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ORIGINAL RESEARCH ARTICLE

Red orange and lemon extract prevents the renal toxicity induced by ochratoxin A in rats

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Abstract

In this work, we investigated the effects of red orange and lemon extract (RLE) on ochratoxin A (OTA)-induced nephrotoxicity. In particular, we analyzed the change in renal function and oxidative stress in Sprague–Dawley rats treated with OTA (0.5 mg/kg body weight, b.w.) and with RLE (90 mg/kg b.w.) by oral administration. After OTA treatment, we found alterations of biochemical and oxidative stress parameters in the kidney, related to a severe decrease of glomerular filtration rate. The RLE treatment normalized the activity of antioxidant enzymes and prevented the glomerular hyperfiltration. Histopathological examinations revealed glomerular damages and kidney cortex fibrosis in OTA-rats, while we observed less severe fibrosis in OTA plus RLE group. Then, we demonstrated that oxidative stress could be the cause of OTA renal injury and that RLE reduces this effect.

KEYWORDS

anthocyanins, kidney, ochratoxin-A, oxidative stress, toxicity

1 | INTRODUCTION

Ochratoxin A (OTA), a mycotoxin that contaminates various foods (Covarelli, Beccari, Marini, & Tosi, 2012), is produced by fungi *Aspergillus ochraceus* and *Penicillium verrucosum* mainly (Jestoi, 2008). The eradication of OTA from the food chain is very difficult due to the high thermal stability (Duarte, Pena, & Lino, 2009). Its subchronic and chronic toxicity to humans and to several animal species, such as nephrotoxicity, neurotoxicity, teratogenicity, immunotoxicity, mutagenicity, and hepatotoxicity is well known (Marín, Cano-Sancho, Sanchis, & Ramos, 2018; Pfohl-Leszkowicz & Manderville, 2007). The kidney is the target organ of OTA in human and animals (Damiano et al., 2018; Limonciel & Jennings, 2014; Malir, Ostry, Pfohl-Leszkowicz, Malir, & Toman, 2016; Pfohl-Leszkowicz, Petkova-Bocharova, Chernozemsky, & Castegnaro, 2002). In fact, the International Agency for Research on Cancer classified OTA as a possible human carcinogen (group 2B; IARC, 1993). Anyway, its mechanisms of toxicity remain elusive. Antioxidants can protect against injuries caused by OTA, in particular against the oxidative stress induced by this mycotoxin (Ringot, Chango, Schneider, & Larondelle, 2006; Sorrenti et al., 2013). In fact, high levels of reactive oxygen species (ROS) could cause reductions of the main cellular antioxidant enzymes responsible for the removal of free radicals. The free radicals, through the production of malondialdehyde (MDA), could damage several components of the cell (Fusi et al., 2010; Kim, Choi, Ham, Jeong, & Lee, 2013). Identify compounds that can reduce their toxic effects and better understand the multiple mechanisms of action with which these secondary metabolites affect the health of human and animal species is one of the main objectives in mycotoxin research. It is well demonstrated that anthocyanins (ANT) have anti-inflammatory, antioxidant, and antiobesity activities (Meng et al., 2019) and they are present in high concentration in fruits and vegetables (Li, Wang, Luo, Zhao, & Chen, 2017). In nature, they give the red tonalities of many fruits, vegetables, and cereals and are pollination attractants and photoprotective agents (Gould, 2004; Steyn, Wand, Holcroft, & Jacobs, 2002). The ANT exerts a direct

antioxidant action going to exercise, at the cellular level, a defensive action counterbalancing the lipid peroxidation protecting the cell from damage to the cell membranes, proteins, lipids, and DNA (Acquaviva et al., 2003). It has also been hypothesized that ANT can, indirectly, contrast oxidative stress by activating specific detoxification enzymes (Shih, Yeh, & Yen, 2007). Here, we analyzed the effect of a red orange and lemon extract (RLE) natural extract rich in antioxidants, especially the cyanidine 3-glucoside (C3G; Damiano et al., 2019) in OTA-treated rat animal model. Part of the mechanism of action of C3G has been attributed to the ability to chelate ions of bivalent metals, necessary to generate ROS through the Fenton reaction (Amorini et al., 2001). In vivo studies demonstrated that the OTA toxicity could be reduced by C3G by inducing improved dimethylarginine dimethylaminohydrolase (DDAH) and inducible nitrogen oxide synthase (iNOS) activation and in this way counterbalancing nitrosative stress (Sorrenti et al., 2012). Thus, the focus of the present work was to investigate the antioxidant effect of the RLE on OTA-treated Sprague–Dawley rats. The renal function, in vivo, was assessed by clearance of inulin to measure glomerular filtration rate (GFR), urea nitrogen (BUN), serum creatinine (CREA), and the weight gain. Lipid peroxidation and antioxidant enzyme activities were evaluated to assess the contribution of ROS in renal damage.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

OTA was purchased from Sigma-Aldrich (Milan, Italy); the RLE was obtained by Council for Agricultural Research and Economics, Research Centre for Olive, Citrus and Tree Fruit, Acireale, Italy). SOD (Item No. 19160), MDA (Item No. MAK085), GSH (Item No. 38185), and CAT (Item No.CAT100) assay kits were purchased from Sigma-Aldrich (Milan, Italy). The establishment that supplies the animals was Charles River Laboratories (Milan, Italy).

2.2 | Ethics statement

The guidelines used in this work are in accord with current European directive. The approval number by the Italian Ministry of Health is 487/2018-PR.

2.3 | Animals and treatments

In this study, male Sprague–Dawley rats, 10 weeks old (240 ± 20 g), were allocated randomly into four experimental groups (six rats for the group) with 22°C temperature and 12 hr day/night cycles. The animals received a standard diet ad libitum. Animals were treated daily for 14 days by gavage as follows: (a) Controls (1 ml solution of sodium bicarbonate); (b) OTA (0.5 mg/kg b.w.); (c) RLE (90 mg/kg b.w.); (d) OTA (5 mg/kg b.w.) + RLE (90 mg/kg b.w.). OTA and RLE were dissolved in 1 ml solution of sodium bicarbonate. The levels of biologically active compounds of RLE is standardized and constant (Damiano et al., 2019). The dose of RLE and OTA to be administered was a choice based on our previous experiment (Damiano et al., 2018; Damiano et al., 2019). Body weights were monitored at three-time points: before the treatment (time 0), 7 and 14 days. Blood samples were centrifuged at 3,000 rpm for 20 min at +4°C and blood urea nitrogen (BUN) and creatinine (CREA) levels were measured after 14 days of treatment by an autochemistry analyzer (PKL PPC 125, Paramedical srl, Salerno, Italy). Samples of kidney were taken on ice and stored at −80°C until analyzed for the activity of enzymes involved in oxidative stress, lipid peroxidation, and partially prepared for routine histopathology.

2.4 | Clearance of inulin

The anesthetized animals were prepared for the clearance of inulin to measure the GFR expressed as ml/min 100 g body weight (b.w) as before described in our study (Damiano et al., 2018).

2.5 | Renal antioxidant enzymes

The superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH) activity in kidney samples was evaluated by a spectrophotometer (Glomax Multi detection system, Promega, Milano, Italy) according to previous studies (Akerboom & Sies, 1981; Sinha, 1972; Sun, Oberley, & Li, 1988). The SOD, CAT, and GSH activity was expressed as the unit for milligrams of proteins (U/mg of proteins).

2.6 | Lipid peroxidation assay

The MDA concentration used to measure the degradation of lipids that occurs a result of oxidative damage, was evaluated with a commercially available kit from Sigma using by system colorimetric at 532 nm and calculated and was expressed as pmole of MDA for milligrams of proteins.

2.7 | Histopathological studies

Kidneys collected during necropsy were fixed in Bouin solution for 24 hr, dehydrated in ethyl alcohol, and embedded in paraffin. Four-micrometer sections were stained with hematoxylin and eosin and with Masson's trichrome stain and were examined and photographed with a light microscope (Nikon Eclipse E600 Tokyo, Japan) associated to a microphotography system Nikon digital camera (DMX1200). For each case, the severity of glomerular damages, tubular damages, inflammation and the presence of proteinaceous material in Bowman's spaces, and tubules lumen were scored from 0 to 3

(0 = absent; 1 = mild; 2 = moderate; 3 = intense). The severity of the fibrosis was evaluated on Masson's trichrome stained section with the ImageJ software (National Institutes of Health) in 10 40×-high-power-field (HPF) randomly photographed with Nikon digital camera (DMX1200). For each photo, the area occupied by connective tissue was evaluated measuring the area occupied by blue pixel using the Color Threshold function (HUE 150-190; Saturation 0-255; Brightness 0-180) and for each case, the mean value in 10 40×-HPF was calculated.

2.8 | Statistical analysis

All the results are expressed as mean ± standard deviation. Analysis of variance tests followed by a Tukey's test was used to analyze the differences (GraphPad Software 3.00, San Diego, CA). Each animal group consisted of six rats and the experiment was made in triplicate. Values of $p < .05$ were considered significant.

3 | RESULTS

3.1 | Effect of RLE on body weight of rats

Bodyweight expressed in grams (g), in rats at 0, 7, and 14 days of treatment with OTA, RLE, and RLE plus OTA is shown in Table 1. A significant time-dependent reduction in body weight was found in the OTA group compared to the control group at 7 and 14 days of treatment (−15.5% and −19%, respectively). No significant change in the bodyweight of rats was found in the OTA plus RLE group compared to the OTA group (Table 1).

3.2 | Biochemical analyses

In the OTA group, we have observed a significant increase in the BUN and CREA levels compared to the control group of +41% and +44%, respectively (Table 2). The RLE plus OTA causes a good restore in BUN and CREA parameters compared to the control group. The BUN and CREA increase were only 7.1% and 22%, respectively (Table 2; $^{*}p < .05$ vs. Control group and $^{\#}p < .05$ vs. OTA group).

TABLE 1 Bodyweight (B.W) expressed in grams (g) in the different groups of rats to 0, 7, and 14 days of treatment

Groups	B.W (g) 0 days	B.W (g) 7 days	B.W (g) 14 days
Control	245.7 ± 4.4	281.7 ± 7.2	298.6 ± 6.2
RLE	247.3 ± 5.1	279.3 ± 6.4	297.5 ± 6.1
OTA	242.1 ± 5.6	241.8 ± 4.8*	241.3 ± 5.1*
RLE + OTA	248.4 ± 4.9	250.8 ± 6.3	255.3 ± 5.4

Note: Control group (control); red orange and lemon extract (RLE); ochratoxin A (OTA); RLE plus OTA group (RLE + OTA). Data are expressed as mean ± standard deviation of n = 6 rats.
*$p < .05$ vs. control group.

TABLE 2 Serum biochemical parameters: Blood urea nitrogen (BUN) and creatinine (CREA) expressed in mg/dl concentrations values in the different groups of rats.

Groups	BUN (mg/dl)	CREA (mg/dl)
Control	50.20 ± 2.35	0.27 ± 0.05
RLE	53.25 ± 2.75	0.28 ± 0.17
OTA	60.75 ± 0.05*	0.39 ± 0.11*
RLE + OTA	54.15 ± 0.03$^{\#}$	0.30 ± 0.05**

Note: Control group (Control); red orange and lemon extract (RLE); ochratoxin A (OTA); RLE plus OTA group (RLE + OTA) after 14 days of treatment. Data are expressed as mean ± standard deviation of $n = 6$ rats.
*$p < .05$ vs. control group.
**$p < .05$ vs. OTA group.

3.3 | Clearance of inulin

Figure 1 shows the effect of RLE and OTA treatment on GFR. OTA group significantly decreased GFR respect to control rats (0.97 ± 0.08 Control rats to 0.51 ± 0.07 OTA group ml/min; ***$p < .001$). RLE in association with OTA completely prevented this renal hemodynamic alteration. In fact, the GFR value was 0.86 ± 0.08 ml/min in OTA plus RLE group ($^{\#\#\#}p < .001$). RLE did not induce any change in GFR respect to Control rats (0.93 ± 0.07 vs. 0.97 ± 0.08 control rats).

3.4 | Antioxidant enzymes SOD, CAT, and GSH activity

No differences were found in SOD and CAT activity in the OTA group and OTA plus RLE respect to the Control group after 14 days of

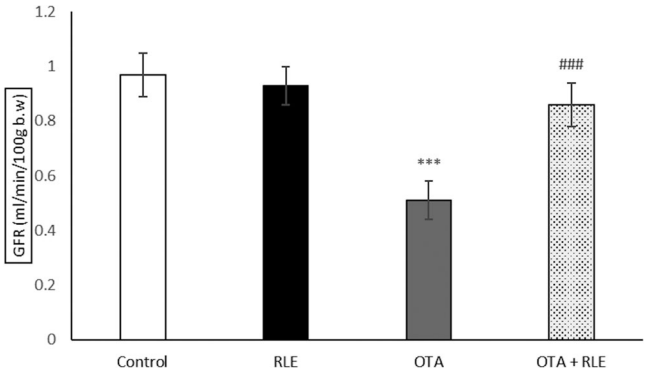

FIGURE 1 Effects of RLE on glomerular filtration rate (GFR). Control group (Control); red orange and lemon extract (RLE); ochratoxin A (OTA); OTA plus RLE group (OTA + RLE) after 14 days of treatment. Data are expressed as mean ± SD of $n = 6$ rats. OTA treatment significantly decreased GFR, while coadministration with RLE significantly restored this effect (***$p < .001$ vs. control group; $^{\#\#\#}p < .001$ vs. OTA group)

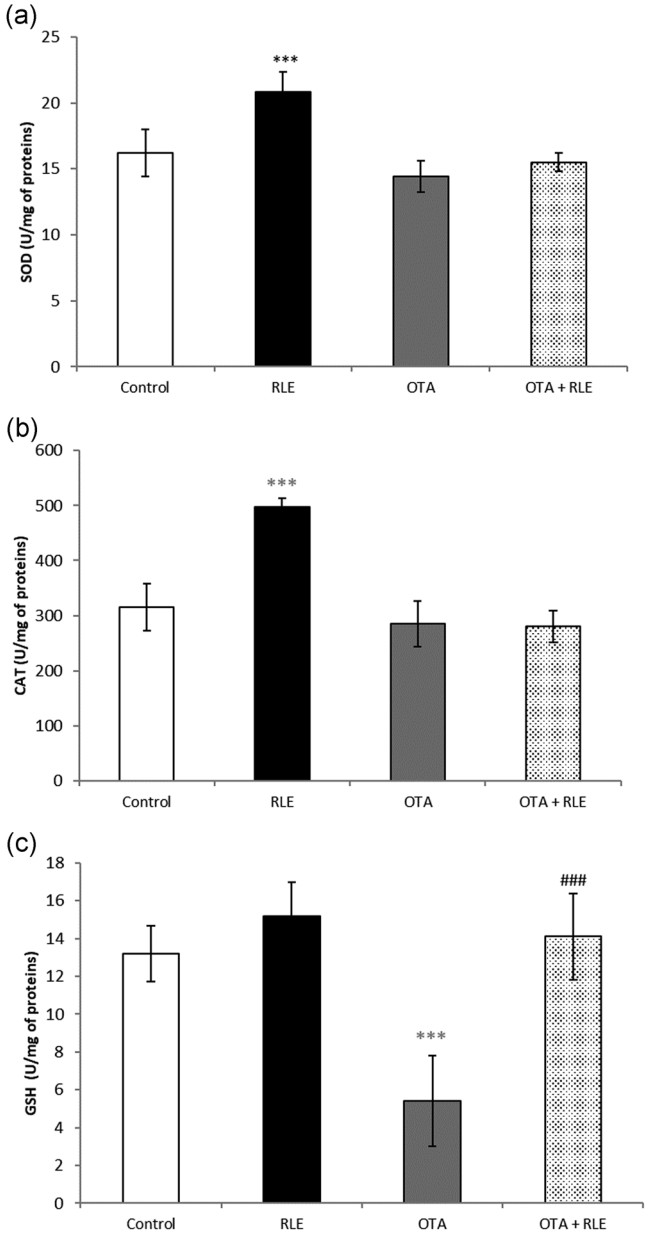

FIGURE 2 Effects of red orange and lemon extract (RLE) on renal antioxidant enzymes. (a) Renal sodium oxide dismutase activity. (b) Renal catalase activity. (c) Renal GSH activity. Control group (Control); RLE; ochratoxin A (OTA); OTA plus RLE group (OTA + RLE) after 14 days of treatment. Data are expressed as mean ± standard deviation (*SD*) of $n = 6$ rats. OTA treatment significantly decreased glutathione enzyme activity, while coadministration with RLE significantly restored this effect (values mean ± *SD*; $^{***}p < .001$ vs. control group; $^{###}p < .001$ vs. OTA group)

treatment (Figure 2). In fact, the SOD value was 16.22 ± 1.8 in Control group respect to 14.4 ± 1.2 in OTA and 15.5 ± 0.7 in OTA plus RLE group. The SOD value in RLE group was 20.84 ± 1.5, showing a significant increase in SOD enzyme activity compared to control group ($^{***}p < .001$ vs. control group; Figure 2a); CAT level in Control group was 315.3 ± 43.1 respect to 285.2 ± 41.14 in OTA group and 280.1 ± 28.25 in the OTA + RLE group. A significant

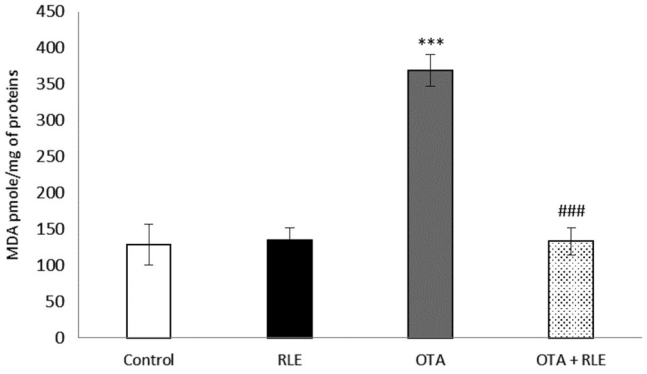

FIGURE 3 Effects of red orange and lemon extract (RLE) on lipid peroxidation. Effects of the ochratoxin A (OTA) and red orange and lemon extract (RLE) used alone or in association on lipid peroxidation measured by malondialdehyde (MDA) test in rat kidney after 14 days of treatment. OTA treatment significantly increased MDA levels, while coadministration with RLE partially prevent this effect. Results are expressed as mean ± standard deviation of $n = 6$ rats. ($^{***}p < .001$ vs. control group; $^{###}p < .001$ vs. OTA group)

increase in CAT enzyme activity was found when RLE was used alone (498.2 ± 15.54 U/mg of proteins; $^{***}p < .001$ vs. control group; Figure 2b). OTA significantly reduced GSH activity (13.2 ± 1.48 Control to 5.3 ± 2.40 OTA; $^{***}p < .001$). Any change in GSH activity respect to Control group we found when RLE was utilized alone. However, a significant restore on GSH activity respect to the OTA group was observed in RLE plus OTA group. In fact, the GSH values shifted from 5.3 ± 2.40 (OTA) to 14.1 ± 2.28 (RLE + OTA; $^{###}p < .001$; Figure 2c).

3.5 | Lipid peroxidation

The renal MDA level in rat kidney is shown in Figure 3. OTA treated group increased respect to the control group (128.4 ± 2.8 control group respect to 368.6 ± 22.0 OTA ($^{***}p < .001$). Groups that received RLE with OTA showed a significant decrease in MDA levels compared with the OTA group. Indeed, the MDA values switched from 368.6 ± 22 (OTA) to 133.3 ± 19 (OTA+RLE; $^{###}p < .001$ respect OTA group). Any variation on lipid peroxidation was observed when RLE was used alone (134.6 ± 17 RLE group).

3.6 | Histopathological examination

In Figure 4, the kidneys from the Control animals and RLE treated groups appeared normal with only mild accumulations of intratubular proteinaceous material. Only in one case of the Control group and in one case of the RLE treated group we observed mild and scattered lymphocytic infiltration in the interstitial space. No differences were seen between Control and RLE treated groups. Kidneys from OTA treated group showed a diffuse global reduction of Bowman's space and segmental or global glomerular necrosis. Bowman's space and

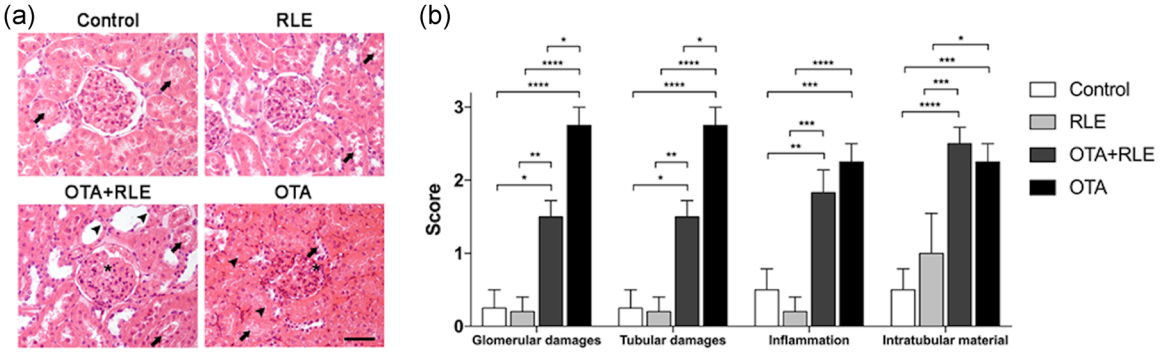

FIGURE 4 (a) Renal histopathological evaluation of study groups. Control group (Control); red orange and lemon extract (RLE); ochratoxin A (OTA); OTA plus RLE (OTA + RLE) after 14 days of treatment. Kidneys were sectioned and stained with hematoxylin–eosin, 40× (scale bar = 50 μm). Rats of the Control and RLE groups showed a small amount of intratubular proteinaceous material (arrow). Rats of the OTA+RLE group showed focal glomerular necrosis (asterisk) and abundant proteinaceous material in Bowman's spaces and tubules lumen (arrows) associated with severe tubular epithelial cells atrophy and necrosis (arrow heads). Rats of the OTA group showed a severe global reduction of Bowman's space (asterisk), abundant proteinaceous material in Bowman's spaces and tubules lumen (arrows) associated with severe tubular epithelial cells atrophy and necrosis (arrow heads). (b) Severity scores of glomerular damages, tubular damages, inflammation and the presence of proteinaceous material for each group. Asterisks denote statistically differences between groups (*$p < .05$; **$p < .01$; ***$p < .001$; ****$p < .0001$)

tubules lumen contained abundant proteinaceous material and rare erythrocytes and few necrotic epithelial cells. Tubular epithelial cells were often atrophic, degenerated, or necrotic. The interstitium was multifocally expanded by a moderate lymphocytic infiltrate and severe fibrosis (Figure 4a). The rats of OTA + RLE group showed a significant reduction of the glomerular damages ($p = .0121$), of tubular damages ($p = .0121$) compared with OTA group. No significant differences were seen regarding inflammation ($p = .7147$) and proteinaceous casts ($p = .9199$) respect to OTA treated group. Moreover, OTA + RLE group showed more severe glomerular damage ($p = .0121$), tubular damages ($p = .0121$), inflammation ($p = .0066$), and proteinaceous casts ($p < .0001$) compared with Control group (Figure 4b). The fibrosis was more evident in OTA and OTA + RLE groups compared with control and RLE groups. Masson's trichrome stain showed more severe fibrosis in the OTA group compared with control ($p = .0013$) and RLE ($p = .001$) groups. The fibrosis was still more severe in the OTA+RLE group compared with control ($p = .0208$) and RLE ($p = .0172$) groups. Furthermore, we observed less severe fibrosis in the OTA+RLE group compared with the OTA group but the difference was not statistically significant ($p = .2826$; Figure 5).

4 | DISCUSSION

The specific target site of OTA toxicity is the kidney (Gan et al., 2018; Lee, Pyo, Shin, Ryu, & Lee, 2018; Rašić et al., 2018). However, the exact mechanism of OTA-induced nephrotoxicity has not yet been completely understood. The results obtained in this study, in accordance with in vitro and in vivo studies (Abdel-Wahhab, Aljawish, El-Nekeety, Abdel-Aziem, & Hassan, 2017; Ciarcia et al., 2016; Costa et al., 2016; Periasamy, Kalal, Krishnaswamy, & Viswanadha, 2016), confirmed that the toxic effect of OTA on the kidney found both on the functional level and from the histopathology of view is mediated by oxidative stress. It has been shown that OTA acts by inhibiting the nuclear factor, which would affect both the synthesis and the recycling of glutathione and also the activity of oxidoreductases thus making the tissue more vulnerable to oxidative stress (Jennings, Limonciel, Felice, & Leonard, 2013; Limonciel & Jennings, 2014). Effects of OTA on oxidative stress production have been demonstrated in other research. In fact, in vitro and in vivo experiments have shown that OTA induces the increase of lipid peroxidation (Abdel-Wahhab, Abdel-Galil, & El-Lithey, 2005; Klarić, Pepeljnjak, Domijan, & Petrik, 2007; Ozçelik, Soyöz, & Kilinç, 2004) and also induces a decrease in glutathione (GSH)

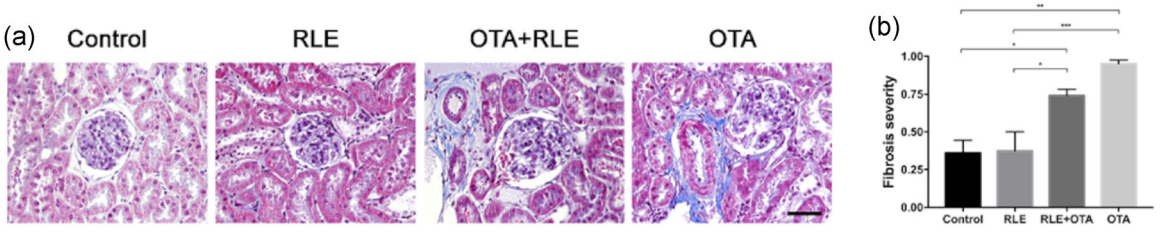

FIGURE 5 (a) Renal histopathological evaluation of study groups. Control group (Control); red orange and lemon extract (RLE); ochratoxin A (OTA); OTA plus RLE (OTA + RLE) after 14 days of treatment. Kidneys were sectioned and stained with Masson's trichrome stain, 40× (scale bar = 50 μm). Rats of the Control and RLE groups show thin branches of fibrous connective tissue (blue) in the interstitium. Rats of the OTA + RLE and OTA groups show a severe interstitium thickening by abundant fibrous connective tissue (blue). (b) Severity of fibrosis evaluated on Masson's trichrome stained sections for each group. Asterisks denote statistically differences among groups (*$p < .05$; **$p < .01$; ***$p < .001$)

levels (Klarić et al., 2007; Meki & Hussein, 2001). Moreover, the increase in ROS induced by OTA is dose-dependent (ROS; Baldi et al., 2004) and it is well demonstrated, both in vitro and in vivo study, an oxidative DNA damage (Arbillaga, Azqueta, Ezpeleta, & López de Cerain, 2007; Kamp et al., 2005). In according with these data, we have demonstrated in our recently paper (Damiano et al., 2018) that OTA treatment induced the increase of ROS production and this value is related to a strong reduction of GFR and absolute fluid reabsorption (Jv) with consequent significant increase in blood pressure. Consistent, we noted in the kidney of rats treated with OTA, an increase in MDA and dihydroethidium production and a reduction of the activity of the GSH. For this reason, in recent years, vegetable-based diets rich in antioxidant and anti-inflammatory substances, in particular, rich in ANT, have been tested to prevent the negative effects of oxidative stress in various chronic diseases, including metabolic diseases (Barone et al., 2018; Jiang et al., 2019). Therefore, this work has shown that C3G, which is the main component of the ANT contained in our RLE extract, effectively neutralized the deleterious effects of OTA thanks to its antioxidant properties. In fact, in the kidney, the restore in GFR induced by RLE is probably due to the washing of hydroxyl lipid radicals and to the increase of glutathione peroxidase, which uses GSH to catalyze the reduction of hydroperoxides. SOD, CAT, and GSH analyzed in this work are the main cellular antioxidant enzymes used for the removal of free radicals. The enzyme activity of SOD and CAT, in the OTA group, showed no significant changes compared to both, the Control and OTA plus RLE group. Instead, CAT and SOD levels were significantly increased when RLE was used alone. These data, according to our previous works (Damiano et al., 2018, 2019) and to Palabiyik et al. (2013) and Domijan et al. (2007) suggest that the restoring potential of RLE on OTA nephrotoxicity is related to its direct scavenging in activity on ROS, rather than its modulation of cellular enzymatic activities. It is interesting to note, in this study, in line with the study conducted by Bertelli et al. (2005), the GSH enzymatic activity in OTA group was reduced compared to the control group instead of in the OTA and RLE group the GSH levels returned to baseline values. This data is probably because OTA is transforming into a more toxic species, OTQ 6, that would provide a basis for the toxins' ability to promote oxidative stress (Dai et al., 2002). We have considered that the raising of ROS levels could cause a decrease in the activity of GSH, which would lead to a significant increase in renal MDA levels OTA animal model. Instead, the combined treatment with RLE and OTA restored the enzyme activities and significantly reduced the concentration of MDA. The reduction in the concentration of MDA in the kidneys of RLE-treated rats could be due to the marked antioxidant properties of the C3G contained in the extract. The CRE and BUN increases in OTA rats are consistent with the reduction in GSH activity and with the increase in MDA concentration. The histologic observation of glomerular damages is compatible with OTA-induced damages already described in rats. The proteinaceous material present in the Bowman's spaces and in the tubules can be a morphological change due to the reported OTA-depended damage of the secondary foot processes of the podocytes (Abdu, Ali, & Ansari, 2011). Some authors attribute the OTA-related damage of the tubular epithelial cells to the production of ROS (e.g., superoxide anion, hydroxyl radical, and peroxide) and other authors reported a role of the cytochrome P450 and of its ability to stimulate OTA-dependent lipid peroxidation mediated by OTA-Fe3+/2+ complexes (Mattace Raso et al., 2013). Antioxidants, such as RLE, may counteract these mechanisms (Sorrenti et al., 2013). The OTA-induced tubulointerstitial fibrosis has been documented in rats both morphologically and by the expression of fibrosis-related genes in the renal cortex (Gagliano et al., 2005). Our results show less severe fibrosis in OTA + RLE respect to OTA, suggesting a protective role of RLE also on the development of fibrosis. We hypothesize that the OTA-related morphological alterations observed were due to the OTA-induced oxidative damage. This hypothesis is also supported by the less severe morphological alterations observed in subjects treated with both OTA and RLE. Our results confirmed that oxidative stress is involved in the mechanism of nephrotoxicity of OTA. The addition of RLE to feed could reduce OTA contamination and limit animal exposure to this toxic mycotoxin and protect animal and human health.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

S.D., S. F., A. G., and R. C., conceived and designed the experiments. S. D., V. I., F. P., A. A., C. S., N. M., M. A., and R. C. performed the experiments. All authors read and gave their approval for the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data sets used in the current study are available from the corresponding authors.

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