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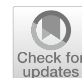
RESEARCH ARTICLE



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 organisms primarily aquatic species. Resveratrol (Res) is a phytoalexin that exhibits multiple bioactivities as anti-oxidative, anti-inflammatory, cardiovascular protective, and anti-aging 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 lipid peroxidation, oxidative stress, and DNA damage 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 30 days and to three different concentrations of Res (5, 10, and 20 mg/kg) together with Pyra. Pyra administration increased liver enzyme parameters and malondialdehyde (MDA) levels whereas decreased glutathione (GSH) levels and activities of superoxide dismutase (SOD) and catalase (CAT). Also, Pyra treatment increased pro-apoptotic (*Bax*), apoptotic (*Caspase-3*, *Caspase-8*, and *Caspase-9*), pro-inflammatory (*NFκB*), cancer (*CYP2E1*), and cell regulatory (*p53*) gene expressions and decreased anti-apoptotic (*Bcl-2*) gene expression in the liver. Furthermore, DNA damage in blood and histopathological changes in the liver and kidney were observed with Pyra administration. In contrast, Res administrations in a dose-dependent manner improved Pyra-induced lipid peroxidation, oxidative and DNA damages, expression levels of these genes in the liver, and histopathological changes in the liver and kidney. Consequently, the treatment of Res, known for its anti-oxidant and protective properties, exhibited a protective effect on Pyra-induced lipid peroxidation, oxidant/anti-oxidant status, gene expressions, and DNA damage in rats.

Keywords Pyraclostrobin · Resveratrol · Oxidative stress · DNA damage · Rat

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Introduction

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, pyraclostrobin (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 (Bartholomaeus 2003; Elbaz et al. 2009). Studies have shown that Pyra increases the tolerance to drought in plants, is strongly held by soil components, and is also used as a building material for wall covering (Capanoglu 2010; Cabrera et al. 2014; Tuttle et al. 2019). Additionally, Pyra is especially used in rice cultivation in the field of agriculture (Guo et al. 2017). On the other hand, Pyra has shown toxic effects on water fleas (*Daphnia magna*), zebrafish embryos (*Danio rerio*), earthworms (*Eisenia fetida*), amphipods (*Hyalella azteca*), and freshwater

clams (*Lampsilis siliquoidea*) (Bringolf et al. 2007; Morrison et al. 2013; Cui et al. 2017; Li et al. 2018). It also induces oxidative stress and DNA damage, and detriments the mitochondrial structure in zebrafish (Zhang et al. 2017; Li et al. 2019), and these effects in zebrafish occur causing apoptosis via mitochondrial pathways in cells (Jiang et al. 2019; Yang et al. 2021). Moreover, Pyra exhibits in vitro genotoxic and cytotoxic effects in human peripheral blood lymphocytes, a significant decrease in lifespan in old bees (*Apis mellifera*), and damage by accumulating in the gills of fish (*Oreochromis niloticus*) (Cayir et al. 2014; Luz et al. 2018; da Costa Domingues et al. 2020; Li et al. 2021).

Resveratrol (3,4,5-trihydroxystilbene) is a stilbene phytoalexin synthesized from grape, soybean, peanut, and peanut products (Burns et al. 2002). Res has the ability to capture both superoxide and hydroxyl radicals and so inhibits lipid peroxidation (LPO) and strengthens the anti-oxidant defense system in organisms (Yazir et al. 2015). Many research showed that Res as a powerful anti-oxidant proves protective effects against mycotic, cardiovascular, and cancer diseases and has apoptotic effects on some tumor cell lines (Crowell et al. 2004; Sayın et al. 2008; Lin et al. 2011). Besides these studies, until now in vivo protective effect of Res against strobilurin toxicity has not come across. Therefore, in this study, it was aimed to determine the protective effect of Res against Pyra-induced oxidative stress in rats. To that end, the role of three different amounts of Res on Pyra-induced oxidative stress, DNA damage, expression levels of various genes, and histopathological changes were investigated in this study.

Materials and methods

Chemicals

Pyra (pyraclostrobin; methyl N-[2-[[1-(4-chlorophenyl) pyrazol-3-yl]oxymethyl]phenyl]-N-methoxycarbamate) and Res (resveratrol; 3,5,4′-trihydroxy-trans-stilbene) 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-month male Albino Wistar rats (300–350 g) were 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: Res_5 (5 mg/kg) and Pyra (30 mg/kg), Group 5: Res_{10} (10 mg/kg) and Pyra (30 mg/kg), and Group 6: Res_{20} (20 mg/kg) and Pyra (30 mg/kg). The experimental period was maintained 30 days. Pyra and Res were dissolved in corn oil and administration with gastric gavage. Also, doses of Pyra and Res 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 anesthesia (xylazine/ketamine). Thereafter, biochemical, molecular, and histopathological analyses were performed to detect the effect of Pyra and Res on tissues.

Biochemical analyses

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, CA, USA). 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 hemoglobin levels which is used to measure anti-oxidant enzyme activities of rat erythrocytes. MDA levels were determined for LPO according to Ohkawa et al. (1979) in the blood and Draper and Hadley (1990) in the tissue. GSH level was measured in blood and tissue according to Beutler et al. (1993). Activities of SOD and CAT were measured according to Sun et al. (1988) and Sinha (1972) in the erythrocyte and tissue, respectively. Also, the protein content of the tissues was determined using the colorimetric method of Lowry et al. (1951). All biochemical parameters were measured with a spectrophotometer (Shimadzu 1601 UV-Vis, Tokyo, Japan).

Molecular analysis

DNA damage analyses

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.

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 website, mRNA sequences of *β-actin*, *p53*, *Bcl-2*, *NFκB*, *Caspase-3*, *Caspase-8*, *Caspase-9*, *Bax*, and *CYP2E1* genes 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).

Histopathological analysis

Liver and kidney tissues of rats were fixed in 10% formalin solution. Fixed tissues were dehydrated by graded alcohol solutions (70–100%). Afterward, 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 CI, Tokyo, Japan).

Statistical analyses

Data obtained from experimental animals were expressed as means and standard deviation (±SD). Before the statistical analyses with SPSS 20.0, the homogeneous distribution of data was determined, and then, statistical analyses were performed using one-way analysis of variance. Afterward, significances between the groups were determined by the Duncan post hoc test and $p < 0.05$ was considered to be significant.

Results

Effect on biochemical, lipid peroxidation, and oxidative stress parameters

Pyra administration caused an increase in AST, ALT, and ALP values as liver enzymes (Table 2), whereas Res treatment, in a dose-dependent manner, decreased these levels in the plasma of rats ($p < 0.001$). When MDA values were

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

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

Table 2 Effects of pyraclostrobin (Pyra; 30 mg/kg) and three different doses of resveratrol (Res; 5, 10, and 20 mg/kg) on plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in rats

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	92.45 ± 7.02 c	57.34 ± 4.20 e	62.83 ± 4.99 d
Corn oil	92.83 ± 7.76 c	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 ab	127.58 ± 14.62 a
Pyra + Res_{10}	129.69 ± 8.41 b	85.48 ± 8.70 bc	109.1 ± 10.34 ab
Pyra + Res_{20}	123.02 ± 9.02 b	76.16 ± 6.05 d	94.56 ± 10.46 c

Mean ± standard deviation; $n = 8$

Values with different letters in the same column are statistically significant ($p < 0.001$)

evaluated in blood (Table 3), liver (Table 4), and kidney (Table 5), it was found that the highest value was in the Pyra group compared to the control group, and this value decreased depending on the increase in doses of Res ($p < 0.001$). In contrast, GSH levels, SOD, and CAT activities were found to be low levels in the Pyra group compared to the control and these values increased and approached the control value depending on the increase in doses of Res ($p < 0.001$). Also, these parameters were not significantly changed in the oil group compared to the control group.

Effect on DNA damage

In terms of DNA damage, the amount of ssDNA (Fig. 1) was determined to be at the highest level in the Pyra-treated

Table 3 Effects of pyraclostrobin (Pyra; 30 mg/kg) and three different doses of resveratrol (Res; 5, 10, and 20 mg/kg) on malondialdehyde (MDA) and glutathione (GSH) levels in the blood and superoxide dismutase (SOD) and catalase (CAT) activities in the erythrocyte of rats

Groups	MDA (nmol/mL)	GSH (nmol/mL)	SOD (U/mgHb)	CAT (U/mgHb)
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; $n = 8$

Values with different letters in the same column are statistically significant ($p < 0.001$)

Table 4 Effects of pyraclostrobin (Pyra; 30 mg/kg) and three different doses of resveratrol (Res; 5, 10, and 20 mg/kg) on levels of malondialdehyde (MDA) and glutathione (GSH) and activities of superoxide dismutase (SOD) and catalase (CAT) in liver tissues of rats

Groups	MDA (nmol/g tissue)	GSH (nmol/g tissue)	SOD (U/μg protein)	CAT (U/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; $n = 8$

Values with different letters in the same column are statistically significant ($p < 0.001$)

Table 5 Effects of pyraclostrobin (Pyra; 30 mg/kg) and three different doses of resveratrol (Res; 5, 10, and 20 mg/kg) on levels of malondialdehyde (MDA) and glutathione (GSH) and activities of superoxide dismutase (SOD) and catalase (CAT) in kidney tissues of rats

Groups	MDA (nmol/g tissue)	GSH (nmol/g tissue)	SOD (U/μg protein)	CAT (U/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 ab	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; $n = 8$

Values with different letters in the same column are statistically significant ($p < 0.001$)

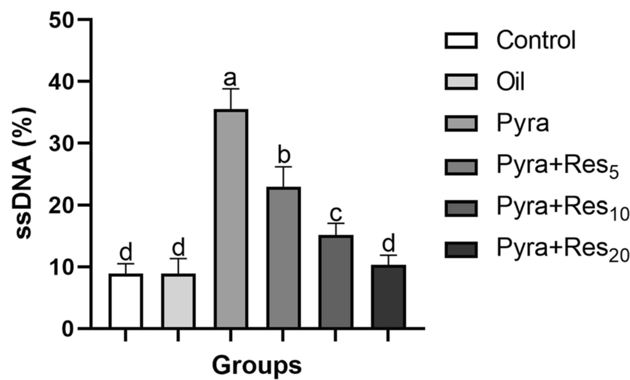

Fig. 1 Effect of Pyra (30 mg/kg) and Res at doses of 5, 10, and 20 mg/kg on DNA damage in blood of rats ($p < 0.001$). Mean values are ± standard deviations ($n = 8$). Values with different letters indicate statistical significance ($p < 0.001$)

group (35.51 ± 3.28) when compared to the control group (8.93 ± 1.60) ($p < 0.001$). On the other hand, in the groups given 5, 10, and 20 mg/kg Res together with Pyra, the amount of ssDNA was determined as 23.09 ± 2.41, 15.13 ± 1.90, and 10.32 ± 1.56, respectively ($p < 0.001$). It was determined that the amount of ssDNA (8.95 ± 2.42) in the oil-given group was not statistically different from the control group.

Effect on gene expression levels

Pyra administration upregulated liver mRNA expression levels of the cancer-associated *CYP2E1* gene; pro-apoptosis-associated *Bax* gene; apoptosis-associated *Caspase-3*, *Caspase-8*, and *Caspase-9* genes; cell regulatory-associated *p53* gene; and pro-inflammatory-associated *NFκB* gene, whereas decreased the anti-apoptosis-associated *Bcl-2* gene expression level ($p < 0.001$). In contrast, administration of increasing doses of Res with Pyra reversed the Pyra-induced mRNA expression levels of these genes (Fig. 2) ($p < 0.001$).

Effect on histopathological changes

Pyra administration at 30 mg/kg for 30 days resulted in vacuolar degenerations in the pericentral regions of hepatocytes, sinusoidal dilatation, and hyperemia in the livers of rats (Fig. 3A3). Also, Pyra administration caused vacuolization in glomeruli, vacuolar degenerations in tubular epithelial cells, and enlargement of Bowman's capsule in glomeruli in kidney tissue of rats (Fig. 4A3). However, 5-, 10-, and 20-mg/kg Res treatment with Pyra alleviated tissue damage caused by Pyra in the liver (Fig. 3A4–6) and kidney (Fig. 4A4–6) in rats. In addition, the liver and kidney tissue architecture of the oil group (Fig. 3A2 and 4A2) was not changed compared to control (Fig. 3A1 and 4A1) tissues. Also, the statistical histopathological evaluation is shown in Table 6 and values indicated damage scores as 0: none, 1: mild, 2: moderate, and 3: severe.

Discussion

Exposure to the fungicides affected liver enzymes; to illustrate, the levels of ALT and AST, the liver function enzymes, increased in the sera of mice and rats administered with fungicide (Sakr and Saber 2007). Lavric et al. (1990) reported that high doses of bithionol sulfoxide (>500 mg/kg), possessing anthelmintic, fungicidal, and insecticidal properties, caused hepatotoxicity, including an increase in serum AST levels in rats and mice. Besides, azoxystrobin, a strobilurin fungicide, was given to rats at 117.25 mg/kg (as 1/20 of LD_{50}) with sesame oil (5 mL/kg) for 14 days and azoxystrobin increased serum AST, ALT, and ALP levels, but sesame oil as an anti-oxidant improved the alterations of liver function biomarkers (Ziada et al. 2020). Similarly, in our study, AST, ALT, and ALP values increased with Pyra administration, and that Res, which is known to have anti-oxidant activity, decreased these values depending on the increase in dose.

Up to now, the effects of strobilurin fungicides on mammals have been limited; however, it has been reported that they cause oxidative stress by enhancing lipid peroxidation and decreasing anti-oxidant status according to the amount and duration of exposure, as a result of studies on model organisms as adult zebrafish and daphnia (Li et al. 2018; Jiang et al. 2019; Mao et al. 2020). Also, strobilurin fungicides such as trifloxystrobin induce oxidative stress in earthworms. According to in vivo study by Ziada et al. (2020), administration of azoxystrobin at a dose of 117.25 mg/kg to rats for 14 days caused oxidative stress due to high MDA and low GSH values. In contrast, sesame oil, as an anti-oxidant, reduced oxidative stress by bringing these values closer to control in rats. Consistent with these studies conducted, Pyra caused an increase in MDA levels and a decrease in GSH level, SOD, and CAT activities due to the formation of reactive oxygen species in this study. On the other hand, Res treatment in a dose-dependent manner improved these values due to its anti-oxidant activity.

In this study, Pyra treatment to rats caused in DNA damage by occurring a high level of blood ssDNA. Similarly, some studies showing the effect of Pyra on DNA damage suggested that Pyra caused highly DNA damage in worms (*Eisenia fetida*), leukocytes isolated from whole blood, aquatic algae (*Chlorella vulgaris*), and fish (*Australoheros facetus*) (Ku-Centurión et al. 2016; Liu et al. 2018; Ma et al. 2019; Cobanoglu et al. 2019; Crupkin et al. 2021). Conversely, natural compounds could have a potentially protective effect on DNA damage in organisms. One of these

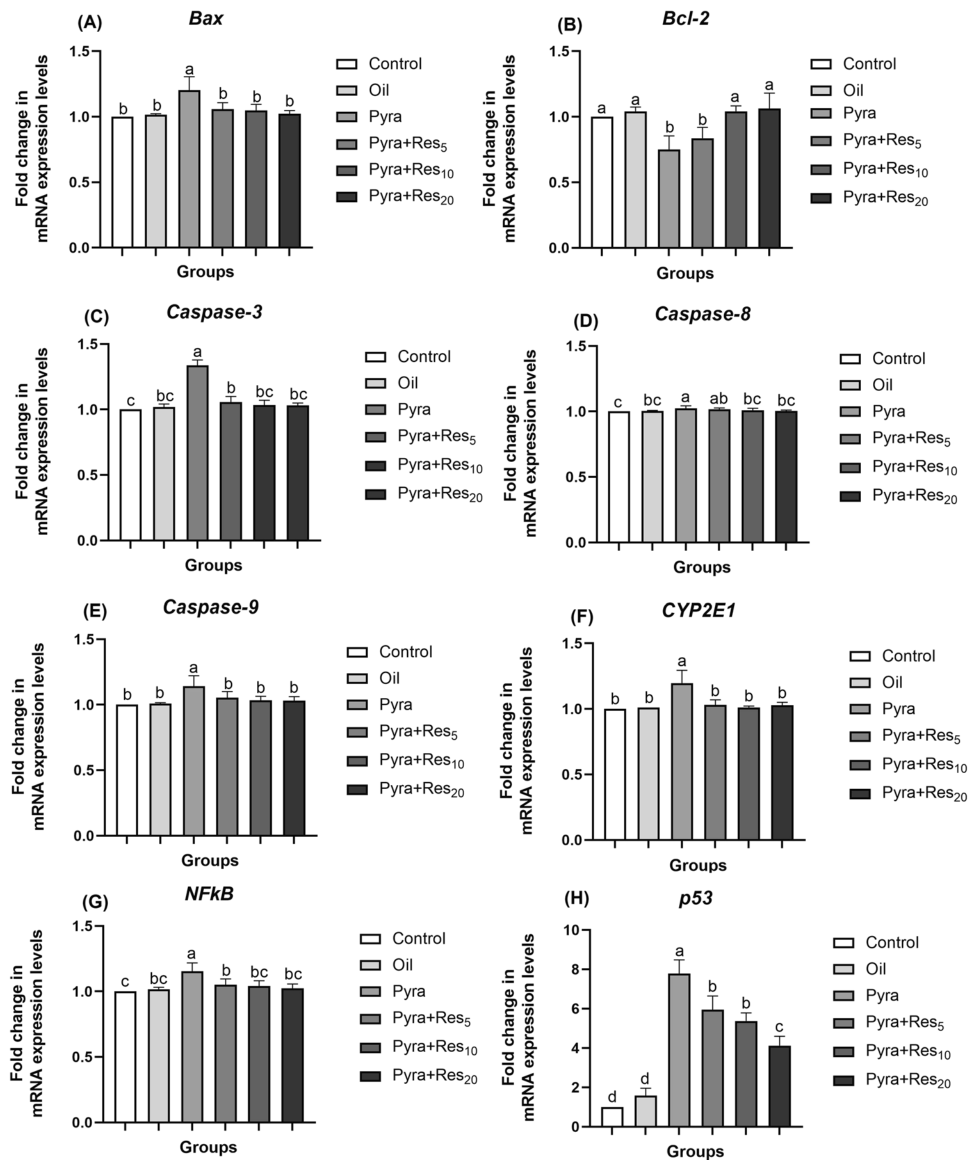

Fig. 2 Effect of Pyra (30 mg/kg) and Res at doses of 5, 10, and 20 mg/kg on the expression levels of *Bax* (**A**), *Bcl-2* (**B**), *Caspase-3* (**C**), *Caspase-8* (**D**), *Caspase*-9 (**E**), *CYP2E1* (**F**), *p53* (**G**), and *NFκB* (**H**) genes in rat liver tissues. Mean values are ± standard deviations ($n = 8$). Values with different letters indicate statistical significance ($p < 0.001$)

compounds, Res could prevent ROS accumulation, oxidative DNA damage, and accumulation of DNA breaks in cell or cell lines exposed to oxidative agents in vitro and in vivo (Sgambato et al. 2001; Quincozes-Santos et al. 2007; Branco et al. 2010). Depending on the properties above of Res, it is observed that especially high doses of Res treatment protected the rats against Pyra-induced DNA damage in this study.

Studies showing the molecular effects of strobilurin fungicides have focused on model organisms and fish. Kumar et al. (2020) reported that 0.1, 10, and 100 µg/L azoxystrobin and Pyra were exposed to zebrafish embryos and expressions of *GST*, *Caspase-9*, *p53*, and *Bax* were found to be high levels. Also, Pyra, trifloxystrobin, and picoxystrobin were applied to zebrafish embryos at doses of 61, 55, and 86 µg/L, respectively, and these strobilurins caused significant upregulation of *IFN* and *CC-chem* and differently changed expressions of *TNF-α*, *IL-1β*, *C1C*, and *IL-8*, suggesting that these fungicides caused immunotoxicity during zebrafish embryo development (Li et al. 2018). In addition, 77.75 ppb of Pyra treated to the zebrafish (*Danio rerio*) during 96 hpf increased the *CYP24A1* gene expression level. This situation has been clarified with our study since no study has been found on the gene expressions of Pyra in mammals so far. In this study, Pyra treatment to the rats increased the gene expression levels of *Bax*, *CYP2E1*, *Caspase-3*, *Caspase-8*, *Caspase-9*, *NFκB*, and *p53* and decreased the *Bcl-2* gene expression level in the liver. In this situation, Pyra has affected apoptosis and inflammation pathways as well as cell regulatory and xenobiotic metabolism. Some studies reported that Res suppresses the expression of anti-apoptotic gene products (e.g., *Bcl-2*, *Bcl-xl*, *Xiap*, and *survivin*),

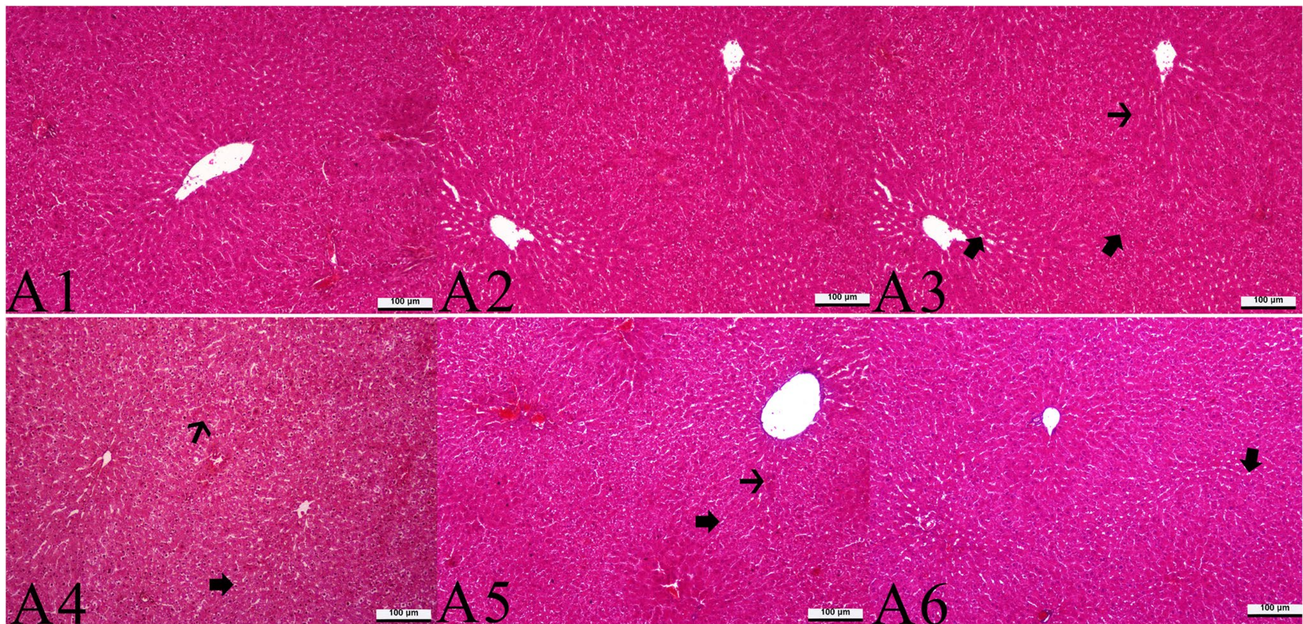

Fig. 3 Histopathological appearance of male rat's liver stained by H&E (dimensions 20 × 100 µm; n = 8). Thick arrow, degenerative changes in hepatocytes in pericentral regions; thin arrow, sinusoidal dilatation and hyperemia. Groups: A1—control, A2—oil (1 mL/kg), A3—Pyra (30 mg/kg), A4—Res_5 (5 mg/kg) and Pyra (30 mg/kg), A5—Res_{10} (10 mg/kg) and Pyra (30 mg/kg), A6—Res_{20} (20 mg/kg) and Pyra (30 mg/kg)

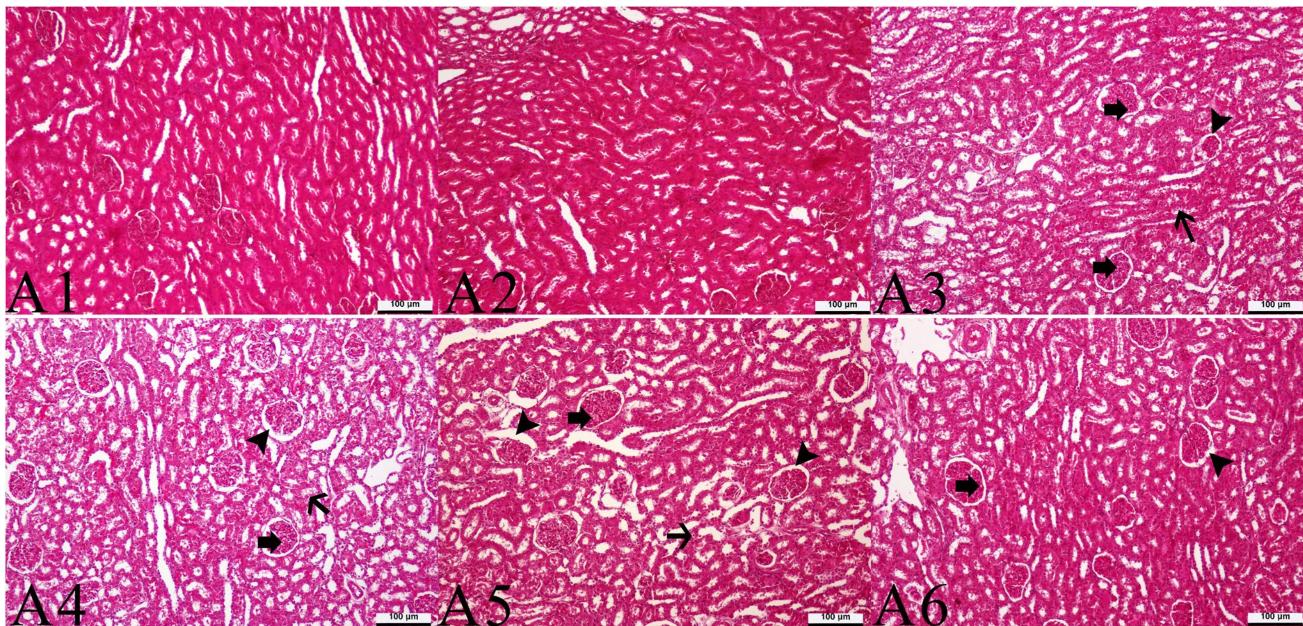

Fig. 4 Histopathological appearance of male rat's kidney stained by H&E (dimensions 20 × 100 µm; n = 8). Thick arrow, formations of vacuolization in glomeruli; thin arrow, degenerative changes in tubular epithelial cells; arrowhead, enlargement of the Bowman's capsule in the glomeruli. Groups: A1—control, A2—oil (1 mL/kg), A3—Pyra (30 mg/kg), A4—Res_5 (5 mg/kg) and Pyra (30 mg/kg), A5—Res_{10} (10 mg/kg) and Pyra (30 mg/kg), A6—Res_{20} (20 mg/kg) and Pyra (30 mg/kg)

and inhibits the expression of cell cycle regulatory genes (e.g., *p53*, *Rb*, *Pten*, *cyclins*, and *CDKs*) and apoptotic genes (e.g., *Bax and Caspase-3*) (Harikumar and Aggarwal 2008; Eleawa et al. 2014; Zhou et al. 2018). Similarly, in this study, Res treatment which is a regulatory role on gene expressions, in a dose-dependent manner, regulated Pyra-induced

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

Tissue	Histopathological changes	Control	Corn oil	Pyra	Pyra + Res_5	Pyra + Res_{10}	Pyra + Res_{20}
Liver	Vacuolar degenerations in hepatocytes	0.00 ± 0.00 d	0.00 ± 0.00 d	2.28 ± 0.74 a	1.75 ± 0.85 ab	0.96 ± 0.21 b	0.15 ± 0.04 c
	Sinusoidal dilatation and hyperemia	0.00 ± 0.00 d	0.00 ± 0.00 d	2.11 ± 0.63 a	1.41 ± 0.84 ab	1.13 ± 0.36 b	0.15 ± 0.07 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 ab	0.86 ± 0.25 b	0.36 ± 0.09 c
	Vacuolar degenerations in tubular epithelial cells	0.00 ± 0.00 d	0.00 ± 0.00 d	1.78 ± 0.53 a	1.40 ± 0.25 ab	0.86 ± 0.25 b	0.20 ± 0.05 c
	Enlargement of Bowman's capsule in glomeruli	0.00 ± 0.00 d	0.00 ± 0.00 d	1.61 ± 0.56 a	1.25 ± 0.78 ab	0.70 ± 0.12 b	0.10 ± 0.02 c

Mean ± standard deviation; $n = 8$

Values indicated damage scores as 0: none, 1: mild, 2: moderate, and 3: severe

Values with different letters in the same line are statistically significant ($p < 0.001$)

gene expressions of *Bax*, *Bcl-2*, *CYP2E1*, *Caspase-3*, *Caspase-8*, *Caspase-9*, *NFκB*, and *p53* in this study.

Pyra (30 mg/kg) administered to rats caused cellular dysfunction due to LPO, oxidative stress, and DNA damage and end up with damage in the liver and kidney determined by histopathological examination in this study. Similarly, azoxystrobin, one of the strobilurin fungicides, was given to rats at 117.25 mg/kg for 14 days, which caused degeneration of some tubular epithelial cells and hemorrhage in the kidney, and severe inflammatory cell infiltration in the liver (Ziada et al. 2020). Also, Ibtissem et al. (2017) reported that one-time intraperitoneal administration of methyl-thiophanate at 300 or 500 mg/kg, a fungicide, to rats caused necrosis, leukocyte infiltrations, and vacuolization of hepatocytes in the liver and narrowing the Bowman's capsule, causing occlusion of the vessels in the glomeruli and between the tubules in the kidneys. Selmanoglu et al. (2001) stated that the administration of fungicide carbendazim to rats at 600 mg/kg for 15 weeks resulted in congestion, an enlargement of the sinusoids, an increase in the number of Kupffer cells, mononuclear cell infiltration, and hydropic degeneration in liver, and congestion, mononuclear cell infiltration, tubular degeneration, and fibrosis were also observed in the kidney. Similar to these studies, Pyra treatment caused histopathological damage in liver and kidney tissues of rats whereas Res, administered using a dose-dependent approach, reversed Pyra-induced changes due to the protective effect on cells and tissue (Akbel et al. 2018; Turkmen et al. 2019).

Conclusion

This study determined that Pyra causes lipid peroxidation, oxidative stress, and DNA damage; changed apoptotic, inflammatory, cell regulator, and cancer-associated gene expressions; and histopathological changes in male rats. Nevertheless, Res, given in a dose-dependent manner, successfully prevented Pyra-induced alterations in rats. After this, in the light of this information, expectations for experimental and/or clinical use of Res as a preventive agent against exposure to pesticides, especially fungicides, will increase rapidly.

Author contribution 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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Data availability Not applicable.

Declarations

Ethics approval This study was conducted with the approval of the local ethics committee for animal experiments, Afyon Kocatepe University, Afyonkarahisar, Turkey (Approval Number: 49533702/135).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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