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The effects of resveratrol on SIRT2, SIRT3 expression levels and oxidative DNA damage in fumonisin-induced hepatotoxicity in BALB/c mice

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ABSTRACT

Oxidative stress, which is characterized by disruption of the oxidant/antioxidant balance, causes pathological processes, including toxicities induced by certain mycotoxins. The present study was designed to investigate the effects of resveratrol on sirtuin deacetylases (SIRT2 and SIRT3), nitric oxide (NO), reduced glutathione (GSH) and malondialdehyde (MDA) in fumonisin B1-induced hepatotoxicity. Regarding the experimental design, forty BALB/c mice were divided into four groups corresponding to the control, resveratrol (10 mg/kg, i.p), fumonisin B1 (2.25 mg/kg, i.p) and resveratrol + fumonisin B1 (10 mg/kg + 2.25 mg/kg) groups. At the end of the 14 day-treatment, expression levels of SIRT2 and SIRT3 protein in the serum and liver were revealed by western blotting and antioxidant/oxidant activity analysis. SIRT2 and SIRT3 expression levels in the liver were significantly decreased by fumonisin B1 in comparison to the control. However, resveratrol supplementation coupled with fumonisin B1 increased the expression levels of SIRT2 and SIRT3, in relation to the fumonisin B1 treatments alone, but did not exhibit significant differences from those of the control group. As substantial indicators of stress and damage, the 8-OH-2-deoxyguanosine, NO and MDA levels of the liver tissue were assayed, and were higher in the fumonisin B1-treated groups, in relation to the control. As expected, resveratrol treatment significantly reduced the levels of NO and MDA in comparison to the fumonisin B1 treatments alone. Also, resveratrol attenuated the liver 8-OH-2- deoxyguanosine levels in the resveratrol + fumonisin B1 group. In conclusion, the findings revealed that resveratrol might possess protective effects against fumonisin-induced hepatotoxicity through modulation of the expression of sirtuin proteins, and by protecting the cell from oxidative/nitrosative stress.

Key words: mycotoxins; hepatoprotection; dietary phenolic compounds; sirtuins; nitric oxide; lipid peroxidation

Introduction

Mycotoxins, which are fungal metabolites toxic to humans and animals, are commonly found as contaminants of food and animal feed. Fumonisins are mycotoxins produced mainly by *Fusarium*

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verticillioides and are mostly found in corn crops worldwide, inducing toxic effects on farm and laboratory animals, but their effects are dose-, sex- and age-dependent (CAST, 2003). Of the fumonisins identified, Fumonisin B1 (FB1) has been revealed to be the most toxic and abundant, and it has been shown to possess hepatotoxic and nephrotoxic effects in rodents. The mechanism of FB1 toxicity has been significantly attributed to the disruption of sphingolipid biosynthesis. Due to its structural similarity with the sphingoid bases, FB1 interferes with their incorporation into ceramide by inhibition of ceramide synthase, leading to intracellular accumulation of sphingoid bases (sphinganine and sphingosine). However, the mechanism of how the disruption of sphingolipid metabolism leads to oxidative stress has not been completely shown, and the downstream toxic mechanisms involved with fumonisins might be complex. FB1 inhibits biosynthesis of macromolecules, and induces lipid peroxidation in rat hepatocytes. Also, it increases the expression levels of inducible nitric oxide synthase (iNOS) (MARY et al., 2012). Of the toxic mechanisms of the relevant mycotoxin discovered so far, FB1 has been reported to modify DNA methylation, modulate autophagy, activate endoplasmic reticulum (ER) stress and MAPKs, and induce oxidative stress by disturbing sphingolipid metabolism (LIU et al., 2019). In this regard, *in vivo* studies are particularly needed to determine whether these pathways or molecules are effective targets to prevent FB1-mediated toxicity.

Nutritional intervention has been addressed in recent reports rather than pharmacological treatments. One of the most common dietary phenolic compounds, resveratrol (3,5,4′-trihydroxy-trans-stilbene), is a natural phenol and phytoalexin found in grapes, red wine, basil, artichokes, and mulberries. The antioxidant activities of the relevant compound have been clearly revealed in a number of experimental and clinical studies mainly performed under pathological conditions (CARRIZZO et al., 2013). Resveratrol indirectly decreases the level of reactive oxygen species (ROS), and prevents superoxide anion radical mediated decomposition of NO produced by eNOS. It also directly scavenges free radicals through stimulating the activities of some antioxidant enzymes, or down-regulating the expression levels of iNOS, which prevents nitrosative stress (XIA et al., 2014a)

Sirtuin (SIRT) is a protein of the nicotinamide adenine dinucleotide (NAD^+)-dependent class III histone deacetylase. Sirtuins have been associated with many physiological processes, including cell cycle, aging, apoptosis, inflammation and cancer. Therefore, they affect the relevant physiological processes by directly or indirectly regulating antioxidant system components. The seven homologs in the mammalian sirtuin family (SIRT1-7) exhibit variations depending on tissue specificity, sub-cellular localization, enzymatic activity and targets. Due to the recently revealed vital roles related to their regulatory actions in the metabolism and processes of various diseases, sirtuins have attracted significant interest (LIN et al., 2018). One of the sirtuins characterized, SIRT2, a cytosolic deacetylase, has been associated with many cellular processes. SIRT3, a mitochondrial deacetylase, has been reported to activate mitochondrial enzymes related to beta oxidation, the electron transport chain, and antioxidant defense (MENDES et al., 2017). However, the exact action mechanisms of SIRT2 and SIRT3 have not yet been completely revealed regarding cellular damage and oxidative stress, but a relationship between sirtuins and iNOS system has been recognized according to the limited number of reports (XIA et al., 2014b).

Therefore the present study aims to present experimental evidence related to the potential role of SIRT2 and SIRT3, and the antioxidant/oxidant system parameters associated with sirtuins in fumonisin-induced hepatotoxicity. The effect of resveratrol, which is also known as a SIRT1 activator (XIA et al., 2014b), on SIRT2 and SIRT3 expression levels and 8-OH-2-Deoxyguanosine, malondialdehyde, reduced glutathione and nitric oxide levels, was investigated in response to fumonisin B1 induced-hepatotoxicity in BALB/c mice.

Materials and methods

Chemicals. The Bradford solution, protein marker, 4 x LaemmLi buffer and and ECL Western blotting substrate were purchased from BioRad

(Bio-Rad Laboratories, Inc., Hercules, CA, USA). Fumonisin B1 was purchased from AdooQ Bioscience (AdooQ Bioscience, US&Canada). Resveratrol was purchased from Cayman Chemical (Cayman Chemical Company, Michigan, USA). Rabbit SIRT3 and GAPDH primary antibodies were obtained from Cell Signaling Technology (Cell Signaling Technology, Inc., Danvers, MA, USA).

The mouse 8-OH-2-deoxyguanosine (8-OHdG) ELISA kit (Cat. No: E-EL-0028) was purchased (Elabscience Biotechnology Inc., Houston, US). All the other chemicals and reagents were analytical grade products from Sigma (St. Louis, MO, USA).

Animals. The required permission was obtained from the Local Ethics Committee of Burdur Mehmet Akif Ersoy University Animal Experiments (MAE-2017-328). Housing, maintenance, and experimental procedures were carried out at the Burdur Mehmet Akif Ersoy University Experimental Animal Production and Experimental Research Center. During the experiment, the mice were placed in cages in a 12-hour light and 12-hour dark cycle and with food and water *ad libitum*. The mice were housed in plastic cages with chip shafts with 5 mice in each cage during the trial period.

Experimental design. Forty BALB/c female and male mice weighing 25 – 35 g were used in the study. For the experiments, forty mice were randomLy distributed into four groups with an equal number of females and males in each group, and the mice were weighed for dose calculations. The groups were as follows: Control (n = 10), Resveratrol (n = 10), Fumonisin B1 (n = 10), and Resveratrol + Fumonisin B1 (n = 10). During the experiment, saline was administered intraperitoneally to the control group daily for 14 days. Regarding the experimental groups, the FB1 group (2.25 mg/kg intraperitoneal), the Resveratrol group (10 mg/kg intraperitoneal) and the Resveratrol + Fumonisin B1 group (10 mg/kg + 2.25 mg/kg intraperitoneal) were treated once a day for 14 days. After 14 days of the experimental period, the mice were sacrificed by cervical dislocation under isoflurane anesthesia, and the intra-cardiac blood samples from animals were centrifuged at 3000 rpm and 4 °C for 15 minutes to separate the serum. The liver tissue were stored to be used in subsequent analyses.

Western blot analysis. The total protein from liver tissues was homogenized using cold NP-40 lysis buffer containing 10 mg/mL aprotinin. The protein concentrations were measured by BRADFORD's method (1976), using bovine serum albumin (BSA) as the standard, by recording the absorbances at 595 nm. Standards with a range of 2-12 µg/mL were prepared from 0.2 mg/mL BSA stock solution. After 10 min incubation at room temperature with the addition of the Bradford reagent, protein concentrations of tissue samples were quantified according to the BSA standard curve at 595 nm. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted according to the method of LAEMMLI (1970). The protein samples were loaded onto the SDS-PAGE gel (10%), separated electrophoretically, and transferred to a nitrocellulose membrane. Membranes were blocked with 5% skimmed milk powder for 1 hour at room temperature. Immunoblots were incubated with a primer antibody against SIRT2 (1:1000) and SIRT3 (1:1000) at 4 °C overnight. After washing with tris buffered saline with Tween 20, antibody binding was detected using HRP-conjugated secondary antibody (1:2000 dilution) for 1 hour and ECL Western blotting substrate. Immunoblots were visualized using a chemiluminescence imaging system (iBrightCL 1000, Invitrogen, California, USA) and quantified using Image J software and normalized with GAPDH (1:1000) as an internal control. To control sampling errors, the ratio of band intensities to GAPDH was obtained to quantify the relative protein expression level.

8-OH-2-deoxyguanosine assay. The 8-OHdG analysis for DNA damage detection in liver samples was performed using enzyme-linked immunosorbent assay (ELISA) kit. This ELISA kit uses the competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with 8-OHdG. After adding samples and standards to the pre-coated plate with mouse 8-OHdG monoclonal antibody, 8-OHdG antibodies were labeled with biotin and combined with streptavidin-HRP to form an immune complex. Following the washing process, the 3,3',5,5'-tetramethylbenzidine

substrate solution was added to each well and the reaction was terminated by the addition of stop solution. A positive correlation between the color intensity and 8-OHdG level was shown. The relevant findings were quantified by reading the absorbance at 450 nm in comparison to the blank, and plotting the standard-curve.

Reduced glutathione assay. GSH analysis of the liver tissue was performed according to the method of BEUTLER et al. (1963). According to this method, all proteins not carrying the sulfhydryl (-SH) group in the samples were precipitated. The absorbance of the yellow complex resulting from the reaction of the proteins containing the -SH group with 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) was read at 412 nm in a spectrophotometer (PowerWave XS, BioTek, Vermonts, USA). From the test tubes marked as blank, standard and sample, 200 µL of the sample tube and 200 µL of the standard solution were taken. 1.8 mL of distilled water and 3 mL of DTNB solution were added to it. Then, 1.2 mL of DTNB solution was added in 800 µL of distilled water to the blank. After mixing the tubes, they were kept on ice for 5 minutes and centrifuged at 3000 rpm and 4 °C for 10 minutes. The new tubes were mixed by adding 2 mL of supernatant and 8 mL of phosphate solution. After adding 1 mL of DTNB, the absorbances of the standard and samples were read at 412 nm against the blank sample.

Nitric oxide assay. Nitric oxide concentrations in serum and liver tissue were determined using a spectrophotometer (PowerWave XS, BioTek, Vermonts, USA) by the method of MIRANDA et al. (2001). Briefly, relevant standards of 100, 50, 25, 12.5, 6.25, and 3.125 µM were prepared from 1 mM stock nitrite and nitrate solutions. The samples were deproteinized with 10% zinc sulphate, and then total NO concentrations (nitrate and nitrite) were calorimetrically determined by the acidic Griess reaction, involving the reduction of nitrate to nitrite by vanadium (III) chloride. The absorbances of the samples were recorded at 540 nm. The nitrate and nitrite concentrations from the standard curve were added together and the nitric oxide concentration was quantified.

Lipid peroxidation estimation. Lipid peroxidation was determined by measuring malondialdehyde concentrations in serum and liver tissue according to MIHARA and UCHIYAMA's method (1978). This method is based on the reaction between MDA and thiobarbituric acid. MDA forms a pink-colored complex with thiobarbituric acid. The absorbance of the solution containing the complex was read at 532 nm in order to estimate the lipid peroxidation.

In this regard, from the tubes marked test and blank, 0.5 mL of sample was added to the test tube, then 3 mL of 20% trichloroacetic acid was added to the blank tube and 2.5 mL to the test tube. Then, 1 mL of thiobarbutyric acid was put into both tubes and the tubes were incubated in a 90 °C water bath for 30 minutes, cooled and 4 mL of n-butanol was added. After the tubes were centrifuged at 3000 rpm for 10 minutes, the n-butanol layer was transferred to another tube and the absorbance of the test against the blank was determined at 535 nm.

Statistical analysis. The results were the mean of three replicates and expressed with their standard deviation (mean ± SD). The relevant experimental data were subjected to one-way variance analysis to compare the means, which were then separated by using Duncan's multiple range test at 5% probability level ($P< 0.05$) (SPSS 16.0).

Results

The expression levels of SIRT2 and SIRT3 proteins in the groups are presented in Fig. 1. In comparison to the control, the liver SIRT2 and SIRT3 protein levels increased significantly in the resveratrol group, while these proteins decreased in the FB1 group ($P<0.05$). The SIRT2 and SIRT3 protein levels in the control and FB1 + Resveratrol groups were significantly higher than those of the FB1 group ($P<0.05$), but no statistical differences were noted between control and FB1 + Resveratrol groups.

The liver 8-OHdG level, an indicator of DNA damage, increased significantly in the FB1 group in comparison to the control and resveratrol groups ($P<0.05$) (Fig. 2A). In the group treated with resveratrol and fumonisin B1, 8-OHdG levels were not significantly different from the values

in the control but there was also no significant difference between resveratrol+fumonisin B1 and the fumonisin only group, indicating normalization in terms of fumonisin induced-DNA damage. Similarly, the values of the control and resveratrol groups did not statistically differ.

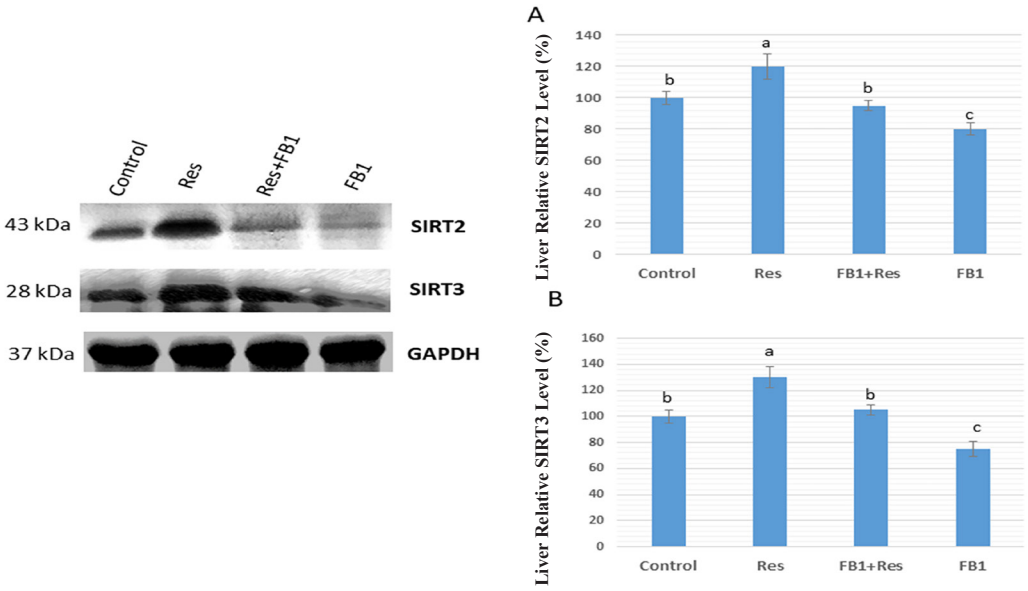

Fig. 1. The effects of resveratrol on expressions of liver SIRT2 and SIRT3 protein in fumonisin-induced hepatotoxicity in mice. The protein levels of A: SIRT2 and B: SIRT3 were determined via Western blotting. Each sample was normalized to GAPDH. Representative protein bands are presented at the top of the histograms. Data (n=3) are expressed as mean±SD values. Values bearing different superscripts (a,b,c) in the same column are significantly different (P<0.05).

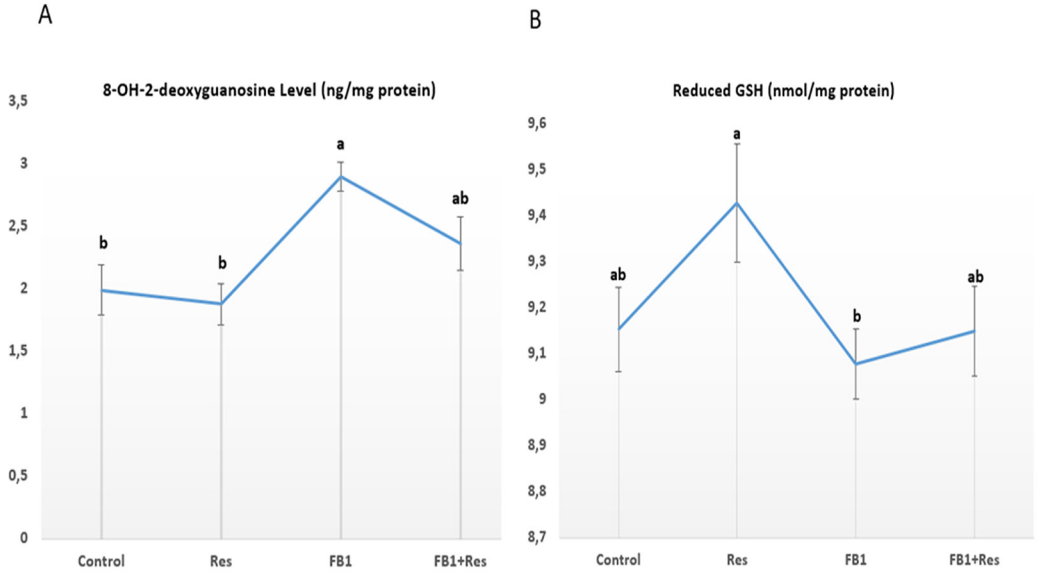

Fig. 2. The effect of resveratrol on 8-OH-2-Deoxyguanosine and reduced glutathione levels in fumonisin-induced hepatotoxicity in mice. A: 8-OH-2-Deoxyguanosine levels in the liver. The 8-OHdG analysis for DNA damage detection was performed using an ELISA kit. B: Reduced GSH levels in the liver. Reduced GSH levels were determined according to BEUTLER et al. (1963). The difference between the groups in different columns (a,b) was statistically significant (P<0.05).

Although the liver GSH level significantly increased in the resveratrol group when compared with the other groups, no difference was observed between the FB1, Resveratrol + FB1 and control groups (Fig. 2B).

Serum NO levels increased significantly in the FB1 group in comparison to the resveratrol group ($P<0.05$) (Fig. 3A). No significant differences were noted regarding NO levels between the FB1 + Resveratrol and control groups.

The liver NO level in the FB1 group increased significantly when compared to all the other groups. While the liver NO level in FB1 + Res group was significantly lower than in the FB1 group, but NO level in FB1 + Res group was higher than in the control and resveratrol groups. There was no significant difference in the NO level between the control and resveratrol groups, and this parameter in the control and resveratrol groups was significantly lower than in the FB1 and Resveratrol + FB1 groups.

Serum MDA levels, an indicator of lipid peroxidation, increased significantly in the FB1 group compared to the control and resveratrol groups ($P<0.001$) (Fig. 3B). The MDA level in the resveratrol group was significantly lower than in the FB1 and FB1 + Res groups. The liver MDA levels were statistically different between the groups. The highest MDA level of the liver was observed in the FB1 group, while the lowest level was observed in the resveratrol group. The liver MDA level in the FB1 + Res group was significantly lower than in the FB1 group.

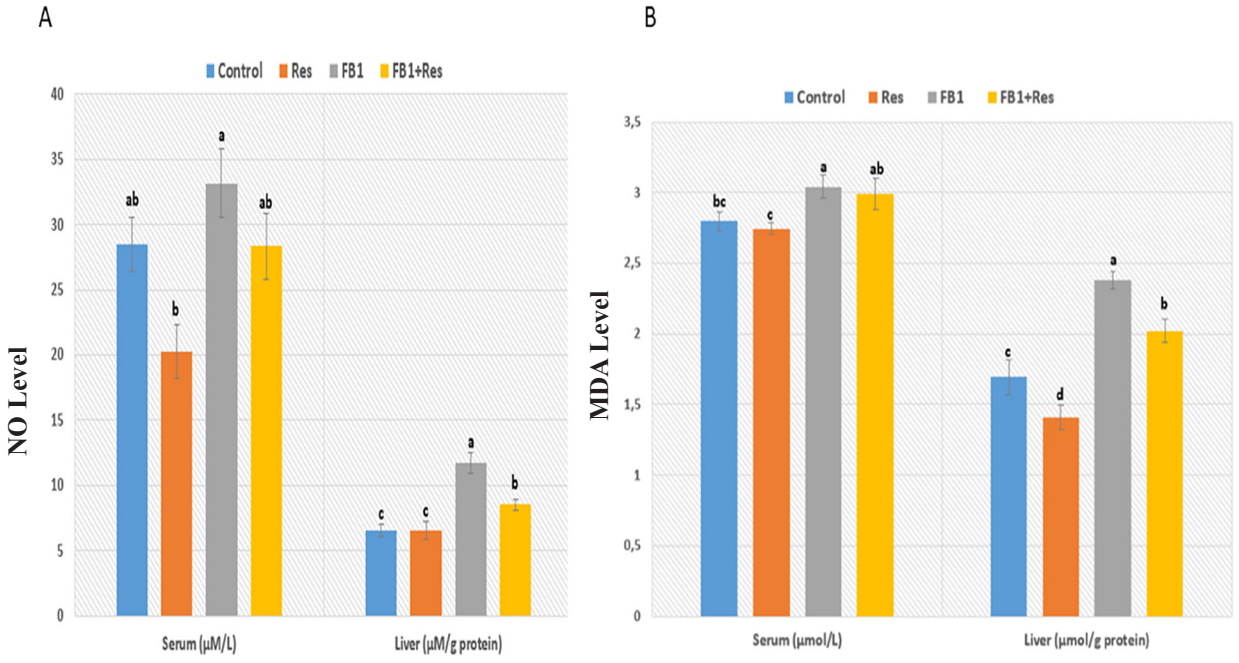

Fig. 3. The effect of resveratrol on nitric oxide and malondialdehyde levels in fumonisin-induced hepatotoxicity in mice. A: Serum and liver nitric oxide levels. The nitric oxide concentrations were determined using a spectrophotometer by MIRANDA et al. (2001) B: Serum and liver malondialdehyde levels. Lipid peroxidation was determined by measuring malondialdehyde (MDA) concentrations by MIHARA and UCHIYAMA's method (1978). The difference between the groups in different columns (a,b,c) was statistically significant (A and B, $P<0.05$ and $P<0.001$, respectively).

Discussion

Oxidative stress, characterized by excessive production of free radicals or reduced antioxidant capacity, causes many pathological processes, including toxicities induced by certain mycotoxins (ABEL and GELDERBLOM,1998). In this study, the effect of resveratrol on SIRT2, SIRT3 protein expressions, 8-OHdG, reduced GSH, nitric oxide and MDA levels were investigated in fumonisin-induced hepatotoxicity in BALB/c mice. It has been reported that exposure to FB1 disrupts the redox-system and exerts this effect by increasing reactive oxygen species (ROS), lipid peroxidation, oxidative DNA damage and reducing antioxidant GSH content, GSH-Px and SOD levels (ABEL and GELDERBLOM, 1998; EL-NEKEETY et al., 2007; KRUPASHREE et al., 2018; MARY et al., 2012). The antioxidant approaches in *in vivo* and *in vitro* studies generally aim to reduce the toxic effects of FB1. It was reported that alpha-tocopherol inhibits oxidative stress in FB1-induced cytotoxicity (ABEL and GELDERBLOM, 1998). Combined administration of coenzyme Q10 (CoQ10), L-carnitine, vitamin E (a-tocopherol) and selenium has been reported to significantly reduce FB1-induced liver injury and DNA damage (ATROSHI et al., 1999).

It was reported that fumonisins (68% FB1, 21% FB2 and 11% FB3, 200 mg/kg for 3 weeks) decreased GSH-Px and SOD levels and increased ALT, AST, triglycerides, cholesterol, HDL, LDL, creatinine and uric acid levels in rats. The authors reported that fumonisins caused biochemical, histological and histochemical changes, and royal jelly had a protective effect by suppressing lipid peroxidation and free radical formation, and increasing GPX formation (EL-NEKEETY et al., 2007). MARY et al. (2012) reported that Aflatoxin B1 (AFB1) and FB1 increased the oxidation of proteins, lipids and DNA. The authors showed that ROS production was related to mitochondrial complex I, CYP450 and the NADPH oxidase system. KRUPASHREE et al. (2018) reported that SOD, CAT, GSH-Px, and GSH levels decreased and MDA levels increased in hepatotoxicity induced by FB1 (2.25 mg) in BALB/c mice.

According to previous studies, the potential mechanism of the toxic effect of FB1 is that FB1 disrupts sphingolipid metabolism, modifies DNA methylation, modulates autophagy, activates endoplasmic reticulum stress and MAPKs, and induces oxidative stress (LIU et al., 2019). Understanding the mechanism of FB1-induced toxicity will provide evidence of effective approaches to reduce its negative health effects. One of the possible causes of FB1 related toxic alterations is associated with the induction of oxidative stress, but how it causes redox imbalance is not yet clear.

Resveratrol has numerous important bioactivities, including hepatoprotective, neuroprotective, cardioprotective, vasculoprotective, antioxidant and anti-inflammatory effects (ATMACA et al., 2014; CHEN et al., 2013; MALLEBRERA et al., 2017; ZOU et al., 2003). Resveratrol, a phenolic compound, is known to scavenge hydroxyl, superoxide, metal-induced radicals and hydrogen peroxide. For the relevant functional compound, weak direct and relatively stronger indirect antioxidant effects have been attributed to the compound, but the reported effects of the compound are weaker than well-known antioxidants such as ascorbate and cysteine.

Therefore, the protective effect of resveratrol against oxidative damage is thought to be more likely to act by regulating endogenous cellular antioxidant systems rather than direct ROS scavenging activity (LI et al., 2012). Reactive oxygen species leads to cellular damage and death, damaging intracellular macromolecules by attacking the double bonds of lipids, proteins and DNA bases (ABDU and ALBOGAMI, 2019; GULMEZ and ATAKISI, 2020). The damage of free radicals is detected by measuring oxidative products such as MDA, protein carbonyl and 8-hydroxyguanine derivatives in body fluids and various tissues. In this study, it was determined that FB1 increased liver DNA damage ($P<0.05$) in fumonisin-induced hepatotoxicity in mice, while resveratrol normalized this damage, by bringing 8-OH-2-deoxyguanosine levels to those of the control.

While liver MDA levels increased in the FB1 group compared to other groups, resveratrol

supplementation decreased the liver MDA level ($P<0.05$). Similarly, this level also decreased in the serum, but the difference was not statistically significant. Considering the level of reduced GSH, it was observed that the reduced GSH level was low in the fumonisin group and high in the resveratrol group ($P<0.05$). Resveratrol supplementation caused decreases in the level of reduced GSH, but the change was not statistically significant. The reduced glutathione and glutathione-associated metabolism is known to play an important role in protecting cells from oxidative stress (KART et al., 2016; SOMEYA et al., 2010). It has been demonstrated that resveratrol (10 mg/kg/b.wt) reduced fumonisin-induced oxidative stress but its protective effects depends on the dose or duration of administration. AZUKA and CHINWE (2018) reported that GSH, SOD and CAT levels did not change in cisplatin-induced lipid peroxidation in both resveratrol (5, 10 and 20 mg/kg/b.wt) and cisplatin groups compared to the control.

While the MDA level was found to be significantly high in the cisplatin group, 20 mg/kg resveratrol administration decreased the MDA level. Resveratrol has been reported to increase GSH levels and decrease MDA levels in liver fibrosis induced with dimethyl nitrosamine in rats (ABDU and AL-BOGAMI, 2019).

Nitric oxide and its oxidant products modify catalytic cysteine residue, and these modifications are reversed by the action of sulfhydryl reducing agents (STRUMILLO et al., 2018). It remains to be determined whether nitric oxide plays a protective or a harmful role in tissue damage. The number of reactive nitrogen oxide species (N_2O_3 and $ONOO^-$ e.g.) can alter critical cellular components under high local concentrations of NO. However, NO can also decrease the oxidation chemistry mediated by reactive oxygen species (H_2O_2 and O_2^- e.g). In addition to the antioxidant chemistry, nitric oxide acts both as a direct catcher of ROS and as an antioxidant system inducer. In the first case, NO would perform the functions of the antioxidant system and so prevent its activation. The other case NO would trigger antioxidant gene expression or activate antioxidant enzymes by posttranslational modifications. In this study, it was observed that the serum NO level was high in the fumonisin group and low in the resveratrol group ($P<0.001$). NO levels in the control and Resveratrol + FB1 groups were lower than in the fumonisin group. The liver NO level was high in the fumonisin group and low in the Resveratrol + FB1 group ($P<0.001$). Resveratrol stimulates the production of NO from eNOS, with upregulation of eNOS expression, stimulation of eNOS enzymatic activity, and reversal of eNOS uncoupling mechanisms. Resveratrol provides NO inactivation by superoxide by enhancing NO bioavailability (XIA et al., 2014a). JALILI et al. (2018) reported that administration of resveratrol (2, 8 and 20 mg/kg) in malathion-induced renal toxicity in rats decreased serum nitric oxide, serum creatinine and kidney MDA levels ($P<0.001$). KIMBROUGH et al. (2015) found that resveratrol reduced hepatocyte iNOS expression and activation, and reduced hepatic injury and inflammation in rats. HONG et al. (2010) also showed that resveratrol's inducible nitric oxide, inflammatory mediators, such as tumor necrosis factor-alpha and interleukin-1 beta, inhibit mRNA expression. In another report, inducible NO was reported to have a protective effect against FB1 (2.25 mg/kg/day) hepatoxicity in mice by regulating sphingosine kinase. Fumonisin B1 induced sphingosine kinase activity by iNOS induction, iNOS deficiency prevents iNOS deficiency sphingosine 1-phosphate formation, depriving cells of their protective effects (SUZUKI et al., 2007).

Mitochondria are the main source of ROS, and ROS are a natural byproduct of many processes generating ATP. SIRT1 and SIRT3 play an important role in counteracting mitochondrial stress by promoting antioxidant defense. Resveratrol is a well-known activator of SIRT-1, but its effect on other sirtuins is not yet clear. As deduced from previous reports, SIRT3 has been shown to exhibit antioxidant defense through SOD2 under physiological and pathological conditions. Elevated expression of SIRT3 under mitochondrial stress increases FOXO3A-dependent gene expression, such as SOD2 and catalase, subsequently decreasing ROS accumulation under mitochondrial stress (PAPA and GERMAIN, 2014). It was also found that resveratrol alleviates cadmium-induced

mitochondrial damage and exerts a mitochondrial protective effect via the SIRT3-FoxO3a-dependent mechanism. However, the biological function of SIRT2 is unclear with respect to cellular damage and oxidative stress. SIRT2, a cytosolic deacetylase, also resides in the mitochondria and can directly bind to energy-metabolism-related proteins to deacetylate them. One study showed that SIRT2 knockout mice showed an increase in the level of mitochondrial ROS, indicating that it was involved in the antioxidant defense system (LIU et al., 2017). In a previous study, it was determined that dimethyl hydrazine decreased liver, kidney and colon SIRT2 and SIRT3 protein expressions in mice, whilst oxidative stress index, 8-OH-2-deoxyguanosine, and TOC levels increased, and TAC and reduced GSH levels decreased.

In this study, fumonisin administration decreased SIRT2 and SIRT3 protein levels while these proteins were increased by resveratrol. Thus, resveratrol treatment in the FB1 + Resveratrol group increased the levels of SIRT2 and SIRT3 protein when compared to the fumonisin group ($P<0.05$). It may be indicated that resveratrol might exhibit this effect by regulating the ROS level, or by protecting the cell from oxidative stress via transcription factors. The molecular pathways underlying these effects of resveratrol have been reported to be associated with the NAD+-dependent, class III histone deacetylase sirtuin 1 (SIRT1), the AMP-activated protein kinase (AMPK), the nuclear factor-erythroid-derived 2-related factor-2 (Nrf2), and estrogen receptors (ER) (XIA et al., 2014b). Although the molecular mechanism underlying the induction of antioxidant enzymes by resveratrol is not fully understood, it was reported that SIRT1 and the nuclear factor-E2-related factor-2 (Nrf2) play an important role in this process. Nrf2 is induced by resveratrol, a transcription factor that regulates most ROS detoxification enzymes (LI et al., 2012). Resveratrol was also reported to reduce oxidative stress by reducing the activity of NADPH oxidases (NOX), one of the major mechanisms that produce ROS (FORSTERMANN, 2010). According to a fairly limited number of studies, there is a relationship between sirtuins and the iNOS system. SIRT1 enhances eNOS activity as a result of direct deacetylating eNOS at lysines 496 and 506 (BRUNET et al., 2004), and transcriptional activation of eNOS by resveratrol upregulating through FOXO factor-mediated mechanisms (PACHOLEC et al., 2010). FOXO factors are also involved in the resveratrol-induced, SIRT1-mediated upregulation of antioxidant enzymes (DAITOKU et al., 2004; GIANNAKOU and PARTRIDGE, 2004). Further studies are needed to clarify the mechanism between sirtuins and oxidative/nitrosative stress.

Conclusion

It may be concluded that fumonisin administration decreases SIRT2 and SIRT3 protein expression levels, and increases oxidative stress leading to lipid peroxidation in the liver. The relevant part, regarding the toxic effects of fumonisin, might be attributed to iNOS-derived NO levels. These processes might trigger DNA damage in fumonisin related toxicity cases. Resveratrol, which increases or induces the expression of SIRT2 and SIRT3 proteins, was able to decrease the level of nitrogen species and lipid peroxidation, and protect the cell from oxidative/nitrosative stress via various transcription factors. Although resveratrol's ability to reduce oxidative stress is due to the polyphenol hydroxyl groups present in its structure, further studies are needed to explain its exact mechanism. Therefore, determining the molecular relationship between sirtuin proteins and oxidative stress will contribute to the development of new treatment options in the future.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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SAŽETAK

Oksidacijski stres, koji obilježava poremećaj ravnoteže oksidansa i antiksidansa, uzrokuje patološke procese, uključujući toksičnost potaknutu određenim mikotoksinima. U ovom je radu istražen učinak resveratrola na sirtuin-deacetilazu (SIRT2 i SIRT3), dušikov oksid (NO), sniženi glutation (GSH) i malondialdehid (MDA) kod hepatotoksičnosti izazvane fumonizinom B1. Istraživanje je postavljeno tako da je 40 BALB/c miševa podijeljeno u četiri skupine: kontrolnu, skupinu koja je dobivala resveratrol (10 mg/kg, ip.), skupinu koja je dobivala fumonizin B1 (2,25 mg/kg, ip) i skupinu koja je dobivala resveratrol i fumonizin B1 (10 mg/kg+2,25 mg/kg). Nakon 14 dana određena je razina ekspresije proteina SIRT2 i SIRT3 metodom *western blotting* te analiza aktivnosti antioksidansa i oksidansa u serumu i jetri. Razina ekspresije SIRT2 i SIRT3 u jetri bila znakovito smanjena u skupini s fumonizinom B1 u usporedbi s kontrolnom skupinom. U skupini s dodatkom resveratrola i fumonizina B1, međutim, povećana je razina ekspresije SIRT2 i SIRT3 u usporedbi sa skupinom koja je dobivala fumonizin B1, no bez znakovite razlike između tih skupina i kontrolne skupine. Analizirani su ključni pokazatelji stresa i oštećenja, razine OH-2-deoksigvanozin, NO i MDA u tkivu jetre, koje su bile veće u skupini s fumonizinom B1, u usporedbi s kontrolnom skupinom. Kao što se očekivalo, primjena resveratrola znakovito je smanjila razine NO i MDA u usporedbi sa skupinom kojoj je primijenjen samo fumonizin B1. Također, resveratrol je smanjio razinu 8-OH-2- deoksigvanozina u jetri u skupini kojoj su dani i resveratrol i fumonizin. Rezultati pokazuju da bi resveratrol mogao imati zaštitni učinak u slučaju hepatotoksičnosti uzrokovane fumonizinom putem modulacije ekspresije sirtuin proteina i zaštite stanice od oksidacijskog/nitrosativnog stresa.

Ključne riječi: mikotoksini; hepatoprotekcija; dijetni fenolni sastojci; sirtuini; dušikov oksid; lipidna peroksidacija