidney tissues were first washed with 0.9% sodium chloride to remove the blood on them, then their moisture was removed with blotting paper, and stored at -20°C until the study day.

Homogenization of kidney tissues

The kidney tissues of the rats removed from the freezer (-20°C), and were allowed to gradually thaw on ice until they reached room temperature. Phosphate buffer containing 1 mmol/L EDTA and 50 mM Potassium Dihydrogen Phosphate (KH2PO4) (pH 7.4) was used for extraction [12]. 5 mL of cold buffer was added to 500 mg of tissue and the tissues were thoroughly crushed with a glass baguette. The tissue in the glass tube placed in the ice-filled plastic container was homogenized with the help of a mechanical homogenizer at 16000 rpm for 3 minutes. The obtained homogenate was centrifuged at 10000 rpm for 30 minutes at 4°C without a delay. Oxidative stress and inflammatory parameters were measured in the supernatant obtained from kidney tissue.

Serum renal function parameters

Urea, creatinine and uric acid levels of serum samples were measured by enzymatic method using commercial kit in Cobas Integra 800 autoanalyzer (Roche Diagnostics GmbH, Manheim, Germany). The results were expressed as mg/dL.

Oxidative stress and inflammatory parameters

Malondialdehyde (MDA) level: The method of Ohkawa et al. [13] was used to determine lipid peroxidation. The reaction mixtures were comprised of 0.15 M tris-HCl buffer (pH: 7.4), 10 mM KH2PO4 and tissue extract in a total volume of 2 mL and incubated at 37°C for 20 minutes with constant shaking. Addition of 1% trichloroacetic acid (TCA) stopped the reaction. Thiobarbituric acid (TBA) was taken in the tubes, which were then heated in a boiling water bath for 20 minutes. The colour formed was measured at 532 nm after centrifugation of tubes [13]. MDA concentration of the samples was measued as nmol/mg protein.

Catalase activity: The method reported by Aebi [14] was used to measure catalase activity. The basis of the method is to monitor the enzymatic degradation of the H2O2 substrate by catalase at 240 nm [14]. The obtained results were expressed as U/mg protein.

Superoxide dismutase (SOD) activity: The method reported by Sun et al. [15] was used to estimate superoxide dismutase activity. The measurement principle of SOD enzyme activity is based on measuring the color absorbed by nitroblue tetrazolium (NBT) at 560 nm of superoxide radicals released by xanthine oxidase in the presence of xanthine [15]. The activities were expressed as U/mg protein.

Reduced glutathione (GSH) level: GSH analysis was performed according to the method reported by Beutler et al. [16]. In this method, the yellow complex formed by the -SH groups in the homogenizer with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) is measured colorimetrically at a wavelength of 412 nm [16]. The obtained results were expressed as nmol/mg protein.

Nitric oxide (NOx) level: The production of NOx was estimated by the Griess method of Miranda et al. [17]. In a 96-well microplate, 50 µL of the supernatant of homogenate of kidney tissue was applied to each well in triplicate. Then, Griess reagent (1% sulfanilamide in 5% phosphoric acid, and 0.1 percent N - (1-naphthyl) ethylenediamine dihydro chloride in bidistilled water) was added. The measurement was performed at a wavelength of 550 nm [17]. The obtained NOx levels were expressed as nmol/mg protein.

Protein carbonyl (PCO) level: This method is based on measuring the color of hydrazone compounds formed by the reaction of 2,4-dinitrophenylhydrazine with carbonyl groups spectrophotometrically [18]. The obtained PCO levels were expressed as nmol/mg protein.

Glutathione peroxidase (GSH-Px) activity: Enzymatic glutathione peroxidase was estimated according to the method reported by Paglia and Valentine [19]. The reaction mixture containing buffer, sodium azide, required amount of enzyme, hydrogen peroxide, reduced glutathione and water was taken to a final incubation volüme, and were incubated for 10 minutes at 30°C. The reaction was termşnated by addition of TCA. The residual GSH content was determined by removing the supernatant by centrifugation by adding precipitating reagent and DTNB reagent. The colour was measured spectrophotometrically at a wavelength of 412 nm, and compared with a blank prepared by using sodium dihydrogen phosphate and DTNB reagent (1.0 mL) [19]. The obtained activities were expressed as U/mg protein.

Glutathione reductase (GSH-Rx) activity: Glutathione reductase was analyzed according to the method reported by Goldberg et al. [20]. Glutathione reductase converts oxidised glutathione to its reduced form by using nicotinamide adenine dinucleotide phosphate (NADPH) [20]. The obtained results were expressed as U/mg protein.

Paraoxonase (PON) and arylesterase (ARE) activities: PON activity was investigated by measuring the formation of p-nitrophenol formed as a result of enzymatic hydrolysis of paraoxon at 412 nm by using 100 mM Tris-HCl buffer (pH= 8.0) containing 2 mM CaCl2 and 4 mM paraoxon [21]. ARE activity was investigated by measuring the formation of phenol formed as a result of enzymatic hydrolysis of phenylacetate at 270 nm

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by using 100 mM Tris-HCl buffer (pH= 8.0) containing 2 mM CaCl2 and 10 mM phenylacetate [21]. The obtained results were measured as U/mg protein.

Prostaglandin E2 (PGE2) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels: PGE2 and 8-OHdG levels were measured using an ELISA kit (Lifespan Biosciences Inc., Seattle, Washington, USA) in accordance with the manufacturer's instructions. The obtained results were expressed as ng/g protein and ng/mg protein for PGE2 and 8-OHdG levels, respectively.

Total protein level: Total protein levels of kidney tissues were measured in order to specify enzyme activities as specific activities. Around water was added to a 0.01 mL sample, which was then combined with alkaline copper reagent and at room temperature, left for 10 minutes before adding 0.5 mL of Folin's phenol reagent. The formed blue colour after 20 minutes was spectrophotometricalls measured at a wavelength of 640 nm. Protein levels in tissues were measured according to the method of Lowry et al. [22], which were expressed as mg protein/mL.

Histopathological evaluation

Kidney tissues were fixed in 10% formaldehyde. After tissue treatment, the samples were embedded in paraffin. Sections of 4 µm thickness were obtained from the samples taken from the kidneys of each rat, stained with hematoxylin-eosin dye, visualized and photographed using a light microscope (Olympus-BX43) [23].

Statistical Analysis

Statistical analyzes were performed using SPSS for Windows v16.0 package program. ANOVA was used to test for statistical differences between groups for all parameters, and Tukey's multiple comparison tests were used for subgroup comparisons. deviation values are given as The descriptive statistics were expressed as Mean±standard deviation. A $p<0.05$ value was considered as statistically significant difference.

Results

Serum Biochemical Parameters

MLT administration increased serum urea and creatinine levels ($p<0.05$). Both concentrations of MYC and QRS decreased serum urea level, while both concentrations of MYC and only high concentration of QRS decreased serum creatinine levels ($p<0.05$). Both doses of MYC and QRS failed to cause a statistically significant decrease in serum uric acid levels ($p>0.05$) (Table 1). There was no statistically significant difference between the doses of MYC and QRS ($p>0.05$).

Table 1. Serum biochemical parameters

	Control	MLT	MLT+MYC100	MLT+MYC200	MLT+QRS100	MLT+MYC100
Urea (mg/dL)	31.6±0.4	32.8±0.4a	31.2±0.3b	31.4±0.3b	31.5±0.4b	31,5±0.3b
Creatinin (mg/dL)	0.31±0.01	0.42±0.02a	0.33±0.02b	0.32±0.01b	0.41±0.02	0.36±0.02b
U. Acid (mg/dL)	2.3±0.2	2.34±0.2	2.32±0.2	2.31±0.2	2.28±0.2	2.27±0.2

[a] Statistically significant compared to the control group ($p<0.05$)
[b] Statistically significant compared to MLT group ($p<0.05$)
(MLT: Malathion; MYC: Myricetin; QRS: Quercetin; U. Acid: Uric acid)

Oxidative Stress Parameters

MLT administration increased the MDA level in the kidney tissue, while both concentrations of MYC decreased the MDA level ($p<0.05$) (Figure 1A). MLT caused an increase in the level of protein carbonyl in the kidney tissue, while both doses of MYC decreased the protein carbonyl level ($p<0.05$) (Figure 1B). An increase in the level of 8-OHdG were observed after MLT intoxication, but both doses of MYC and QRS decreased the 8-OHdG level ($p<0.05$) (Figure 1C). MLT exposure caused an increase in kidney NOx and PGE2 levels, while administration of low and high doses of MYC reversed this situation ($p<0.05$)

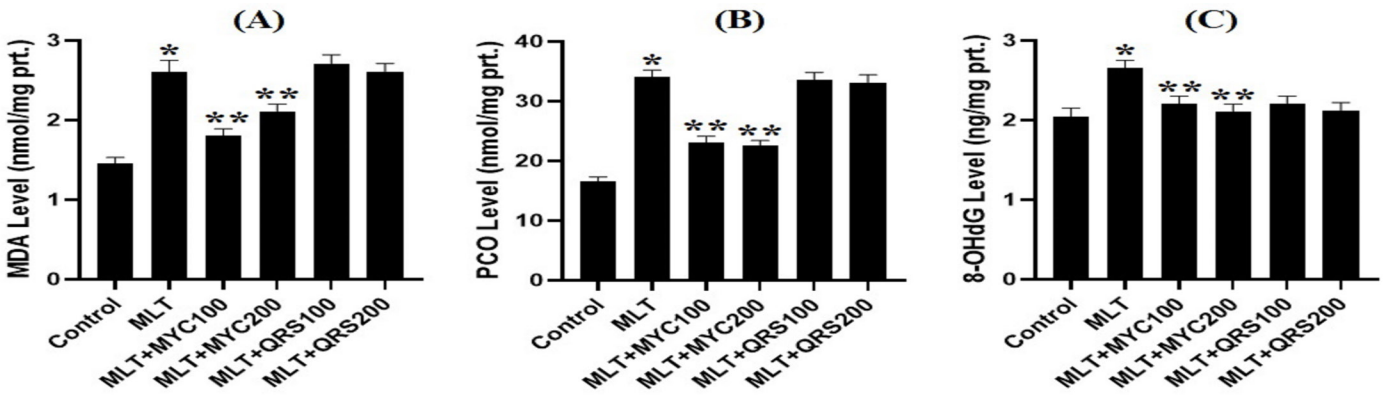

Figure 1. MDA, PCO, and 8-OHdG levels in kidney tissue. (MDA: Malondialdehyde; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; PCO: Protein carbonyl; MLT: Malathion; MYC: Myricetin; QRS: Quercetin)
* Statistically significant compared to the control group ($p<0.05$)
** Statistically significant compared to MLT group ($p<0.05$)

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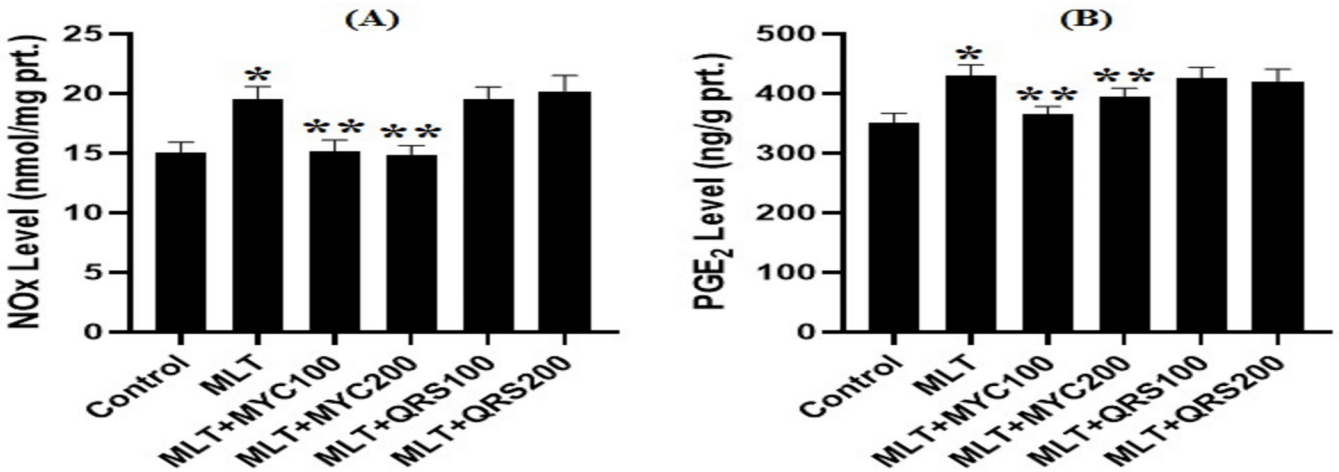

Figure 2. NOx and PGE2 levels in kidney tissue. (NOx: Nitric oxide; PGE2: Prostaglandin E2; MLT: Malathion; MYC: Myricetin; QRS: Quercetin)
* Statistically significant compared to the control group (p<0.05)
** Statistically significant compared to MLT group (p<0.05)

(Figure 2A and 2B, respectively). There was no any statistically significant difference between doses of MYC and QRS (p>0.05).

SOD, catalase, PON and ARE activities in the kidney tissue decreased after MLT administration (p<0.05) (Figure 3). However, high-dose MYC application increased SOD activity (Figure 3A), both doses of MYC application increased catalase activity (Figure 3B), both concentrations of MYC and QRS increased PON activity (Figure 3C), and both doses of MYC and high-dose QRS application increased ARE activity (Figure 3D) in the kidney tissue (p<0.05). There was no statistically significant difference between MYC and QRS concentrations (p>0.05).

While exposure to MLT caused a decrease in GSH level and GSH-Px activity, it did not cause any change in GSH-Rx activity (p<0.05) (Figure 4). While both doses of MYC caused an increase in GSH level (p<0.05) and GSH-Px activity (p<0.05 and p<0.05, respectively) (Figure 4B), QRS application did not cause any change in these parameters (p>0.05). In addition, MYC and QRS treatments did not cause a change in GSH-Rx activity (p>0.05) (Figure 4C). No statistically significant difference was observed between concentrations of MYC and QRS (p>0.05).

Histopathological Analysis

No pathological findings were determined in the histological sections of the kidneys of the rats in the control group (Figure 5A). There was no degeneration in the tubular epithelial cells, cystic dilation in the tubules and hyaline cylinders in the tubule lumen, hyperemia, inflammation and glomerular lesions were not observed. After MLT application, tubular degeneration, cystic dilation in the tubules and hyaline cylinders, bleeding, inflammation and glomerular lesions in the tubular lumen were detected in the kidneys of the rats (Figure 5B). Low-dose MYC treatment decreased the hyperemia in the kidney tissue, but tubular epithelial cell degeneration, cystic dilation in the tubules, and hyaline cylinders were detected in the tubule lumen (Figure 5C). On the other hand, high-dose MYC administration abolished all pathological findings and normalized the histology of the kidney tissue (Figure 5D). After low-dose QRS treatment, degeneration of some tubular epithelial cells, narrowing of glomeruli, mononuclear cell infiltration, and hyaline cylinders in the tubular lumen were detected in the kidneys of rats (Figure 5E). While almost all pathologies caused by MLT intoxication were eliminated with high-dose QRS administration, degeneration in the tubules was still present (Figure 5F).

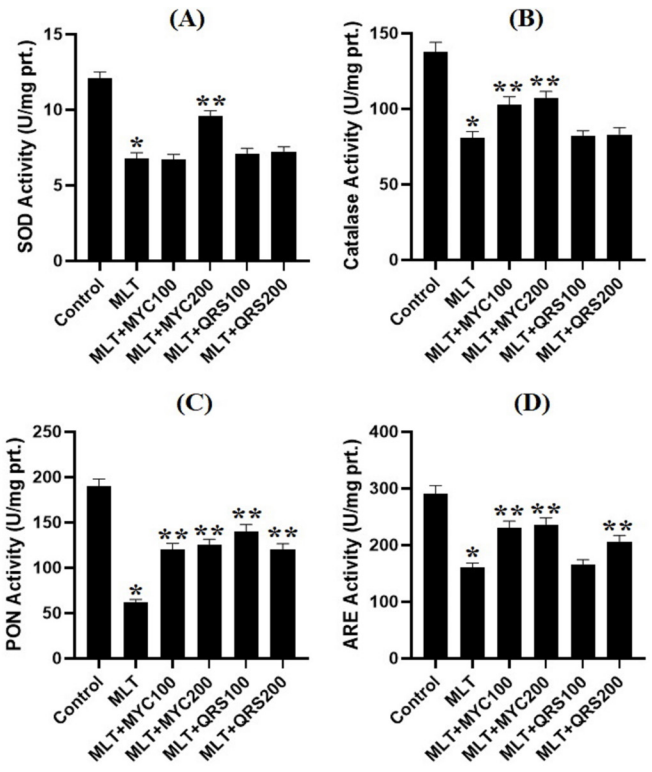

Figure 3. SOD, catalase, PON, and ARE activities in kidney tissue. (SOD: Superoxide dismutase; PON: Paraoxonase; ARE: Arylesterase; MLT: Malathion; MYC: Myricetin; QRS: Quercetin)

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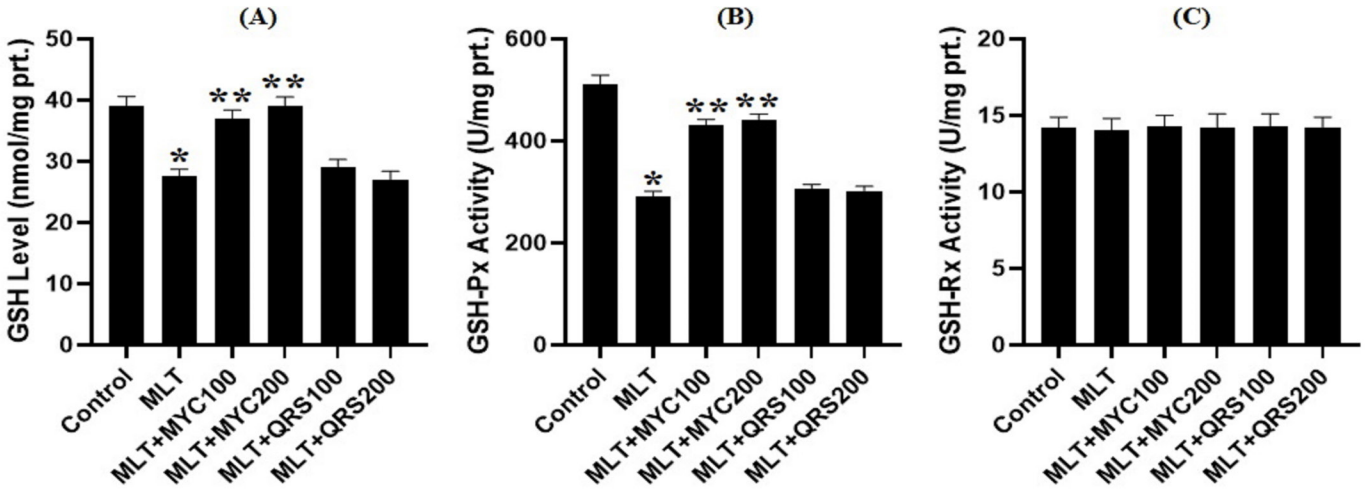

Figure 4. GSH level and GSH-Px and GSH-Rx activities in kidney tissue. (GSH: Reduced glutathione; GSH-Px: Glutathione peroxidase; GSH-Rx: Glutathione reductase; MLT: Malathion; MYC: Myricetin; QRS: Quercetin)
* Statistically significant compared to the control group ($p<0.05$)
** Statistically significant compared to MLT group ($p<0.05$)

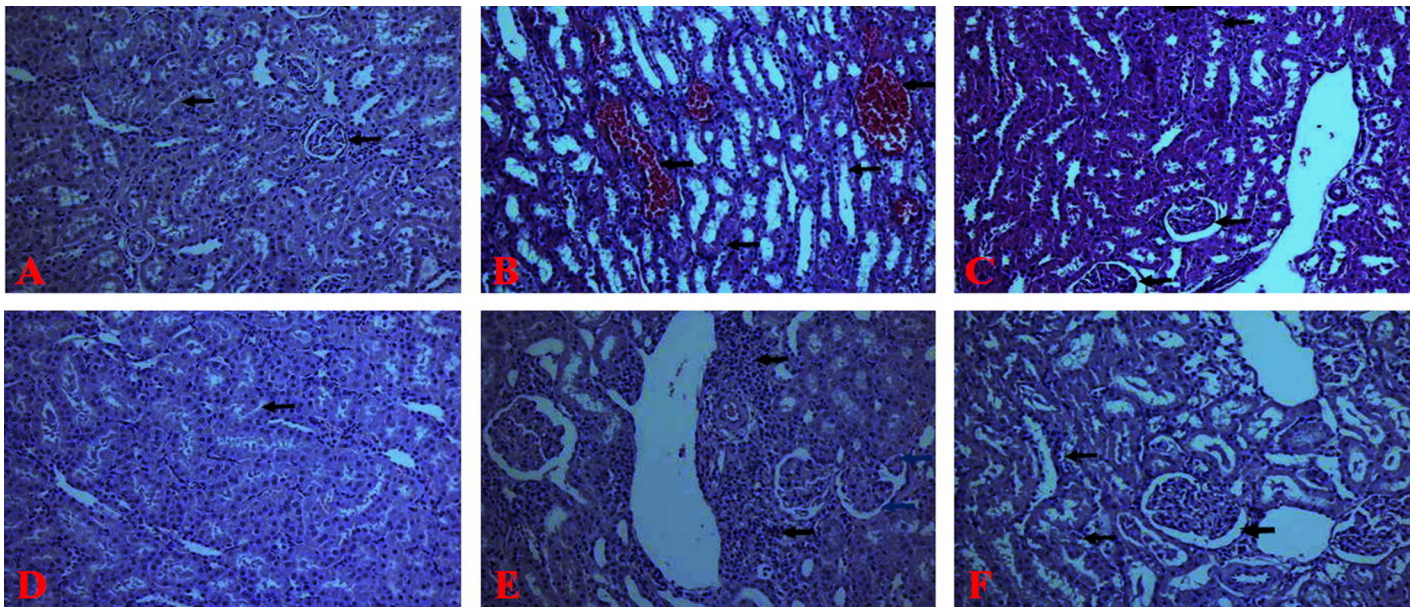

Figure 5. Histological alterations of kidney tissue (Hematoxylin and eosin staining with x40 magnification). (A: Control group, B: MLT group, C: MLT+MYC100 group, D: MLT+MYC200 group, E: MLT+QRS100 group, F: MLT+QRS200 group) (MLT: Malathion; MYC: Myricetin; QRS: Quercetin)

* Statistically significant compared to the control group ($p<0.05$)
** Statistically significant compared to MLT group ($p<0.05$)

Discussion

Pesticides, which cause environmental pollution to a large extent, are used unconsciously and randomly, thus causing various problems. Residues of organophosphate pesticides have been detected in soil, aquatic organisms, vegetables, seeds and various food products. MLT, which is an effective insecticide among organophosphate pesticides, is widely used in many countries. It is used for the control of internal or external parasites of farm animals and pets as well as insect control of houses and open spaces [4]. MLT is rapidly and well absorbed from the gastrointestinal tract, skin, lungs and mucous membranes. MLT is excreted from the body through bile, urine and respiration. The toxic effect of MLT is activated in the liver by converting it to its metabolite, malaoxone [24]. Due to the low solubility of MLT in water, it was dissolved in corn oil and administered to rats in the experiments. Oral LD50 dose of MLT was determined as 1080 mg/kg in male rats [25]. In the current study, 108 mg/kg of MLT, which is 1/10 LD50 concentration of MLT, was administered to rats by gavage for 28 days.

MLT and its metabolites are genotoxic, cytotoxic, hepatotoxic, embryotoxic, thyrotoxic, neurotoxic and adrenotoxic in humans and in various animal species. It has also been shown to have

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toxic effects on hematological and immunological systems. The liver and kidney are the most damaged tissues by MLT as these pesticides are biotransformed in the liver, and the excretion process takes place in kidneys in general. Since the kidneys are more sensitive to endothelial destruction and intravascular volume changes than other organs, they are among the organs which are adversely affected by MLT intoxication. High oxygen consumption, metabolic activity of the kidneys, as well as reactive oxygen species in infiltrating cells and tubulin cells cause the kidney to exceed its own antioxidant protection mechanism, thus the tissue damage occurs accordingly. In many studies, it has been stated that ROS causes multiple organ toxicity, especially nephrotoxicity [24]. For this reason, we aimed to observe the biochemical changes caused by MLT in the kidney tissue and the effects of MYC and QRS treatments, which were performed at 100 and 200 mg/kg/day doses for MYC [26] and QRS [27], respectively.

Urea and creatinine amounts are important parameters that provide information about the functions of the kidneys. Many studies report that elevated serum urea and creatinine levels are evidence of renal dysfunction [28]. Sarhan et al. [28] showed a decrease in glomerular filtration as evidence of nephrotoxicity. They stated that this was determined by the increase in serum urea and creatinine levels. MLT exposure and MYC or QRS treatments did not cause any change in serum uric acid levels, and it is thought that this is a result of the study procedure as the period was not long enough to cause a change in uric acid level.

There are many studies in the literature indicating that short or long-term exposure to pesticides causes damage to biomolecules by increasing oxidative damage in various tissues, especially in the kidney. The redox imbalance observed during pesticide intoxication leads to oxidative stress, organ damage and endothelial dysfunction. In a study, the effects of organophosphates were examined and it was stated that antioxidant enzyme levels decreased and lipid peroxidation increased. It was stated that toxicity occurred in the liver, kidney, spleen and brain of rats administered subletal dose of chlorpyrifos, and that chlorpyrifos caused changes in biochemical parameters in rat tissues [29]. A study conducted with methyl parathion showed oxidative stress in the kidneys, gills, and liver tissues of mrigal carp (Cirrhinus mrigala) caused by this substance [30]. In another study, rats were orally administered with chlorpyrifos, which resulted in increased MDA levels and erythrocytes counts as well as decreased SOD, catalase and GST levels [31]. In our study, it was observed that MLT administration increased MDA level in kidney tissues of rats, decreased SOD, catalase, GSH and GSH-Px levels, but did not change GSH-Rx levels. These results show that MLT intoxication induces oxidative stress by increasing free radical formation and suppressing antioxidant defence mechanism.

Various preservatives have been examined against organ damage as a result of pesticide intoxication, and positive results have been obtained in some studies. Gur and Kandemir [32] reported that when malathion was administered orally to rats at a dose of 100 mg/kg together with rutin at 50 mg/kg and 100 mg/kg concentrations, it reduced the oxidative stress caused by malathion. Also, Abdel-Daim et al. [33] found that when thymoquinone and diallyl sulphide were applied to rats together with malathion, these phytochemicals prevented the nephrotoxicity, hepatotoxicity, and neurotoxicity in rats caused by this pesticide. In our study, it was observed that MDA levels in the kidney tissues of MLT-treated rats decreased with MYC administration, SOD activity increased only in the presence of high-dose MYC, GSH level, GSH-Px and catalase activities increased with MYC application, and GSH-Rx activity was not affected by any phytochemical application. These results show that especially MYC can reduce oxidative stress resulting from MLT intoxication.

PON and ARE are synthesized by the liver and capable of hydrolyzing aromatic carboxylic acid esters and organophosphates in plasma. The most important tasks of PON and ARE are to play active role in reducing the toxic effects of organophosphate compounds. It was stated that serum PON and ARE decreased significantly in organophosphate poisoning compared to the control group. It has been stated that low serum PON and ARE may cause an increase in ROS levels [34]. Our study revealed that MLT application has a decreasing effect on PON and ARE activities in rat kidney tissue, MYC has an increasing effect PON and ARE activities at both concentrations. While QRS increased PON activity at both concentrations, it increased ARE activity at only high concentrations.

DNA damage resulting from oxidation mostly damages the purine and pyrimidine bases in the structure of DNA, and 8-OHdG, one of the most important markers of this damage, is widely used in both in vivo and in vitro studies [35]. In the current study, we determined that 8-OH dG level, which is one of the most important parameters showing DNA oxidation, was increased after MLT application and decreased with MYC and QRS application. This result shows that both of the studied phytochemicals can prevent DNA damage. Protein carbonyl level is the most important parameter in determining protein oxidation [36]. In this investigation, it was observed that MLT application increased the protein carbonyl level in rat kidneys. Both concentrations of MYC reduced the protein carbonyl level in kidney tissue. This result showed that MLT intoxication has an increasing effect on protein oxidation in kidney tissue, which can be prevented by MYC treatment

Experimental studies have shown that chronic administration of organophosphates increases inflammation in the brain, Langerhans islet cells, macrophages and serum TNF-α levels of rats [37]. Aboubakr et al. [38] reported that inflammation in the brain increased with acute administration of chlorpyrifos. There are few studies showing the effect of MLT administration on the distal inflammatory mediators; NOx and PGE2 levels [33]. In our study, it was observed that MLT application caused an increase in both NOx and PGE2 levels, also the increase in NOx and PGE2 levels decreased as a result of treatment of MYC at

both doses. This result shows that MYC application can suppress inflammation caused by MLT intoxication.

Pesticides usually cause necrosis, tubular degeneration, glomerular atrophy and infiltration in the kidneys. Necrosis and glomerular atrophy are accepted as indicators of impaired renal hemostasis and severe damage to the kidneys [24,39]. In this study, degeneration of tubular epithelial cells, cystic dilation in the tubules, hyaline cylinders, mononuclear cell infiltration, hemorrhage, inflammation and glomerular lesions were observed in the kidney tissues of the rats as a result of MLT intoxication, which was concluded as damaging effect of MLT on the histological structure of the kidney tissue. Our results were in accordance with those reported in the study by El Okle et al. [39]. These results demonstrated that MYC and QRS treatments reduced necrosis, tubular degeneration, glomerular atrophy and infiltration. It was observed that even the low dose of MYC returned the kidney tissue to an almost normal morphology. These results demonstrate that MYC and QRS treatments have been effective against kidney damage caused by MLT intoxication.

Conclusion

In conclusion, MLT caused an increase in oxidative stress and inflammatory response with impaired kidney functions in rats with an exposure for a period of four weeks. In the present study, it was also investigated to what extent MYC and QRS treatment could prevent the resulting kidney damage. It was observed that MYC and QRS especially at high-dose administrations can prevent nephrotoxicity in rats. However, the use of MLT should be limited as much as possible, and if to be used, the required precautions should be taken at a maximum extent.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

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Ethical approval

The ethical approval of the study was obtained from Van Yuzuncu Yil University Ethical Commission for Animal Experiments (Decision number: 2020/06-06, Date: 25.06.2020).

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