RESEARCH ARTICLE

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Investigation of Possible Protective Effects of Resveratrol on Oxidative Stress and Ferroptosis in PFOA Exposure in HepG-2 Cells

ABSTRACT

Objective: Exposure to perfluorooctanoic acid (PFOA) is linked to adverse health effects, including cancer and hepatic diseases. PFOA induces reactive oxygen species generation in human hepatic cells, causing oxidative stress and cell death. Resveratrol (RSV) has garnered attention for its protective effects against xenobiotic-induced damage, yet its impact on PFOA-induced oxidative stress and ferroptosis in the liver remains understudied. This study investigates RSV's protective mechanisms against oxidative stress and ferroptosis in HepG2 cells exposed to PFOA.

Method: HepG2 cells were cultured in DMEM with 10% FBS and 1% penicillin/streptomycin in a 5% CO2 incubator at 37°C. PFOA was added to the cells at concentrations ranging from 0 to 450 µM and incubated at 37°C for 24 hours. The IC50 was determined to be 457 µM. To examine RSV's protective effects, cells were treated with 60 µM RSV. Following treatment with PFOA, RSV, and the combination of PFOA+RSV, cell lysates were prepared for analysis. Oxidative stress and ferroptosis parameters were measured spectrophotometrically using ELISA.

Results: In the PFOA+RSV group, antioxidant capacity increased, and ferroptosis was suppressed compared to the control. Conversely, the PFOA group showed decreased antioxidant capacity, increased oxidant capacity, and induced ferroptosis compared to the control and RSV-treated groups.

Conclusion: PFOA exposure heightens oxidative stress and ferroptosis, whereas RSV treatment significantly reduces hepatic oxidative stress and protects against ferroptosis during PFOA exposure.

Keywords: PFOA, Resveratrol, HepG2, Oxidative Stress, Ferroptosis.

PFOA Maruziyetinde Resveratrol’ün Oksidatif Stres ve Ferroptosis Üzerindeki Olası Koruyucu Etkilerinin HepG2 Hücrelerinde Araştırılması

ÖZET

Amaç: Perflorooktanoik asit (PFOA) maruziyeti, kanser ve karaciğer hastalıkları dahil olmak üzere çeşitli olumsuz sağlık etkileri ile ilişkilendirilmiştir. PFOA, insan karaciğer hücrelerinde reaktif oksijen türlerinin oluşumunu indükleyerek oksidatif strese ve hücre ölümüne neden olur. Son yıllarda, Resveratrolün (RSV) ksenobiyotiklerin neden olduğu hasarlara karşı koruyucu etkisi üzerine çalışmalar önem kazanmıştır. Ancak, RSV'nin karaciğerde PFOA'nın neden olduğu oksidatif stres ve ferroptozis üzerindeki koruyucu etkileri hakkında yeterli çalışma bulunmamaktadır. Bu çalışmada, PFOA maruziyeti sonucu HepG2 hücrelerinde oluşabilecek oksidatif stres ve ferroptozis üzerine RSV'nin koruyucu etki mekanizmalarını araştırmayı amaçladık.

Yöntem: HepG2 hücreleri, %5 CO2 inkübatöründe 37°C'de %10 FBS ve %1 penisilin/streptomisin içeren DMEM'de kültürlendi. PFOA, 0-450 µM konsantrasyonlarında hücrelere eklendi ve 37°C'de 24 saat inkübe edildi. IC50, 457 µM olarak belirlendi. RSV'nin koruyucu etkilerini değerlendirmek için hücreler 60 µM RSV ile muamele edildi. PFOA, RSV ve PFOA+RSV ile muamele sonrasında, hücre lizatı hazırlandı ve analizler için kullanıldı. Oksidatif stres ve ferroptozis parametreleri, ELISA yöntemiyle spektrofotometrik olarak belirlendi.

Bulgular: PFOA+RSV grubunda, kontrol grubuna kıyasla antioksidan kapasite artmış ve ferroptozis baskılanmıştır. Buna karşılık, PFOA grubunda antioksidan kapasite azalmış, oksidan kapasite artmış ve kontrol ve RSV ile muamele edilen gruplara kıyasla ferroptozis indüklenmiştir.

Sonuç: PFOA maruziyeti oksidatif stresi ve ferroptozisi artırırken, RSV tedavisi karaciğer oksidatif stresini önemli ölçüde azaltır ve PFOA maruziyeti sırasında ferroptozise karşı korur.

Anahtar Kelimeler: PFOA, Resveratrol, Oksidatif Stres, HepG2, Ferroptoz.

INTRODUCTION

Perfluorinated alkylated substances (PFAS) are highly fluorinated organic pollutants, exceeding 4700 chemicals that find in environment (1). These compounds exhibit a unique configuration, comprising a hydrophilic end-group and a hydrophobic alkyl chain, setting them apart from conventional persistent organic pollutants. Their solubility in aqueous solutions surpasses that of traditional persistent organic pollutants, and they exhibit non-biodegradable and bioaccumulative traits (2). Perfluorooctanoic acid (PFOA) serves primarily as a chemical intermediate in fluoroacrylic ester production, while its salts act as processing aids in the manufacture of fluoropolymers, fluoroelastomers, and other surfactants (3). PFOA's stability stems from its eight-carbon chain, housing a hydrophilic functional group and a hydrophobic alkyl chain (4,5). Notably persistent, detectable levels of PFOA are detectable in humans and wildlife due to its slow excretion, with an average half-life in humans ranging from 3 to 5 years and a concentration-dependent elimination rate (6,7). This slow elimination may lead to accumulation, potentially triggering long-term health effects. Human exposure to PFOA can occur via food, water, and workplaces, with the highest distribution in lung, kidney, liver, and blood samples, while lower levels are found in the nervous system. PFOA has been detected in cord blood and breast milk among the general population, accumulating in the liver, kidney, and serum post-absorption, resulting in various toxicities (7). Acute exposure to PFOA may induce mild to moderate toxicity, whereas chronic exposure could lead to severe adverse effects such as hepatomegaly, liver damage, and cardiovascular diseases. Additionally, it has carcinogenic potential on organs like the liver, pancreas, and testes (8).

Studies have linked PFOA exposure to human liver enzymes (9), with the liver identified as the primary site for PFOA accumulation in animals, characterized by hepatocellular hypertrophy and necrosis at different exposure levels. In rodents and non-human primates, high liver concentrations of PFOA correspond with liver enlargement, potentially leading to hepatocellular adenomas in rats. Animal research has suggested that PFOA accumulation may trigger genotoxicity, immunotoxicity, neurotoxicity, and hepatotoxicity (10). Evidence from human studies suggests a positive association between PFOA exposure and elevated liver enzymes without conclusive evidence of liver diseases or cancers (7-9). Xu M. et al. reported that PFOA and PFOS alter antioxidant enzyme activities, induce oxidative stress, leading to cytotoxicity in mouse primary hepatocytes, whereby reduced cell viability, apoptosis, and increased oxidative stress levels were observed. These compounds directly interact with superoxide dismutase, resulting in oxidative stress production and apoptosis (11).

Ferroptosis, a cell death pathway catalyzing unsaturated fatty acids primarily found in cell membranes, triggers lipid peroxidation, inducing apoptosis through divalent iron or ester oxygenase action (12). Immediate exposure to PFOA is suggested to disrupt liver cells and induce cell death, although studiesare are inadequate for detailing the associated cell death mechanisms. Resveratrol (RSV), a polyphenol from grapes and other fruits, offers diverse benefits encompassing chemopreventive, antioxidant, tissue differentiation promotion, adipogenesis modulation, and antiproliferative effects in experimental tumor models (13). Although studies highlight the therapeutic potential of resveratrol in liver damage and chronic liver diseases, research gaps persist concerning its protective effects against PFOA-induced oxidative stress and the mechanisms of cell death associated with PFOA exposure. This study aims to unveil the protective mechanisms of resveratrol against potential oxidative stress and ferroptosis in HepG-2 cells following exposure to PFOA.

MATERIALS AND METHODS

Chemicals and Test Reagents: The ammonium salt form of PFOA, (Cas No. 3825-26-1) of ≥98.0% purity was purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). It was dissolved in dimethyl sulfoxide (DMSO; Cas No. D8418) from Sigma-Aldrich. Resveratrol was purchased from Santa Cruz Biotechnology (Dallas, TX). Lipid peroxidation (MDA) assay kit was purchased from Sigma-Aldrich (Saint Louis, MO; Cat no MAK085). Reduced glutathione (GSH), human glutathione peroxidase 4 (GPx4) and human acyl-CoA synthetase long-chain family member 4 (ACSL4) ELISA kits were purchased from MyBioSource (Cat no MBS727656, MBS2000338 and MBS9331516, respectively). Total oxidant (TOS) and antioxidant (TAS) ELISA Kits were purchased from Rel Assay Diagnostic (Gaziantep, Turkey). Caspase 3 (CASP3) and cytochrome C (CYCS) ELISA kits were purchased from Cloud-Clone Corp (Cat no SEA626Hu and SEA594Hu, respectively).

Cell Culture: HepG2 cell line, (ATCC® HB-8065™) was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). The HepG2 cell line was cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% FBS and 1% penicillin/streptomycin in a %5 CO_2 incubator at 37 °C. For sub-cultivation, the cells were trypsinized and washed with diphosphate-buffered saline (DPBS, pH 7.4), subsequently centrifuged at 2000×g for 5 min, and divided.

Determination of PFOA's IC_{50} Concentration: HepG2 cells were seeded into a 96-well plate with each well of 1 x 10^6 cells. When the confluence of the cells reached 70–80%, PFOA was added to the cells at concentrations of 0 -450 µM and incubated 5% CO_2 at 37 °C for 24 h.

Study Groups:

- ✓ Control group: Untreated HepG2 cells
- ✓ PFOA group: HepG2 cells treated with IC_{50} dose (572 µM) of PFOA
- ✓ RSV group: HepG2 cells treated with 60 µM) of RSV
- ✓ PFOA + RSV group: HepG2 cells treated with 572 µM dose of PFOA (IC_{50}) and 60 µM dose of RSV (572 µM+ 60 µM)

Cell Viability: Cytotoxicity of PFOA was determined with the MTT assay. Briefly, HepG2 cells were seeded into 96-well microplates (10,000 cells/well). After overnight incubation at 37 °C, the growth medium was replaced with fresh medium containing 75, 150, 300, and 450 µmol/ml of PFOA and 75 mol/ml PFOA + 60 µmol/ml RSV, 150 mol/ml PFOA + 60 µmol/ml RSV, 300 mol/ml PFOA + 60 µmol/ml RSV, 450 mol/ml PFOA + 60 µmol/ml RSV in 96 well plates. The cells were incubated for an additional 24 hours. MTT was then added to a final concentration of 0.5 mg/mL and the cells were incubated at 37 °C for 3 h. After 3 h, the medium was removed, and formazan crystals were dissolved with DMSO. The optical density (OD) of the solution in each well was measured at 570 nm by a microplate reader.

Cell Lysate Preparation: Cell lysates for MDA, GSH, TAS, TOS, GPx4, ACSL4, cytochrome C (CYCS), and caspase 3 (CASP3) assays were prepared based on the following method. 527 µM PFOA and/or 527 µM PFOA+60 µM RSV were used in all the assays mentioned above. 24 hours after respective PFOA and/or RSV application, adherent cells were washed with PBS (pH 7.4) then detached with trypsin, and collected by centrifugation at 3000 x g for 10 min at 4°C. The supernatant was discarded and the pellet was collected. Protein levels were measured by the biuret method (14). The prepared cell lysates were stored at -80^{0}C until using for the assays.

TOS and TAS Measurements: TOS and TAS measurements were performed according to the manufacturer's instructions for RelAssay Diagnostic® commercial kits. TOS measurement depends on the oxidation process. The TOS method involves the oxidation of ferrous ions to ferric ions facilitated by various oxidizing agents within an acidic medium, with the subsequent quantification of ferric ions performed using xylenol orange. The results were evaluated as mmol Trolox equiv. /L.

The TAS level in the cell lysate was assessed using an automated method, specifically the Rel Assay (Rel Assay® Diagnostics kit, Mega Tip, Gaziantep, Turkey), which generates a hydroxyl radical. In this assay, a ferrous ion solution from reagent 1 is combined with hydrogen peroxide from reagent 2. The assay results in the formation of sequential radicals, including the brownish dianisidinyl radical cation, produced by the hydroxyl radical, which is a potent radical. This method measures the sample's antioxidative effect against the potent free radical reactions initiated by the hydroxyl radical. The results are expressed in micromolar Trolox equivalents per liter (mmol Trolox equivalents/L).

GSH Measurement: GSH levels were measurements were performed according to the manufacturer's instructions. Briefly, GSH levels in cells were incubated with GSH-horseradish peroxidase (HRP) conjugate in the pre-coated plate for one hour, and then the wells were emptied and washed five times. Then, the wells were incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction formed a blue colored complex. Finally, a stop solution was added to stop the reaction. Following the reaction, the intensity of the color is measured spectrophotometrically in a microplate reader at 450 nm. The results were presented as µg/ml.

Lipid Peroxidation Measurement: MDA is a key parameter that is ordinarily used as a marker of lipid peroxidation. Lipid peroxidation in cell lysates was analyzed at 532 nm colorimetrically using a method based on the reaction of MDA with thiobarbituric acid (TBA) to form a compound. MDA level measurements were performed according to the manufacturer's instructions and were evaluated as nmol/mg protein.

GPx4 and ACSL4 Measurements: GPx4 and ACSL4 proteins were analyzed according to the manufacturer's instructions. The protein concentrations were calculated by measuring absorbance values using a microplate reader at the recommended wavelengths. The results were presented as ng/ml.

CYC and CASP3 Measurements: CYC and CASP3 levels in HepG2 cell lysates were measured using colorimetric commercial kits, pre-coated with an antibody specific to either CYC or CASP3. The cells were lysed depending on the manufacturer's protocol. The enzyme-substrate reactions for CYC and CASP3 were performed according to the manufacturer's instructions and then color changes were determined spectrophotometrically at 450 nm. CASP3 and CYCS levels results are presented as ng/ml and pg/ml respectively.

Statistical Analysis: The experiments were replicated three times for each group, with triplicate samples in each repetation. Statistical analysis was carried out using GraphPad 8, employing one-way analysis of variance (ANOVA) to examine the distribution of values. The data were presented as mean±standard deviation (SD), and statistical significance was determined at p values <0.05.

RESULTS

Cell Viability: The viability of HepG2 cells exposed to various concentrations of PFOA and PFOA+RSV was assessed using the MTT assay, and the results are illustrated in Figure 1A–D. PFOA exhibited toxicity in the micromolar dose range, displaying a variable dose–response curve. PFOA showed dose-dependent toxicity in the micromolar dose range. At a concentration of 450 µM PFOA, cell survival decreased by 50%. PFOA demonstrated a dose-dependent reduction in cell proliferation. However, when RSV was applied at a concentration of 60 µM, cell viability significantly increased, as depicted in Figure 1.

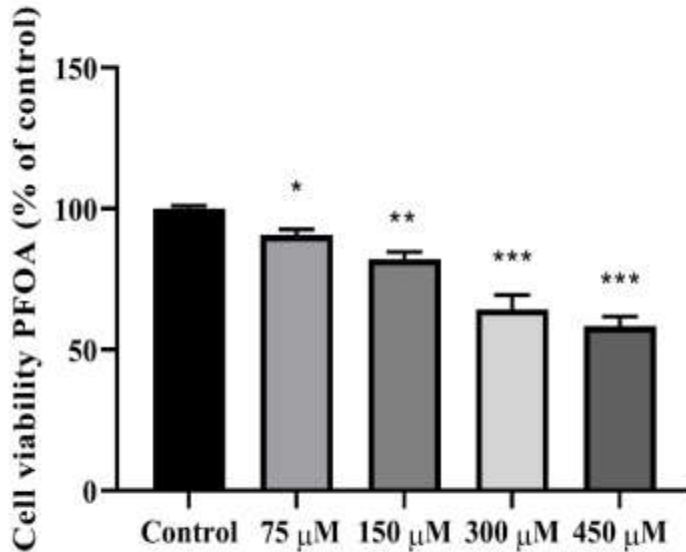

Figure 1. HepG2 cells were cultured with 0-450 µM concentrations of PFOA for 24 h**.** PFOA was toxic in the micromolar dose range showing a variable dose-response curve. At 450 µM PFOA concentration, cell survival was lower by 50%. * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$ vs. control

TOS, TAS, and Oxidative Stress Biomarkers: In the total oxidant status measurements, we observed a 17.71% increase in total oxidant capacity in the PFOA group compared to the control. In the RSV and PFOA+RSV groups, TOS decreased by 14.57% and 9.85%, respectively, relative to the control group. Conversely, TAS decreased by 15.16% in the PFOA group and increased by 13.81% and 8.51% in the RSV and PFOA+RSV groups, respectively (Figure 2 and Figure 3). Notably, there was a 27% increase in the PFOA+RSV group compared to the PFOA group.

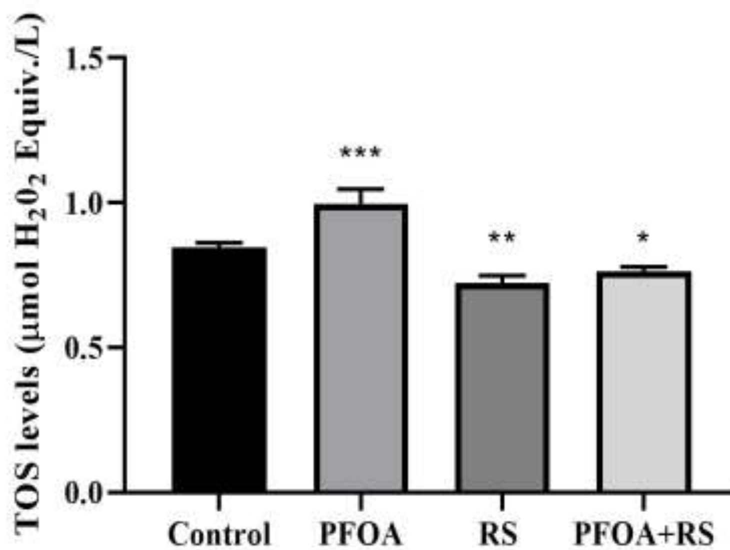

Figure 2. Effect of PFOA and RSV on TOS levels in HepG2 cells. Mean and standard mean values were found 8.4±0.008 in control group, 0.99±0.029 in PFOA group, 0.72±0.014 in RSV group and 0.76 ±0.008 in PFOA+RSV group. * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$ vs. control

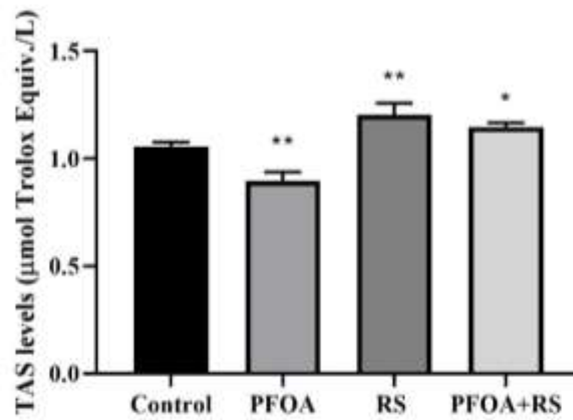

Figure 3. Effect of PFOA and RSV on TAS levels in HepG2 cells. Mean and standard mean values were found; 1.06±0.01 in control group, 0.90±0.02 in PFOA group, 1.20±0.03 in RSV group and, 1.47 ±0.012 in PFOA+RSV group. * $p < 0.05$; ** $p < 0.001$ vs. control

GSH and MDA carbonyl group levels are presented in Figure 4 and Figure 5. Total glutathione (GSH) levels decreased by 28.37% in the PFOA group compared to the control group ($p < 0.05$). In the PFOA+RSV and RSV groups, there was a significant increase by 32% and 62%, respectively, compared to the control group ($p < 0.05$ and $p < 0.0001$). Regarding MDA levels, a significant increase of 32.9% was observed in the PFOA group. In contrast, in the RSV and PFOA+RSV groups, MDA levels decreased by 32.9% and 26.5% compared to the control, respectively (Figure 4 and Figure 5).

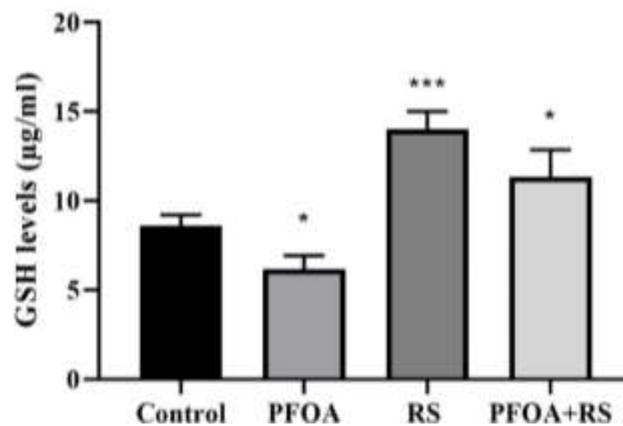

Figure 4. Effect of PFOA and RSV on GSH levels in HepG2 cells. Mean and standard mean values were found; 8.60±0.34 in control group, 6.167±0.44 in PFOA group, 14.0±0.57 in RSV group and 11.33 ±0.88 in PFOA+RSV group. * $p < 0.05$; *** $p < 0.0001$ vs. control

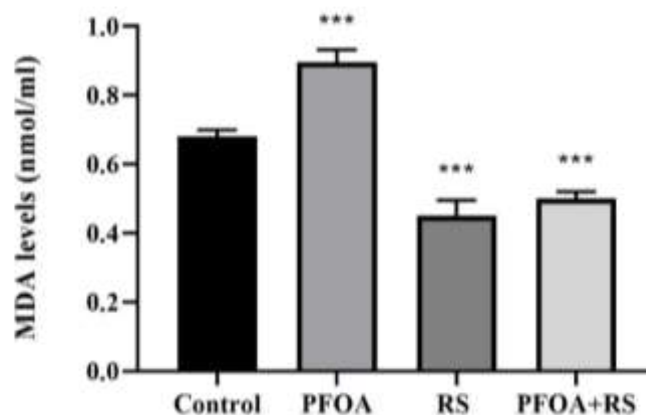

Figure 5. Effect of PFOA and RSV on MDA levels in HepG2 cells. Mean and standard mean values were found; 0.6 ±0.011 in control group, 0.89±0.02 in PFOA group, 0.45±0.02 in RSV group and 0.50 ±0.01 in PFOA+RSV group. *** $p < 0.0001$ vs. control

GPx4 and ACSL4 levels: In the PFOA group, GPx4 levels decreased by 28.69% compared to the control. These levels were found to decrease by 23.71% and 14.9% in the RSV and PFOA+RSV groups, respectively. Moreover, there was a 61.2% increase in the PFOA+RSV group compared to the PFOA group (Figure 6).

ASCL4 levels were significantly higher in the PFOA group, showing a 33.52% increase compared to the control. In the RSV group, an insignificant decrease of 29.41% was observed compared to the control (Figure 7). Additionally, there was a 15.88% decrease in the PFOA+RSV group compared to the control. Importantly, we found a substantial 37% decrease in the PFOA+RSV group compared to the PFOA group.

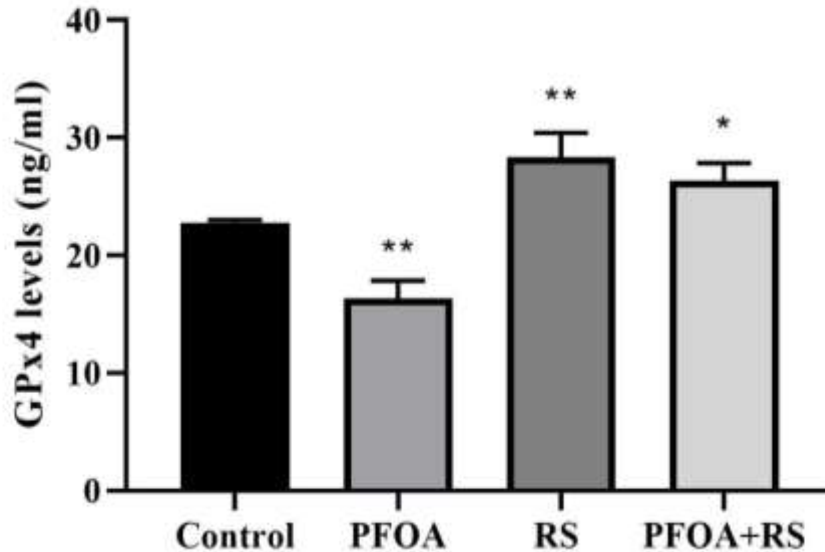

Figure 6. Effect of PFOA and RSV on GPx4 levels in HepG2 cells. Mean and standard mean values were found; 22.77±0.14 in control group, 16.33±0.88 in PFOA group, 28.33±1.2 in RSV group, and 26.33 ±0.86 in PFOA and RSV group. * $p < 0.05$; ** $p < 0.001$ vs. control

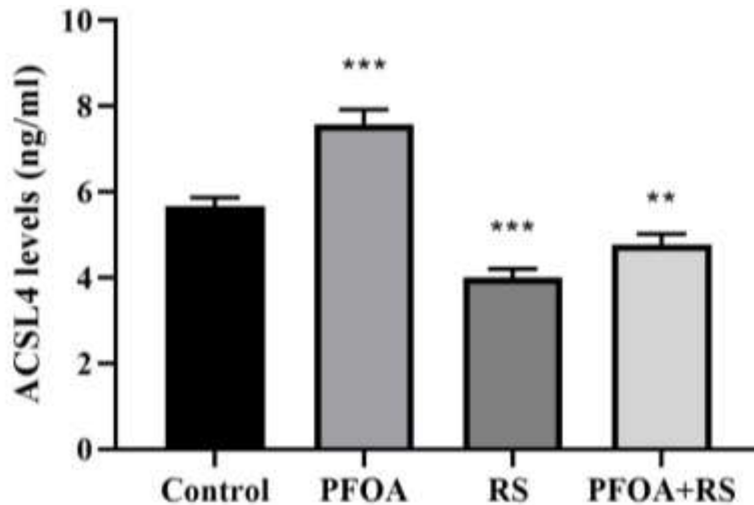

Figure 7. Effect of PFOA and RSV on GPx4 levels in HepG2 cells. Mean and standard mean values were found; 5.67 ±0.12 in control group, 7.6±0.2 in PFOA group, 4.01±0.11 in RSV group and 4.8±0.14 in PFOA+RSV group. ** $p < 0.001$; *** $p < 0.0001$ vs. control

CYC and CASP3 Levels: CYC levels increased in the PFOA group by 37.31% and in the PFOA+RSV group by 10.49% compared to the control group ($p < 0.0001$ and $p < 0.05$). There were no significant changes in the RSV group compared to the control. However, there was a notable 19.54% decrease in the PFOA+RSV group compared to the PFOA group (Figure 8).

For CASP3 activity, decreases were detected in the PFOA group (74%) and PFOA+RSV group (29.1%) compared to the control ($p < 0.0001$ and $p < 0.05$). In the RSV group, there was a 14.45% increase compared to the control. When comparing the PFOA group and the PFOA+RSV group, caspase 3 activity was lower by about 34.81% in the PFOA+RSV group compared to the PFOA group (Figure 9). All results have been summarized in Table 1.

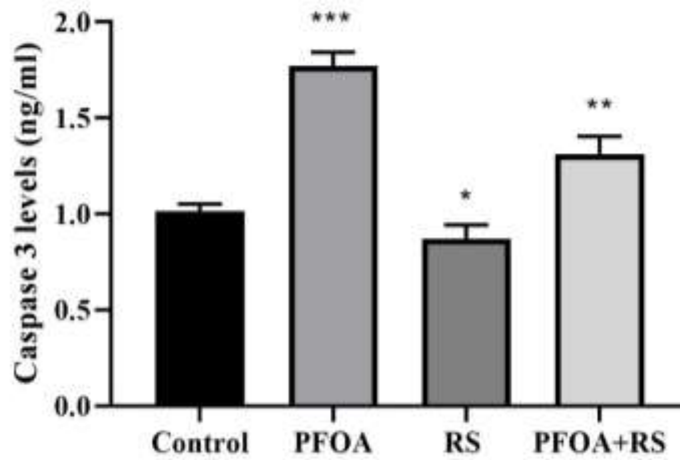

Figure 8. Effect of PFOA and RSV on ACSL4 levels in HepG2 cells. Mean and standard mean values were found; 1.02±0.02 in control group,1.77±0.04 in PFOA group, 0.87±0.04 in RSV group and 1.31±0.05 in PFOA+RSV group. * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$ vs. control

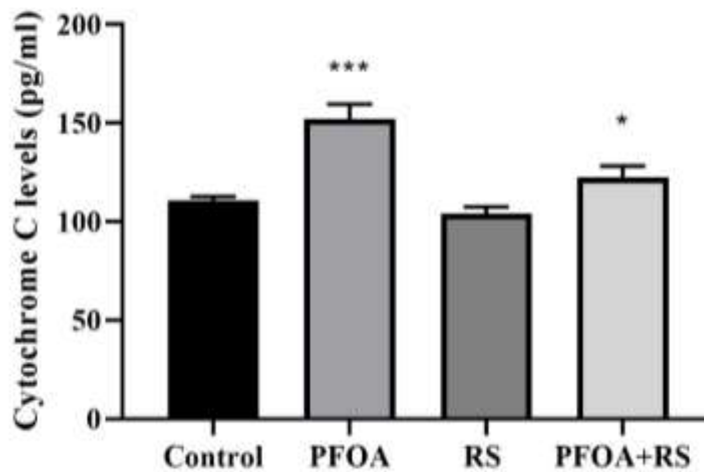

Figure 9. Effect of PFOA and RSV on caspase 3 levels in HepG2 cells. Mean and standard mean values were found; 110.7±1.2 in control group, 152±4.4 in PFOA group, 104±2.1 in RSV group and 122.3±3.4 in PFOA+RSV group. * $p < 0.05$; *** $p < 0.0001$ vs. control.

Table 1. General focus on the results

Parameter	Control	PFOA	RSV	PFOA+RSV
TOS	8.4 ±0.008	0.99 ±0.029^{a}	0.72 ±0.014^{b}	0.76 ±0.008^{c}
TAS	1.06 ±0.01	0.90 ±0.02^{c}	1.20 ±0.03^{b}	1.47 ±0.012^{b}
GSH	8.60 ±0.34	6.167 ±0.44^{c}	14.0 ±0.57^{a}	11.33 ±0.88^{c}
MDA	0,68 ±0,011	0,89 ±0,02^{a}	0,45 ±0,02^{a}	0,50 ±0,01^{a}
GpX4	22.77 ±0,14	16.33 ±0.88^{b}	28.33 ±1.2^{b}	26.33 ±0.86^{c}
ACSL4	5.67 ±0.12	7.6 ±0.2^{a}	4.01 ±0.11^{a}	4.8 ±0.14^{b}
Caspase 3	1.02 ±0.02	1.77 ±0.04$^{'a}$	0.87 ±0.04^{c}	1.31 ±0.05^{b}
Cytochrome C	110.7 ±1,2	152 ±4.4^{a}	104 ±2.1	122.3 ±3.4^{c}

Note. PFOA group, HepG2 cells cultured with IC50 dose of PFOA for 24 h; RSV group, HepG2 cells cultured with 60 µM RSV for 24 h; PFOA+RSV group, HepG2 cells cultured with IC50 dose of PFOA and 60 µM RSV for 24 h. Values are given as mean ± SD of n = 9 experiments and triplicate measurements a, b, c Lines that do not share the same letters (superscripts) are significantly different from each other ($p < 0.0001$; $p< 0.001$; $p < 0.05$, respectively).

DISCUSSION

PFCs are synthetic fluorinated compounds with a carbon backbone, and one of the widely used PFCs is PFOA with an eight-carbon structure (15). PFOA has been identified as resistant to metabolic and environmental degradation, exhibiting bioaccumulation and biopersistence, and has been detected in wildlife and human populations (16). The liver has been recognized as the primary target organ for PFOA-induced toxicity in the body, as demonstrated by in vivo and in vitro studies indicating toxic effects on the liver (17). RSV, a naturally occurring polyphenolic compound found in grapes and other plant sources, possesses potent free radical scavenging and antioxidative properties. RSV has been observed to protect

against proliferation through apoptosis in various cell models, modulating key mediators of the cell cycle and survival. It is known for its antioxidant, anti-inflammatory, and immunomodulatory properties (18). In the present study, the aim was to investigate the protective mechanisms of RSV against potential oxidative stress and cell death in HepG-2 cells resulting from PFOA exposure. The study found that PFOA led to a dose-dependent increase (1.17-fold) in oxidative capacity compared to the control. However, when 60 μM RSV was added along with PFOA at the same concentration (572 μM), a significant decrease in oxidative capacity (0.90-fold) was observed compared to the control group. Additionally, oxidant capacity decreased in the group where only RSV was added (0.80-fold) compared to the control group. In the PFOA+RSV group, total oxidant capacity decreased by 1.3-fold compared to the PFOA group. In antioxidant capacity measurements, PFOA dose-dependently decreased antioxidant capacity compared to RSV-added groups and the control group. In the PFOA group, total antioxidant capacity decreased by 0.84-fold compared to the control. However, there was a 1.08-fold increase in PFOA+RSV and a 1.13-fold increase in the RSV group compared to the control, respectively. GSH levels decreased by 0.28-fold in the PFOA group compared to the control. When RSV was added, these levels increased by 1.63-fold and 1.32-fold in the PFOA+RSV group and RSV group, respectively. MDA levels increased by 1.32-fold compared to the control in the PFOA group, while in the RSV and PFOA+RSV groups, these levels decreased by 0.66-fold and 0.73-fold, respectively (Table 1). The study suggests that PFOA administration increases oxidative stress markers, while RSV treatment significantly reduces hepatic oxidative stress caused by PFOA exposure. This reduction is attributed to the suppression of lipid peroxidation and enhancement of antioxidant enzyme activities, indicating that RSV has a hepatoprotective effect as an antioxidant against PFOA-induced oxidative liver injury. In a related study by Naderi et al., the protective effect of resveratrol against PFOA-induced mitochondrial toxicity in rat liver mitochondria was evaluated. The results indicated that resveratrol has a protective effect against PFOA-induced hepatotoxicity due to its antioxidant activity. The study suggests that RSV can be an effective supplement against oxidative stress and mitochondrial dysfunction in PFOA-induced hepatotoxicity (19).

Studies have demonstrated that PFOA can induce cell death mechanisms (20,21). Conversely, polyphenolic compounds have been suggested to have protective effects against apoptosis and other cell death mechanisms. In the current study, the effects of treatment with PFOA and RSV on cell death mechanisms were investigated. CYC levels were found to increase by 1.37-fold in the PFOA group and by about 1.1-fold in the PFOA+RSV group compared to the control. Conversely, there was a 0.9-fold decrease in the RSV group. Additionally, in the RSV group, there was a 0.86-fold increase versus the control. Regarding CASP3 levels, a 1.74-fold decrease was observed in the PFOA group, and a 1.26-fold decrease in the PFOA+RSV group. Interestingly, this decrease was 1.51-fold in the PFOA+RSV group compared to the PFOA group. These findings suggest that PFOA induces an increase in CYC levels and caspase-3 activity, indicative of apoptosis, while RSV treatment appears to mitigate these effects, as demonstrated by the decrease in CYC levels and CASP3 activity in the PFOA+RSV group compared to the PFOA group. These results underscore the potential of RSV in ameliorating PFOA-induced cellular responses, shedding light on promising avenues for further research in therapeutic interventions.

Ferroptosis is recognized as a distinct form of cell death, separate from apoptosis, autophagy, and necrosis. The current study indicates that PFOA reduces the levels of GPx4 and ACSL4. However, these levels were even lower in the PFOA+RSV group compared to the PFOA group. This suggests that RSV may alleviate the toxic effects of PFOA by further decreasing GPx4 and ACSL4 levels. This mechanism could indicate a potential protective role of RSV against ferroptosis induced by PFOA. In a related study by Wang et al., the role of ferroptosis in deoxynivalenol (DON)-exposed HepG2 cytotoxicity and the antagonistic effect of resveratrol (RSV) were investigated. HepG2 cells were treated with RSV (8 μM) and/or deoxynivalenol (0.4 μM) for 12 hours. The study measured various parameters including cell viability, cell proliferation, expression of ferroptosis-related genes, levels of lipid peroxidation, and Fe (II). The results showed that deoxynivalenol reduced the expression levels of GPX4, SLC7A11, GCLC, NQO1, and Nrf2, while increasing the expression of TFR1, causing GSH depletion, accumulation of MDA, and total reactive oxygen species (ROS). Deoxynivalenol also increased the production of 4-hydroxynonenal (4-HNE), ROS, and excess Fe (II), leading to ferroptosis. However, retreatment with resveratrol reversed these changes, alleviating deoxynivalenol-induced ferroptosis and improving cell viability and proliferation. The study suggested that resveratrol can protect against ferroptosis by activating the SLC7A11-GSH-GPx4 signaling pathway in the HepG2 cell line (22). Together, these findings in both studies imply that resveratrol may have a protective role in mitigating ferroptosis induced by environmental stressors, such as PFOA or deoxynivalenol, through its impact on key molecular pathways involved in ferroptosis regulation.

CONCLUSION

The current study indicates that PFOA decreases antioxidant parameters such as TAS, MDA, and GSH. Conversely, it increases TOS, GPX4, ACSL4, cytochrome C, and caspase 3, particularly in the RSV and PFOA+RSV groups. This suggests that oxidative stress may mediate the onset of ferroptosis, and ferroptosis could play a significant role in PFOA-induced hepatotoxicity. Ferroptosis might be a crucial factor that dictates the detoxification process in response to PFOA exposure. The intricate interplay between oxidative stress, ferroptosis, and the observed changes in antioxidant parameters underscores the complexity of the cellular response to PFOA and highlights the potential involvement of ferroptosis in the liver's reaction to PFOA-induced stress. Further investigation into the molecular mechanisms underlying these interactions could provide valuable insights into the detoxification processes and potential therapeutic strategies for mitigating the hepatotoxic effects of PFOA.

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