Resveratrol Alleviates Pyraclostrobin Induced-Lipid Peroxidation, Oxidative Stress, And DNA Damage In Rats

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Abstract

Pyraclostrobin (Pyra) is a fungicide in the strobilurin class and has proven to be very toxic to aquatic species. Resveratrol (Res) is a phytoalexin that exhibits multiple bioactivities as antioxidative, anti-inflammatory, cardiovascular protective, and anti-aging in animals and is found in plant species such as mulberry, peanut, and grape. This study aimed to determine the protective effect of Res against Pyra-induced oxidative stress in rats. For this purpose, a total of 48 male rats divided into 6 groups - 8 in each group - were exposed to 30 mg/kg Pyra by oral gavage once a day for 4 weeks and to 3 different concentrations of Res (5, 10 and 20 mg/kg) together with Pyra. It was observed that, in groups administered with Pyra, malondialdehyde (MDA) levels increased whereas glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) levels decreased. It was observed that, in the group administered with Pyra, expression levels of *CYP2E1* gene, which is associated with increased cancer risk, pro-apoptotic *BAX* gene, apoptotic *caspase-3, caspase-8* and *caspase-9* genes, *NFκB* gene, which is a pro-inflammatory transcription factor, and *p53* gene, which plays a regulatory role in the cell, increased whereas expression level of anti-apoptotic *bcl-2* gene decreased. It was determined that Res administrations improved Pyra-induced oxidative damage, histopathological changes and expression levels of various genes. According to the ssDNA analysis obtained from the DNA isolated from the blood; when DNA damage and histopathological damage in tissues were examined, it was observed that the highest damage was in the group administered with Pyra and the damage decreased depending on the increase in dose of Res. Consequently, it was observed that Res, known for its antioxidant protective properties, exhibited a protective effect against oxidative stress caused by Pyra.

Introduction

Pesticides are substances that are frequently used to control pests such as insects, weeds, bacteria, mold and fungi, and are known to increase the risk of conditions such as cancer, Parkinson's and reduced fertility in living organisms (Cunha & Fernandes. 2019; Grzywacz et al. 2019). Strobilurins, a new fungicide isolated from fungi, have become the most widely used fungicide group in the world (Balba. 2007; Cui et al. 2017). Classified according to their chemical similarities, Pyra is a member of the strobilurin fungicide group in the chemical form of methyl N-{2-[1-(4-chlorophenyl)-1H-pyrazol-3-yloxymethyl]phenyl}(N-methoxy) carbamate (Elbaz et al. 2019; Bartholomaeus et al. 2003). Studies have shown that Pyra increases the tolerance to drought in plants, is used as a building material for wall covering, and is strongly held by soil components (Capanoglu. 2010; Tuttle et al. 2019; Cabrera et al. 2014). Pyra is especially used in rice cultivation (Guo et al. 2017).

It is reported that Pyras, which have a wide usage area, have toxic effects on water fleas (*Daphnia magna*), zebrafish embryos (*Danio rerio*), earthworms (*Eisenia fetida*), amphipods (*Hyalella azteca*) and freshwater clams (*Lampsilis siliquoidea*) (Cui et al. 2016; (Li et al. 2018; Morrison et al. 2013; Bringolf et al. 2007). It is also stated that Pyra induces oxidative stress and DNA damage on zebrafish (*Danio rerio*) and damages the mitochondrial structure (Zhang et al. 2017; Li et al. 2019). In some studies, it is reported that it shows mitochondrial and developmental toxicity in zebrafish, significantly affects mitochondrial

pathways and causes apoptosis in cells (Yang et al. 2021; Jiang et al. 2019). It is also reported that Pyra is genotoxic and cytotoxic in human peripheral blood lymphocytes *in vitro*, that it induces mitochondrial dysfunction by reducing mitochondrial membrane potential and adenosine-5'-triphosphate (ATP)-dependent respiration, that it causes a significant decrease in lifespan in old bees (*Apis mellifera*), and that it causes damage by accumulating in the gills of fish (*Oreochromis niloticus*) (Cayir et al. 2014; Li et al. 2021; Luz et al. 2018; da Costa Domingues et al. 2020).

Resveratrol (3, 4, 5-trihydroxystilbene) is a stilbene phytoalexin synthesized from grape, soybean, peanut and peanut products (Burns et al. 2002). It is reported that Res is an antimycotic, anti-cardiovascular diseases, anti-cancer and a powerful antioxidant, and has apoptotic effects on some tumor cell lines (Sayın et al. 2008; Crowell et al. 2004; Lin et al. 2011). Some studies show that Res has the ability to capture both superoxide and hydroxyl radicals (Yazir et al., 2015). It has been reported that Res inhibits lipid peroxidation (LPO) and strengthens the antioxidant defense system in rats, preventing oxidative stress caused by glyphosate-based herbicide (Turkmen et al. 2019). In this study, it was aimed to determine the protective effect of Res against Pyra-induced oxidative stress in rats. As a result of the research conducted, no studies have been found on the effect of Res on Pyra. Therefore, the role of three different amounts of Res on Pyra-induced oxidative stress, histopathological and biochemical changes, expression levels of various genes and DNA damage was investigated in this study.

Materials And Methods

Chemicals

Pyra and Res were provided from BASF (Seltima®, BASF, Turkey) and Terraternal (Santa Clara, CA, USA), respectively. In addition, other chemicals of analytical purity were obtained from commercial companies.

Experimental Design

After approval of the Local Ethical Committee on Animal Research (49533702/135), 3–4 months of age male Albino Wistar rats (300–350 g) was obtained from the Afyon Kocatepe University Experimental Animal Implementation and Research Center, Turkey. Animals were kept at suitable conditions (12-h light/dark period, 50–55% humidity, and 25°C), drinking water was given to rats, and they were fed with standard rodent diet. In the experiment, a total of 48 male rats were split into 6 equal groups. Experimental design was carried out as follows: Group 1:control (fed with standard rodent diet); Group 2: corn oil group (1 ml), Group 3:Pyra (30 mg/kg), Group 4:Pyra (30 mg/kg) and Res_5 (5 mg/kg), Group 5: Pyra (30 mg/kg) and Res_{10} (10 mg/kg), Group 6:Pyra (30 mg/kg) and Res_{20} (20 mg/kg). The implementation period of the experimental model was 4 weeks (30 days). Pyra and Res doses dissolved in corn oil that were chosen according to Yoshizawa et al. 2019 and Akbel et al. 2018, respectively. At the end of the administrations, blood, liver and kidney tissues were collected under anaesthesia

(xylazine/ketamine). Additionally, biochemical, molecular and histopathological analyses were performed to detect the effect of Pyra and Res on tissues.

Biochemical Analyses

Preparation of homogenates was carried out according to the procedure of Ince et al. 2014. The method of Winterbourn et al. 1975 was carried out to determine the antioxidant enzyme activities of rat erythrocytes. MDA levels were determined for lipid peroxidation (LPO) according to Ohkawa et al. (1979) in the blood and Draper and Hardley (1990) in the tissue. GSH level was measured in blood and tissue according to Beutler et al. (1993). Activities of SOD and CAT was measured according to Sun et al. (1988) and Sinha (1972) respectively in the erythrocyte and tissue. By the estimation of plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were determined by using commercial kits (Teco Diagnostics assay kit, Teco Diagnostics, Anaheim, California, USA). Spectrophotometric measurements were performed using a Shimadzu 1601 UV-VIS spectrophotometer (Tokyo, Japan).

Histopathological Analysis

For histopathological analysis, liver and kidney tissues of rats were fixed in 10% formalin solution. Fixed tissues were dehydrated by graded alcohol solutions (70–100%). Afterwards, they were objected to xylene and embedded in paraffin blocks which were sliced sections (5 μm) with microtome (Leica, RM 2245). It was stained with hematoxylin–eosin (H&E) and each section was examined under a light microscope (Nikon Eclipse Cl, Tokyo, Japan).

Molecular Analysis

RNA isolation and determination of gene expression by Real-Time PCR

Total RNA of the liver was extracted and reversed transcribed using GeneJet RNA purification kit (Thermo Scientific, USA). Quality of isolated RNAs was measured with Multiskan™ FC Microplate Photometer (Thermo Scientific, USA). DNase-I (Thermo Scientific, USA) was used to remove DNA from RNA and cDNA was synthesized by means of RevertAid H Minus Single Strand cDNA Synthesis Kit (Thermo Scientific, USA). In primer design from NCBI web site, mRNA sequences of *β-actin, p53, bcl-2, NFκB*, *caspase-3, caspase-8, caspase-9, BAX* and *CYP2E1* genes which are unique to *Rattus norvegicus* and computer package program named FastPCR 6.0 (Kalendar et al. 2009). Primer sequences, total base length and gene bank numbers are given in Table.1. CFX Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., USA) was used with a view to identifying the differences in gene expression levels of groups. PCR analysis was conducted with PCR mix, Maxima SYBR Green qPCR Master Mix and ROX Solution (Thermo Scientific, USA). Experiments were performed in 3 replicates. Proportional variety in mRNA expression levels of target genes were calculated by $2^{-\Delta\Delta Ct}$ method based on cycle thresholds (Ct)

of amplification curves obtained following amplification process comprising denaturation, annealing of primer and chain extension steps (Pfaffl 2001).

DNA Fragmentation Analysis

For DNA fragmentation analysis, first DNA was isolated from rat blood in accordance with the ABP/N014-iQuant™ ssDNA Assay KiT protocol, and then the %ssDNA ratio was calculated and charted after measuring the total DNA and ssDNA amount in ng/µl. The iQuant™ ssDNA Assay Kit provides an easy and accurate quantification of ssDNA or oligonucleotides. The kit is highly reliable in detecting ssDNA ranging from 1 to 200 ng. Samples were diluted using the buffer solution for which reagents were provided, and reading was done using the Qubit® Fluorometer. The kit can tolerate common contaminants such as proteins, salts, solvents and detergents very well.

Statistical Analyses

Data obtained from experimental animals were expressed as means and standard deviation of means (± SD).

Homogeneous distribution of data was determined in statistical calculations. Analysis of the data was performed using one-way analysis of variance, followed by the Duncan test on the SPSS 20.0 and $p < 0.05$ was considered to be significant.

Results

Effect on biochemical parameters

According to the results obtained from the biochemical data of our study; when the GSH, SOD and CAT values in liver (Table 2), blood (Table 3), and kidney (Table 4) tissues of Pyra and Res administration were examined, it was observed that the lowest value was seen in the Pyra group, and these values increased and approached the control value depending on the increase in dose of Res ($p < 0.005$). When MDA values were examined, it was found that the highest value was in the Pyra group compared to the control group ($p < 0.005$), and this value decreased depending on the increase in dose of Res. It was found that Pyra administration caused an increase in AST, ALT and ALP values (Table 5), and that Res, which is known to have antioxidant activity, decreased these values depending on increase in dose.

Table 1
Description of polymerase chain reaction primers (*β-Actin, p53, Caspase-3, Bcl-2, NFκB, Caspase-8, Caspase-9, BAX, CYP2E1*), product size, and gene accession numbers

Gene	Primers		Product size (bp)	Gene accession numbers
β-Actin	F	GAGGGAAATCGTGCGTGACAT	452	NC_005111.4
	R	ACATCTGCTGGAAGGTGGACA		
p53	F	TGCAGAGTTGTTAGAAGGCCCA	397	NM_030989.3
	R	GTCACCATCAGAGCAACGCTC		
Caspase-3	F	ACCCTGAAATGGGCTTGTGTA	427	NM_012922.2
	R	GCCATATCATCGTCAGTTCCAC		
Bcl-2	F	GGGTATGATAACCGGGAGATCG	508	NM_016993.1
	R	ACTCAGTCATCCACAGAGCGA		
NFκB	F	TCCCCAAGCCAGCACCCCAGC	334	NM_199267.2
	R	GGCCCCCAAGTCTTCATCAGC		
Caspase-8	F	TTGCTGAACGTCTGGGCAACG	502	NM_022277.1
	R	TCGTCGATCCTTCCCAGCAAGC		
Caspase-9	F	AGAAACACCCAGGCCGGTGGA	327	NM_031632.1
	R	ACCACGAAGCAGTCCAGGGCAC		
BAX	F	AGGACGCATCCACCAAGAAGC	363	NM_017059.2
	R	CAGTGAGGACTCCAGCCACAA		
CYP2E1	F	TGAGATATGGGCTCCTGATCC	293	AF061442.1
	R	ATCTGGAAACTCATGGCTGTC		

Table 2

Effects of Pyra (30 mg/kg) and Res (5, 10 and 20 mg/kg) on malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) levels in liver tissues of rats.

Groups	MDA (nmol/g tissue)	GSH (nmol/g tissue)	SOD (U/µg protein)	CAT (nmol min^{-1}/ µg protein)
Control	4.57 ± 0.83^{d}	28.83 ± 5.28^{a}	7.00 ± 0.59^{a}	7.38 ± 1.41^{a}
Corn oil	4.59 ± 0.77^{d}	26.99 ± 5.19^{ab}	6.81 ± 0.81^{a}	7.06 ± 1.23^{a}
Pyra	18.02 ± 2.12^{a}	9.75 ± 1.76^{d}	1.14 ± 0.15^{d}	0.88 ± 0.12^{c}
Pyra + Res_5	15.61 ± 1.71^{b}	14.04 ± 1.10^{cd}	1.65 ± 0.30^{d}	1.31 ± 0.09^{c}
Pyra + Res_{10}	13.73 ± 1.69^{c}	17.47 ± 2.20^{c}	2.31 ± 0.41^{c}	2.63 ± 0.49^{b}
Pyra + Res_{20}	12.16 ± 1.79^{c}	23.12 ± 4.25^{b}	3.01 ± 0.46^{b}	3.17 ± 0.37^{b}
Mean ± standard deviation; $n = 8$				
$^{a, b, c, d, e}$ Values with different letters in the same column are statistically significant ($p < 0.001$)				

Table 3

Effects of Pyra (30 mg/kg) and Res (5, 10 and 20 mg/kg) on malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) levels in the blood of rats.

. Groups	MDA (nmol/g tissue)	GSH (nmol/g tissue)	SOD (U/µg protein)	CAT (nmol min^{-1}/ µg protein)
Control	7.06 ± 0.94^{c}	71.49 ± 9.84^{a}	18.77 ± 2.64^{a}	15.3 ± 2.06^{a}
Corn oil	8.08 ± 0.87^{c}	69.73 ± 7.68^{a}	17.31 ± 2.08^{a}	13.59 ± 2.03^{a}
Pyra	19.54 ± 3.28^{a}	17.36 ± 1.97^{e}	7.93 ± 1.24^{c}	3.68 ± 0.65^{c}
Pyra + Res_5	17.46 ± 2.5^{a}	24.93 ± 4.02^{de}	9.45 ± 1.51^{c}	5.39 ± 0.80^{c}
Pyra + Res_{10}	14.27 ± 2.58^{b}	29.56 ± 5.04^{cd}	13.78 ± 2.52^{b}	8.11 ± 1.24^{b}
Pyra + Res_{20}	8.25 ± 0.90^{b}	34.93 ± 6.82^{b}	16.64 ± 2.16^{a}	8.55 ± 1.32^{b}
Mean ± standard deviation; $n = 8$				
$^{a, b, c, d, e}$ Values with different letters in the same column are statistically significant ($p < 0.001$)				

Table 4
Effects of Pyra (30 mg/kg) and Res (5, 10 and 20 mg/kg) on malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) levels in kidney tissues of rats.

Groups	MDA (nmol/g tissue)	GSH (nmol/g tissue)	SOD (U/µg protein)	CAT (nmol min^{-1}/ µg protein)
Control	5.07 ± 0.77^{d}	29.33 ± 7.73^{a}	5.26 ± 1.37^{a}	5.18 ± 0.70^{a}
Corn oil	5.7 ± 0.79^{d}	24.07 ± 6.38^{b}	4.80 ± 1.59^{a}	5.16 ± 0.84^{a}
Pyra	22.84 ± 2.39^{a}	8.64 ± 1.38^{e}	1.00 ± 0.20^{c}	0.89 ± 0.13^{d}
Pyra + Res_5	20.01 ± 2.62^{b}	12.84 ± 1.13^{d}	1.04 ± 0.25^{c}	1.12 ± 0.20^{cd}
Pyra + Res_{10}	13.3 ± 1.10^{c}	16.55 ± 2.71^{cd}	1.62 ± 0.28^{bc}	1.52 ± 0.25^{c}
Pyra + Res_{20}	11.56 ± 1.43^{c}	18.49 ± 2.08^{c}	2.27 ± 0.45^{b}	2.21 ± 0.38^{b}
Mean ± standard deviation; $n = 8$				
$^{a, b, c, d, e}$ Values with different letters in the same column are statistically significant ($p < 0.001$)				

Table 5
Effects of Pyra (30 mg/kg) and Res (5, 10 and 20 mg/kg) on serum levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) in plasma of rats.

Groups	AST	ALT	ALP
Control	92.45 ± 7.02^{d}	57.34 ± 4.20^{e}	62.83 ± 4.99^{d}
Corn oil	92.83 ± 7.76^{d}	57.64 ± 3.89^{e}	63.04 ± 5.82^{d}
Pyra	180.52 ± 14.45^{a}	110.28 ± 7.84^{a}	136.95 ± 19.17^{a}
Pyra + Res_5	168.31 ± 12.88^{a}	99.98 ± 7.98^{ba}	127.58 ± 14.62^{a}
Pyra + Res_{10}	129.69 ± 8.41^{c}	85.48 ± 8.70^{c}	109.1 ± 10.34^{ba}
Pyra + Res_{20}	123.02 ± 9.02^{c}	76.16 ± 6.05^{d}	94.56 ± 10.46^{c}
Mean ± standard deviation; $n = 8$			
$^{a, b, c, d, e}$ Values with different letters in the same column are statistically significant ($p < 0.001$)			

Effect on Histopathological Changes

When the histopathological findings were examined; as a result of Pyra administration, degenerative changes in hepatocytes in the pericentral regions of the liver, deterioration in the remark cord structure and formation of binucleated hepatocytes caught the attention whereas vacuolization in glomeruli in kidney tissues, degenerative changes in tubular epithelial cells and enlargement of Bowman's capsule in glomeruli caught the attention. In addition, it was determined that the intensity of these formations decreased depending on increase in the Res administration. The histopathological changes of the rat liver and kidney are given in Fig. 1 and the statistical results of the values obtained based on the histopathological evaluation are given in Table 6.

Table 6
Histopathological evaluation of Pyra (30 mg/kg) and Res (5, 10 and 20 mg/kg) administration in liver and kidney tissues of rats

Organ	Histopathological Finding	Control	Corn oil	Pyra	Pyra + Res_5	Pyra + Res_{10}	Pyra + Res_{20}
Liver	Degenerative changes in hepatocytes in pericentral regions	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	2.28 ± 0.74^{a}	1.75 ± 0.85^{a}	0.961 ± 0.91^{b}	0.05 ± 0.04^{c}
	Sinusoidal dilatation and hyperemia	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	2.11 ± 0.63^{a}	1.41 ± 0.84^{b}	1.13 ± 0.96^{b}	0.00 ± 0.00^{c}
Kidney	Formations of vacuolization in the glomeruli	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	2.28 ± 0.40^{a}	1.58 ± 0.87^{b}	0.86 ± 0.85^{c}	0.36 ± 0.56^{d}
	Degenerative changes in tubular epithelial cells	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	1.78 ± 0.53^{a}	1.40 ± 1.25^{ab}	0.86 ± 0.85^{b}	0.00 ± 0.00^{c}
	Enlargement of Bowman's capsule in glomeruli	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	1.61 ± 0.56^{a}	1.25 ± 0.78^{ab}	0.70 ± 0.62^{b}	0.00 ± 0.00^{c}
Mean ± standard deviation; $n = 8$							
$^{a, b, c, d, e}$ Values with different letters in the same column are statistically significant ($p < 0.001$)							

Effect on Gene Expression Levels Changes

At the end of the study, when the anti-apoptotic *bcl-2* gene expression levels were examined, it was observed that the expression level decreased in the Pyra group compared to the control group, and the gene expression level in the Res groups increased depending on the increase in the dose and approached the control group. When expression levels of the *CYP2E1* gene, which is associated with increased cancer risk (İşcan & Ada, 2017), proapoptotic *BAX* gene, apoptotic *caspase-3, caspase-8 and caspase-9* genes, and *NFκB* gene, which is a pro-inflammatory transcription factor, were examined, it was observed that the

expression levels of all genes in the Pyra group increased when compared to the control group whereas the expression levels in the Pyra and Res groups decreased and approached to the expression levels of the control group. When the expression level of the *p53* gene, which plays a highly significant regulatory role in the cell, was examined, it was determined that the highest increase in expression level (approximately 7.775 times higher when compared to the control group) was in the Pyra group, and there was a significant decrease depending on the increase in Res dose (Fig. 2). ($p < 0.05$).

Effect on DNA Damage Changes

When the DNA damage between the experimental groups was examined at the end of the study, it was observed that the highest DNA damage in terms of ssDNA was in the group administered with Pyra (Fig. 3). It was determined that Res decreased the amount of ssDNA depending on the increase in dose when compared to the control group ($p < 0.005$).

Discussion

It is stated in studies that fungicides are effective on liver enzymes. To illustrate, it is reported that the levels of ALT and AST, the liver function enzymes, increase in the sera of mice and rats administered with mancozeb (Sakr & Saber, 2007; Sakr et al. 2005). In addition, Lavric et al. (1990) reported that bithionol sulfoxide causes hepatotoxicity at high doses, including an increase in serum AST level. As in our study, it was also found that AST, ALT and ALP values increased with Pyra administration, and that Res, which is known to have antioxidant activity, decreased these values depending on increase in dose. It is reported that Pyra causes oxidative stress effects in adult zebrafish liver and embryos and increases SOD, CAT and MDA levels depending on the dose and inhibits GSH activity (Mao et al., 2020; Li et al. 2018). Jiang et al. (2019) It is reported that tebuconazole, a different fungicide, increases SOD, CAT and GPx activities and decreases GSH levels in rats (Othmène et al. 2020). Consistent with the other studies conducted, it was determined in this study that Pyra caused an increase in MDA levels and a decrease in glutathione and antioxidant enzyme activities due to the formation of reactive oxygen species, whereas Res improved these values due to its antioxidant activity.

Prya (30 mg/kg) produced noticeable histopathological changes in the liver and kidney of the rats. Ibtissem et al (2017) reported that administration of methyl-thiophanate, a fungicide, to rats caused necrosis, infiltration of inflammatory leukocyte cells and hepatocyte vacuolization in the liver, narrowing the Bowman's capsule in the kidneys and causing occlusion of the vessels in the glomeruli and between the tubules. Selmanoğlu et al. (2001) stated in their study that the administration of fungicide carbendazim to rats caused congestion in the liver, enlargement of the sinusoids, an increase in the number of Kupffer cells, mononuclear cell infiltration and hydropic degeneration, and obstruction in the kidney tissue, mononuclear cell infiltration, tubular degeneration and fibrosis. It is reported that hypertrophy, separation of epithelium from lamellae, lamellar fusion and epithelial cell necrosis were observed in the tissues of rainbow trout exposed to captan fungicide, and organs affected the most were gills, kidneys and liver (Boran et al. 2012). Similarly, it is determined in this study that deterioration of the

remark cord structure in liver tissue, degenerative changes in hepatocytes in pericentral regions and formations of binucleated hepatocyte increased in rats administered with Pyra and that vacuolization formations in the glomeruli, degenerative changes in tubular epithelial cells and enlargement of the Bowman capsule in the glomeruli in the kidney tissue were increased. In the other words, Res, administered using a dose-dependent approach, protected the liver and kidney tissue of the rats against Prya-induced cellular damage due to the protective effect on cell and tissue.

An excess of pro-apoptotic proteins in the cell indicates that the cell is prone to apoptosis, whereas an excess of anti-apoptotic proteins indicates that the cell is less prone to apoptosis (Adams & Cory, 2001). Similarly to this study, conducted on zebrafish (*Danio rerio*) by Kumar et al. (2020), it was observed that Pyra increased *caspase-9*, *p53* and *BAX* gene expression. In addition, it is reported that during the development of zebrafish embryo, Pyra causes immunotoxicity by changing the innate immune system-related *TNF-α*, *IL-1b*, *C1C* and *IL-8* gene expression levels (Li et al. 2018). It is revealed that tebuconazole fungicide also decreased *bcl-2* gene expression in male rat kidney tissues and increased *BAX* and *caspase-3* gene expression, which trigger apoptosis via *Bax/Bcl-2* and caspase pathway (Othmène et al. 2020). In a study conducted with metalaxyl fungicide, it is revealed that it increases *NFκB*, *TNF*-α and *caspase-8* gene expression levels and caused DNA damage in liver tissue; on the other hand, ginger administration is effective in protecting rats against metalaxyl-induced liver damage with an anti-inflammatory mechanism (Hassa et al. 2018). It was observed that azoxystrobin and Pyra increased the *CYP24A1* gene expression level in zebrafish embryos (Kim et al. 2021). Similarly, it was found in this study that it increased the gene expression levels of *BAX*, *CYP2E1*, *caspase-3*, *caspase-8*, *caspase-9*, *NFκB* and *p53* in groups administered with Pyra when compared to the control group and decreased the *bcl-2* gene expression level, and that Res administration, which is known to be protective against this, reversed these values. In another study, Res was observed to be protective against Cadmium chloride ($CdCl_2$)-induced toxicity in rat testicles. $CdCl_2$ was down-regulated the anti-apoptotic gene *Bcl-2* and up-regulated the expression of the pro-apoptotic genes *p53* and *Bax*. Res was protected against and partially reversed $CdCl_2$ testicular toxicity through upregulation of *Bcl-2* and downregulation of *p53* and *Bax* gene expression (Eleawa et al. 2014) The protective effects of Res against UVB-induced photoaging in HaCaT cells were investigated, and Res upregulated the expression of *HSP27*, decreased the production of pro-apoptotic proteins such as *p65*, *Bax* and cleaved *caspase-3*, and promoted the expression of the anti-apoptotic protein *Bcl-2*. It has been shown to inhibit UVB-induced apoptosis (Zhou et al. 2018). As in our study, it has also been reported that resveratrol suppresses the expression of antiapoptotic gene products (eg *Bcl-2*, *Bcl-XL*, XIAP and survivin), and inhibits the expression of cell cycle regulatory genes (eg *p53*, Rb, PTEN, cyclins and CDKs) (Harikumar et al. 2008). With all these studies, the expectations for clinical use of Res are increasing rapidly. Our review explains Res's potential for protective effects against Prya at the various gene level.

In the study, it was found that Pyra caused DNA damage, whereas the administration of Res, which is known to have antioxidant activity, reduced this damage. Similarly, it is reported some studies that Pyra causes DNA damage in worms (*Eisenia fetida*), leukocytes isolated from whole blood, and aquatic algae

(*Chlorella vulgaris*) (Ma et al. 2019; Cobanoglu et al. 2019; Liu et al. 2018). When studies on different fungicides are examined, it is emphasized, for example, that monceren, a commercial fungicide, causes DNA damage in zebrafish embryos and azoxystrobin (AZX) causes DNA damage in fish (*Australoheros facetus*) (Ku-Centurión et al. 2016; Crupkin et al. 2021). In another study, it was shown that resveratrol can prevent ROS accumulation, oxidative DNA damage and accumulation of DNA breaks in cell lines exposed to oxidative agents such as the particulate phase of cigarette tobacco smoke (TAR) (Sgambato et al. 2001). Cryopreservation of human sperm can cause DNA damage that compromises fertilization and normal embryo development. Res has also been shown to prevent these harms in both fertile and infertile men (Branco et al. 2010). The study by Quincozes-Santos et al. supports that Res prevents DNA damage and is important in health and disease in protecting against DNA damage against oxidative stress caused by hydrogen peroxide in C6 glioma cells (Quincozes-Santos et al. 2007). In this study, Res administered with a dose-dependent approach protected the rats against Prya-induced DNA damage.

Conclusion

The results of this study demonstrate that Res, given in a dosedependent manner, successfully prevented Prya-induced toxicity in rats. This study determined that Pyra causes oxidative stress in male rats, that causes histopathological damage on liver and kidney tissue, that it causes DNA damage, that according to the results of molecular analysis obtained from liver tissue, it reduces the level of anti-apoptotic *bcl-2* gene, that it increases expression levels of the *CYP2E1* gene, pro-apoptotic *BAX* gene, apoptotic *caspase-3, caspase-8 and caspase-9* genes, *p53* gene, which plays a regulatory role in the cell, and *NFκB* gene, which is a pro-inflammatory transcription factor, that it causes pathological damage to liver and kidney tissues, and that, according to these results, Res plays a protective role against Pyra.

Declarations

Author Contributions: F.Z.-N.; writing—original draft preparation, gene expression analysis, DNA damage analysis, statistical analysis, S.İ.; statistical analyses, biochemical analyses and writing—review and editing, H.H.D.; histological analyses, D.A.A.; writing—review, biochemical analyses and project administration U.A.; biochemical analyses and project administration, E.N.-D.; project administration, obtained tissue and rat feeding. All authors have read and agreed to the published version of the manuscript.

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Figures

Figure 1

Histopathological appearance of male rat's liver (A) and kidney (B) (stained by H&E; dimensions 20×100 µm; *n*: 8). Thick arrow; Degenerative changes in hepatocytes in pericentral regions, thin arrow; Deterioration in remark cord structure, arrowhead; Formations of binuclear hepatocyte epithelial cells (Liver) shed in the tubule lumen. Thick arrow; Formations of vacuolization in glomeruli, thin arrow; Degenerative changes in tubular epithelial cells, arrowhead; Enlargement of the Bowman's capsule in the glomeruli (Kidney). Groups; A1, control; A2, oil (5 ml/kg); A3, Pyra 30 mg/kg; A4, Pyra and Res 5 mg/kg (Res_5); A5, Pyra and Res 10 mg/kg (Res_{10}); A6, Prya and Res20 mg/kg (Res_{20}).

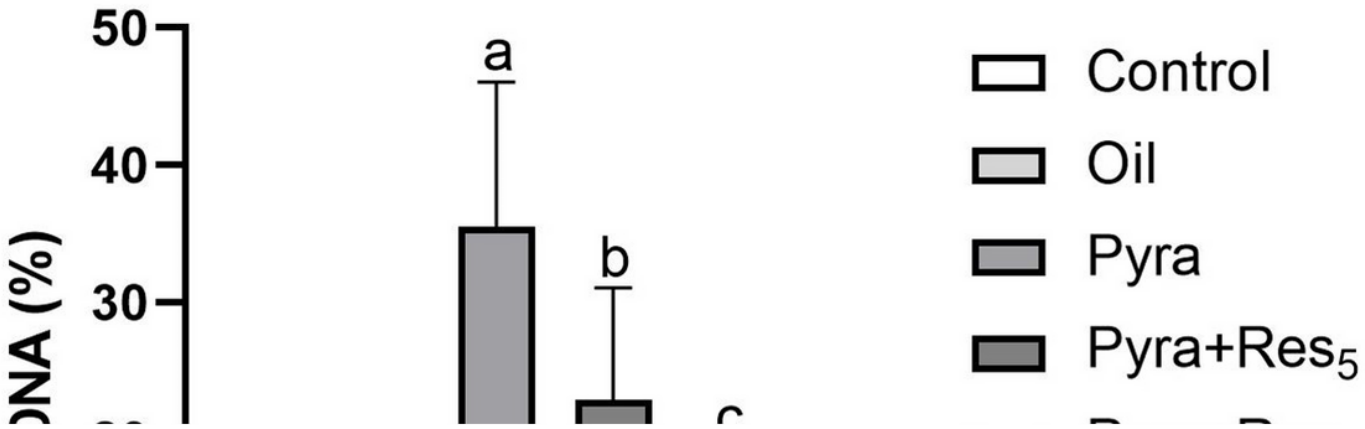

Figure 2

Demonstration of DNA damage levels between Pyra (30 mg/kg) and Res (5, 10 and 20 mg/kg) groups (p<0.05).

Figure 3

Effect of Pyra (30 mg/kg) and Res at doses of 5, 10 and 20 mg/kg on the expression levels of *BAX*, *Bcl-2*, *CYP2E1*, *Caspase-3*, *Caspase-8*, *Caspase*-9, *NFκB* and *p53* genes in rat liver tissues. Mean values are ± standard deviations (n=8). (a, b, c, d, e, f) Values with different letters in the same column indicate statistically significant differences. (p < 0.05).