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Effect of chrysin on oxidative stress, biochemical, and inflammatory alterations in Wistar albino rats exposed to cypermethrin

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Cypermethrin is a broad-spectrum pesticide commonly used in agricultural, veterinary and household applications. Cypermethrin, being a neurotoxic agent and due to its hydrophobic structure, may cause tissue and organ damage by stimulating oxidation in cells and increasing reactive oxygen species. It has a detrimental effect on the immune system and promote inflammation by producing cytokines. The harmful effects of cypermethrin may be alleviated by the intracellular defense system that reduces ROS. Chrysin, a flavonoid, commonly found in vegetables, propolis, honey, fruits and mushrooms, is known to possess antioxidant, antiviral, antidiabetic and anticarcinogenic properties. It provides protection against disease and toxicity through various mechanisms. In this study, we investigated the protective effects of chrysin against oxidative damage in rats exposed to cypermethrin. There is no research on the response of chrysin to the toxicity of cypermethrin in mammals and whether it can be used among treatment options or as a supplement to treatment. A total of thirty 6-8 week old male Wistar albino rats, each weighing 190-240 g, were used and divided into six equal groups, randomly. The groups were formed as control (Gr.I), cypermethrin (25 mg/kg body wt.) (Gr.II), cypermethrin (25 mg/kg body wt.) plus chrysin (50 and 100 mg/kg body wt.) (Gr. III & IV, respectively); and chrysin (50 and 100 mg/kg body wt.) (gr. V & VI, respectively). The indicated doses were given orally for 10 days. Cypermethrin and chrysin were given to the animals alone and together within the specified groups. In blood/tissue samples (brain, heart, kidney, liver, lung and testis), lipid peroxidation/oxidative stress parameters (MDA, NO, CAT, SOD, GSH and GSH-Px) and serum biochemicals (AST and ALT) and inflammatory parameters (TNF-α, IL-1β and IL-6) were evaluated. When a general evaluation is made, compared to the control group, cypermethrin considerably increased the levels of TNF-α, IL-1β, IL-6, ALT, AST in serum and MDA and NO in tissues/plasma, while significantly decreasing the levels/activities of SOD, CAT, GSH and GSH-Px in tissues/erythrocytes (P <0.05). On the other hand, in the groups co-administered with cypermethrin and chrysin, recovery changes were observed in antioxidant status/lipid peroxidation values, and serum biochemical parameters, similar to the control group. Results of this study suggest that chrysin may prevent oxidative damage and inflammation via supporting antioxidants and reducing pro-inflammatory cytokines in cypermethrin-exposed rats.

Keywords: Aspartate aminotransferase, Glutathione, Interleukin, Malondialdehyde, Pesticide toxicity, Tumor necrosis factor

Synthetic pyrethroids are the synthetic form of pyrethrins obtained from the *Chrysanthemum* plant. They are divided into two groups according to their chemical composition: type I, which contains a cyano group, and type II, which does not have a cyano group. Cypermethrin, a type II pyrethroid, is a broad-spectrum pesticide widely used in veterinary, agricultural, and household applications. Cypermethrin is a hydrophobic chemical with high solubility in organic solvents but low solubility in water. Humans are exposed to cypermethrin during pesticide application or consumption of pesticide-contaminated products. This pesticide is metabolized in the body to phenoxy benzoic acid (PBA) and cyclopropane carboxylic acid (CPA) via ester bond cleavage, hydroxylation, and glucuronidation. The mechanism of action of cypermethrin and other pyrethroids is voltage-dependent sodium channels in the nerve cell membrane. When Na^+ channels remain open for a long time, Na^+ transport accelerates and nerve occlusion occurs. Cypermethrin has been reported to be a neurotoxic agent in animals[1]. Due to its hydrophobic structure, cypermethrin may cause tissue and organ damage by stimulating oxidation in cells and increasing reactive oxygen species (ROS)[2]. The harmful effects of cypermethrin may be alleviated by the intracellular defense system that reduces ROS[2,3]. Cypermethrin may also have a detrimental effect on the immune system and promote inflammation by producing cytokines[4].

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Flavonoids are phenylbenzopyrones, a typical class of polyphenolic secondary metabolites found in plant-derived foods and beverages such as apples, onions, tea and red wine and are also specific plant metabolites with properties such as anti-inflammatory, antioxidant, antihepatotoxic, antiviral, anticarcinogenic, vasodilator and spasmolytic. Chrysin is one of the flavonoids commonly found in the passion flowers (*Passiflora caerulea* L. and *Passiflora incarnata* L.), propolis, honey, and Oyster mushroom [*Pleurotus ostreatus* (Jacq.) P. Kumm.] and has analgesic, muscle relaxant, sedative, antioxidant, antiviral, antidiabetic and anticarcinogenic properties. Chrysin provides protection against disease and toxicity through various mechanisms, and its protective effects in general are related to the reduction of cytokines and free radicals. This flavonoid can suppress inflammatory mediators in inflammatory diseases and minimize oxidative damage by increasing antioxidants and reducing free radicals[5].

There are literature reports on the effects of antioxidant and anti-inflammatory activities of natural products[6-8]. In previous studies, phenolic compounds such as curcumin, propolis, epicatechin, quercetin, and baicalin were used as protective agents in rats exposed to cypermethrin[9-14]. There are no radical treatment options for poisoning with pyrethroid group insecticides. The basis of treatment is the elimination of symptomatic findings[1]. Therefore, practices aimed at limiting free radical formation, which is among the mechanisms of poisoning with pyrethroids, and partially preventing the negative effects that may arise from the use of other drugs, may be important in improving the prognosis of poisonings. To date, studies on chrysin in rats exposed to cypermethrin are scarce. In this context, here, we evaluated the beneficial role of chrysin on inflammation, lipid peroxidation, and enzymatic and non-enzymatic antioxidant status in rats exposed to cypermethrin.

Material and Methods

Chemicals and test kits

Chrysin (98%), bovine serum albumin (BSA), sodium carbonate (Na_2CO_3), potassium sodium tartrate tetrahydrate, copper(II) sulfate ($CuSO_4$), 1,1,3,3-tetraethoxypropane, sodium dodecyl sulfate (SDS), acetic acid, 2-thiobarbituric acid, sodium hydroxide (NaOH), xanthine, EDTA.Na_2.$2H_2O$, nitrotetrazolium blue chloride, ammonium sulfate [$(NH_4)2SO_4$], xanthine oxidase from bovine milk, copper(II) chloride dihydrate ($CuCl_2$.$2H_2O$), chloroform, ethanol, L-glutathione reduced, tris hydrochloride, tris base, trisodium citrate, 5,5′-dithiobis(2-nitrobenzoic acid), methanol, sodium phosphate dibasic dihydrate (Na_2HPO_4.$2H_2O$), potassium phosphate (KH_2PO_4), hydrogen peroxide (35%), β-NADPH-Na_4, glutathione reductase from baker's or brewer's yeast (*Saccharomyces cerevisiae*), and sodium azide (NaN_3) were purchased from Sigma and Merck Chemical Companies (USA). Tumor necrosis factor-alpha (TNF-α), interleukin 1-beta (IL-1β), and interleukin-6 (IL-6) rat-specific cytokine kits provided from Elabscience Biotech Co. Ltd, (Wuhan, China).

Animal experiments and design

For this research, thirty 6-8 week old male Wistar Albino rats, each weighing 190-240 g, were used as experimental material. All rats were housed in polyethylene cages under standard laboratory conditions (12 h light/dark cycle at 22±2°C and relative humidity of 45-55%) and provided with *ad libitum* commercial pellet feed (protein 24%, fats 5.09%, cellulose 3.2%, and a total of 3100 kcal/kg metabolic energy) and water throughout the experiment. The experimental protocols were approved by the Erciyes University Local Ethics Committee for Animal Research (Report No: 17/097) and carried out in accordance with international standards. In the experiment, cypermethrin and chrysin to be administered to rats by oral gavage were determined to be 25 mg/kg body wt. and 50-100 mg/kg body wt., respectively, based on literature studies[13,14]. Six groups of five rats each were created[15]. The first group (Gr. I) was kept as a control and only the vehicle (corn oil) was given at a dose of 1 mL/kg/day. Group II was given cypermethrin alone at a dose of 25 mg/kg body wt./day. Groups III & IV were co-administered with 25 mg/kg body wt./day of cypermethrin and 50 & 100 mg/kg body wt./day of chrysin, respectively. Groups V & VI received 50 and 100 mg/kg body wt./day of chrysin, respectively. All applications were performed once a day with an oral gavage and continued for 10 days.

Sample collection and tissue preparation

At the end of the experimental period, blood samples were collected from the rats by cardiac puncture into tubes containing heparin and without anticoagulant under intraperitoneal ketamine (80 mg/kg) and xylazine (10 mg/kg) anesthesia. After blood

collection, the rats were sacrificed by cervical dislocation. Serum was separated from blood samples in anticoagulant-free tubes by centrifugation at 1711 g at 4°C for 10 min and then stored at −80°C for biochemical analysis. Blood samples in heparinized test tubes were centrifuged at 1711×g for 10 min at 4°C, and the plasma-containing supernatant was carefully separated. The buffy coat was removed from the remaining phase and the erythrocytes were washed thrice in normal saline (0.9% NaCl). For this process, the mixture was centrifuged at 760 g for 5 min, and the supernatant was discarded. The erythrocytes were stored at −80°C after being diluted with an equal volume of normal saline. The erythrocytes were haemolyzed with ice-cold normal saline (a ratio of 1:5) prior to analysis. Brain, heart, kidney, liver, lung and testicular tissues were quickly collected and washed with a 0.9% NaCl solution to remove blood clots. The tissues were homogenized (1:5, w/v) in ice-cold phosphate buffer (pH 7.4) with a homogenizer (Silent Crusher M, Heidolph) on ice and then centrifuged for 60 min at 9168×g at 4ºC. Supernatants were transferred into eppendorf tubes and stored in deep freezing (−80°C) until being analyzed for tissue lipid peroxidation and enzymatic or non-enzymatic antioxidant measurements.

The measurement of some biochemical and inflammatory parameters

A Roche Cobas C 8000 autoanalyzer and the same brand kits were used for the determination of serum AST, and ALT enzyme activities. Serum TNF-α, IL-6 and IL-1β contents were measured using commercial ELISA kits on a microplate reader (BioTek Synergy H1, USA) according to the manufacturer's instructions.

Determination of tissue lipid peroxidation and enzymatic/non-enzymatic antioxidants

Protein levels in tissue homogenates were determined by a spectrophotometric method described by Lowry *et al.*[16]. Glutathione peroxidase (GSH-Px) activity in tissues was measured according to the method of Paglia & Valentine[17]. Enzyme activity was described as nmol/min/mg of protein. Superoxide dismutase (SOD) activity was assessed by the method of Sun *et al.*[18] and, it was expressed as U/mg protein. Catalase (CAT) enzyme activity was determined by using the method of Luck[19], and its unit was specified as katal/mg protein. Nitric oxide (NO), reduced glutathione (GSH) and malondialdehyde (MDA) levels of tissue were assayed using the methods of Tracey *et al.*[20], Sedlak & Lindsay[21], Ohkawa *et al.*[22], respectively. These parameters were expressed as nmol per mg protein.

Statistical analyses

The SPSS 21.0 statistics program was used to perform statistical computations on the research data. The data were presented in the form of mean and standard deviation. One-way ANOVA was used to analyze the data, and Tukey's post hoc test was utilized to evaluate the differences between groups. P <0.05 were regarded as statistically significant.

Results

Serum biochemical and inflammatory parameters

The difference between the control and chrysin-treated groups (Gr. V & VI) was not statistically significant. In the cypermethrin-exposed group (Gr. II), AST and ALT enzyme activity, as well as TNF-α, IL-6 and IL-1β levels had increased significantly in comparison to the control group (P<0.05). In the groups (Gr. III & IV) co-administered with chrysin and cypermethrin, IL-6, AST, and ALT levels/activities were similar to those in the control group. The levels IL-1β, and TNF-α in the Group 4 were found to be close to control levels, but not in the Gr. III. Other than ALT activity, changes were dose-dependent (Table 1).

Lipid peroxidation/oxidative stress parameters

Liver tissue

There was no statistically significant difference in liver MDA, NO, GSH, SOD, CAT and GSH-Px levels/activities between the chrysin-treated groups (Gr. V & VI) and the control group. On the other

Table 1 — Effect of chrysin (50 and 100 mg/kg) on serum AST, ALT, IL-1β, IL-6 and TNF-α levels in cypermethrin (25 mg/kg) exposed rats

	Group I	Group II	Group III	Group IV	Group V	Group VI
AST (U/L)	91.16±7.80^{abc}	105.33±8.57^{c}	96.66±9.24^{bc}	91.00±5.0^{abc}	85.00±12.2^{ab}	78.50±4.32^{a}
ALT (U/L)	52.66±2.87^{a}	63.16±6.08^{b}	53.16±10.20^{a}	53.50±3.61^{a}	52.66±3.66^{a}	50.33±2.25^{a}
IL-1β (pg/mL)	226.16±33.03^{a}	319.85±52.58^{c}	311.92±56.61^{bc}	239.50±55.72^{ab}	240.61±25.29^{ab}	208.85±29.98^{a}
IL-6 (pg/mL)	1.26±0.12^{a}	1.64±0.22^{c}	1.46±0.22^{ab}	1.30±0.07^{a}	1.27±0.09^{a}	1.19±0.13^{a}
TNF-α (pg/mL)	57.92±2.33^{a}	118.27±12.98^{c}	86.02±5.52^{bc}	66.33±5.04^{ab}	58.33±19.71^{a}	56.22±21.77^{a}

[The mean ± standard deviation is used to express the data. A statistically significant difference (P <0.05) between groups is indicated by different superscripts (a, b and c) in the same row. Gr. I: Control; corn oil; Gr. II: cypermethrin 25 mg/kg/day; Gr. III & IV: cypermethrin plus chrysin 50 and 100 mg/kg/day, respectively; Gr. VI & VI: chrysin 50 & 100 mg/kg/day, respectively]

hand, MDA and NO levels of the group exposed to cypermethrin (Gr. II) were significantly higher than the control group, but GSH levels were lower (P <0.05). SOD, CAT and GSH-Px activities were shown to be significantly reduced compared to the control group (P <0.05). In the groups receiving chrysin and cypermethrin (Gr. III & IV), the values in all parameters were close to the control group, but in Gr. III, MDA and GSH-Px level/activity were quite different from the control group (Table 2).

Kidney tissue

MDA, NO and GSH levels in the kidney tissue of the chrysin applied groups (Gr. V & VI) did not show any significant change compared to the control group. Similarly, no statistically significant difference was seen in SOD, CAT and GSH-Px activities. When the

Table 2 — Effect of chrysin (50 and 100 mg/kg) treatment on the levels/activities of MDA, NO, GSH, GSH-Px, CAT, SOD in the (A) liver; (B) kidney; (C) brain; (D) heart; (E) testes; and (F) lungs of cypermethrin (25 mg/kg) exposed rats

	MDA (μmol/g-protein)	NO (nmol/g-protein)	GSH (μmol/g-protein)	GSH-Px (nmol/dk/mg-protein)	CAT (katal/g-protein)	SOD (U/g-protein)
Liver						
Gr. I	1.63±0.06^{a}	0.48±0.92^{a}	9.54±0.32^{a}	115.88±10.79^{a}	216.41±15.93^{a}	36.71±1.18^{a}
Gr. II	2.52±0.00^{b}	0.74±0.04^{b}	7.33±0.28^{b}	70.73±9.03^{c}	155.32±9.54^{b}	27.18±0.32^{b}
Gr. III	2.30± 0.38^{b}	0.60±0.08^{ab}	8.22±1.09^{ab}	87.36±6.36^{bc}	184.79±16.74^{ab}	33.38±4.31^{ab}
Gr. IV	2.05± 0.01^{ab}	0.53±0.05^{a}	9.02±0.74^{ab}	106.34±9.97^{ab}	182.96±15.95^{ab}	32.27±1.54^{ab}
Gr. V	1.79± 0.12^{a}	0.56±0.05^{ab}	8.79±0.42^{ab}	113.77±3.46^{ab}	213.58±20.22^{ab}	35.64±3.40^{a}
Gr. VI	1.77± 0.10^{a}	0.55±0.05^{ab}	9.31±0.50^{a}	119.11±15.16^{a}	216.31±23.49^{a}	37.08±2.80^{a}
Kidney						
Gr. I	1.48±0.09^{a}	0.50±0.02^{a}	3.65±0.33^{a}	168.09±6.66^{a}	130.25±6.60^{a}	43.75±0.54^{a}
Gr. II	2.47±0.09^{c}	0.70±0.10^{b}	2.85±0.85^{a}	108.89±20.13^{b}	94.00±18.56^{a}	30.26±10.73^{b}
Gr. III	2.06±0.02^{a}	0.59±0.04^{ab}	3.01±0.49^{a}	150.08±14.13^{a}	124.63±27.27^{a}	39.44±1.61^{ab}
Gr. IV	1.99±0.05^{a}	0.52±0.04^{a}	3.76±0.54^{a}	152.52±10.18^{a}	111.76±32.04^{a}	39.52±2.13^{ab}
Gr. V	1.65±0.07^{a}	0.48±0.00^{a}	3.53±0.65^{a}	171.63±17.15^{a}	133.62±11.86^{a}	43.81±3.39^{a}
Gr. VI	1.69±0.10^{a}	0.49±0.02^{a}	3.38±0.18^{a}	166.59±16.02^{a}	137.07±7.94^{a}	46.04±1.00^{a}
Brain						
Gr. I	2.34±0.22^{a}	0.48±0.06 ^{a}	5.49±0.20^{a}	42.13±1.40^{a}	12.78±1.90^{a}	57.41±1.03^{ab}
Gr. II	4.23±0.45^{c}	0.55±0.05 ^{ab}	3.89±0.09^{b}	30.87±5.69^{b}	5.35±1.53^{b}	46.31±4.05^{a}
Gr. III	3.84±0.50^{bc}	0.69±0.06 ^{bc}	4.31±0.28^{b}	37.60±0.48^{ab}	7.84±1.96^{ab}	50.57±2.82^{ab}
Gr. IV	3.43±0.02^{ab}	0.54±0.02 ^{ab}	4.36±0.21^{b}	40.10±2.34^{a}	9.13±2.89^{ab}	50.84±1.45^{ab}
Gr. V	2.84±0.22^{ab}	0.78±0.09 ^{c}	4.71±0.62^{ab}	39.89±1.83^{a}	10.27±1.27^{ab}	56.61±7.25^{ab}
Gr. VI	2.54±0.65^{a}	0.57±0.07 ^{ab}	4.99±0.66^{ab}	41.92±2.38^{a}	10.37±2.93^{ab}	60.37±7.97^{b}
Heart						
Gr. I	1.60±0.15^{a}	0.52±0.01^{a}	6.18±0.61^{a}	139.52±5.89^{a}	13.96±1.51^{a}	44.72±3.24 ^{a}
Gr. II	2.94±0.17^{c}	0.79±0.02^{b}	3.99±0.20^{b}	106.51±4.96^{b}	6.62±2.41^{b}	34.00±3.76 ^{a}
Gr. III	2.46±0.33^{bc}	0.62±0.08^{a}	4.68±0.91^{ab}	111.41±11.48^{b}	11.52±1.44^{ab}	39.89±5.61^{a}
Gr. IV	2.45±0.23^{bc}	0.57±0.03^{a}	4.84±0.54^{ab}	115.56±6.18^{ab}	13.94±2.02^{a}	45.51±5.93 ^{a}
Gr. V	2.07±0.33^{ab}	0.52±0.04^{a}	4.93±0.52^{ab}	129.75±9.78^{ab}	13.93±2.32^{a}	42.65±3.78 ^{a}
Gr. VI	2.03±0.35^{ab}	0.49±0.08^{a}	5.29±1.32^{ab}	130.06±16.74^{ab}	12.16±3.00^{ab}	43.46±5.41 ^{a}
Testes						
Gr. I	2.16±0.23^{a}	0.51±0.03^{a}	4.18±0.32^{a}	85.09±3.05^{ab}	14.50±1.41^{a}	52.86±1.19^{a}
Gr. II	3.72±0.05^{c}	0.81±0.01^{b}	3.08±0.08^{b}	56.29±0.77^{c}	7.69±3.29^{b}	44.60±1.95^{a}
Gr. III	3.40±0.11^{c}	0.59±0.04^{a}	3.76±0.32^{a}	65.17±11.58^{bc}	8.90±1.37^{ab}	47.35±6.53^{a}
Gr. IV	2.79±0.00^{b}	0.53±0.08^{a}	3.40±0.43^{ab}	77.91±5.16^{ab}	9.35±1.24^{ab}	48.02±3.01^{a}
Gr. V	2.62±0.21^{ab}	0.52±0.03^{a}	3.79±0.72^{ab}	85.03±5.29^{ab}	11.27±1.46^{ab}	55.20±3.17^{a}
Gr. VI	2.39±0.26^{ab}	0.51±0.01 ^{a}	3.96±0.50^{a}	89.57±7.00^{ab}	12.19±2.71 ^{ab}	56.46±9.21^{a}
Lungs						
Gr. I	2.07±0.11^{a}	9.80±0.80^{ab}	8.81±0.46^{a}	98.62±2.11^{a}	13.93±2.15^{a}	47.39±3.12^{a}
Gr. II	3.49±0.05^{b}	17.07±1.84^{c}	5.92±0.12^{b}	60.80±5.86^{b}	7.44±3.66^{b}	39.66±2.15^{a}
Gr. III	3.09±0.07^{b}	11.88±0.11^{ab}	7.75±0.07^{a}	84.84±12.00^{a}	8.85±1.13^{ab}	44.19±2.72^{a}
Gr. IV	3.07±0.32^{b}	12.80±2.65^{b}	7.22±1.33^{ab}	85.87±7.35^{a}	8.30±1.89^{ab}	46.22±6.15^{a}
Gr. V	2.53±0.27^{a}	8.30±0.92^{a}	8.05±0.56^{a}	95.34±5.92^{a}	10.18±0.48^{ab}	48.44±0.47^{a}
Gr. VI	2.21±0.03^{a}	8.65±0.80^{a}	8.05±0.51^{a}	98.03±3.56^{a}	11.75±2.30^{ab}	47.14±3.42^{a}

[The mean ± standard deviation is used to express the data. A statistically significant difference (P <0.05) between groups is indicated by different superscripts (a, b and c) in the same column. Gr. I: Control; corn oil; Gr. II: cypermethrin 25 mg/kg/day; Gr. III & IV: cypermethrin plus chrysin 50 and 100 mg/kg/day, respectively; Gr. VI & VI: chrysin 50 & 100 mg/kg/day, respectively]

group given cypermethrin alone was compared with the control group, a significant increase in MDA and NO levels and a decrease in SOD and GSH-Px activity were observed (P <0.05). Although GSH level and CAT activity were lower than the control group, the difference was not statistically significant. It was observed that all evaluated parameters in the groups receiving cypermethrin and chrysin (Gr. III & IV) were close to the control level (Table 2).

Brain tissue

Measurements of antioxidant status or lipid peroxidation in brain tissue of the chrysin-treated groups were not significantly different from the findings of the control groups. However, there was a significant difference in NO levels in Gr. V and SOD activity in Gr. VI compared to the control (P <0.05). Compared with the control group, MDA levels were significantly increased and GSH levels were decreased in the cypermethrin-administered group (P <0.05). Similarly, GSH-Px and CAT activities in the same tissue also decreased (P <0.05). However, the difference in SOD activity and NO levels between the two groups was insignificant. In terms of values, the cypermethrin and chrysin applied groups (Gr. III & IV) were approaching the control group (P <0.05), but there were still significant differences in MDA, NO and GSH levels in Gr. III and GSH levels in Gr. IV (Table 2).

Heart tissue

In cardiac tissue, there was no statistically significant difference between the group of chrysin treated and the control group. Compared to the control group, the group exposed to cypermethrin (Gr II) showed significantly higher MDA and NO levels in addition to lower GSH levels (P <0.05). Apart from SOD activity, cardiac CAT and GSH-Px activities showed a significant decrease (P <0.05). In groups receiving chrysin and cypermethrin (Gr. III & IV), the GSH-Px activity and the MDA levels in Gr. III was significantly different from the control group (P <0.05). It was found that the data in the rest of the groups was similar to that of the control group (Table 2).

Testis tissue

There was no significant difference between the groups treated with chrysin alone and the control group in all parameters investigated in the testicular tissue. MDA and NO levels increased after the exposure of the cypermethrin, while GSH, CAT and GSH-Px level/activities decreased (P <0.05) whilst changes in SOD activity were insignificant. The GSH levels of the groups (Gr. III & IV) where the cypermethrin and the chrysin were given together, and NO, GSH, SOD, CAT and GSH-Px activities/ levels were similar to the control group, but MDA levels were different (P <0.05, Table 2).

Lung tissue

The groups treated with chrysin alone (Gr. V & VI) were similar to the lung MDA, GSH, GSH-Px, CAT and SOD values of the control group and there was also no statistical difference between the two groups mentioned. When compared with the control group, Gr. II, where the cypermethrin alone was administered, MDA and NO levels showed a statistically significant increasing trend (P <0.05). On the other hand, there was a statistically measurable difference, which was a decrease in GSH, CAT and GSH-Px activities/levels compared to control (P <0.05). The difference in SOD activity was statistically insignificant. The levels of MDA in the groups receiving co-administered cypermethrin and chrysin (Gr. III & IV) remained statistically significant (P <0.05). The data analyzed in the rest of the groups showed a pattern similar to that of the control group (Table 2).

Erythrocytes

In groups treated with chrysin (Gr. V & VI), there was a similarity in blood NO, GSH, GSH-Px, CAT and SOD activities/levels. GSH, CAT and GSH-PX levels/activities in the group given cypermethrin alone have decreased significantly, while NO level has increased (P <0.05). NO, CAT and GSH-Px activities/levels in both the cypermethrin and chrysin applied groups (Gr. III & IV) were close to the control group. On the contrary, GSH levels were statistically different in this group compared to the control group (P <0.05). In all groups, SOD activity was similar to that of the control group (Table 3).

Discussion

Chrysin

Flavonoids can prevent lipid peroxidation. They act as antioxidants by scavenging radicals such as superoxide, lipid peroxyl, hydroxyl and nitric oxide. The free hydroxyl groups of flavonoids enable them to scavenge free radicals, and especially the numereous hydroxyl groups in the B ring increase their antioxidant activity. The double bond between C2-C3 and the carbonyl group at the C4 atom in the chrysin structure contributes to its antioxidant

Table 3 — Effects of chrysin (50 and 100 mg/kg) treatment on the levels/activities of NO, GSH, GSHPx, CAT, SOD in erythrocytes/plasma of cypermethrin (25 mg/kg) exposed rats

	NO (nmol/g-Hb)	GSH (μmol/g-Hb)	GSH-Px (nmol/dk/mg-Hb)	CAT (katal/g-Hb)	SOD (U/g-Hb)
Gr. I	0.61±0.07^{a}	5.78±0.70^{a}	107.29±1.25^{a}	63.44±4.39^{a}	26.64±2.52^{a}
Gr. II	0.91±0.07^{b}	3.43±0.30^{b}	76.32±16.92^{b}	35.13±2.76^{b}	20.80±0.94^{a}
Gr. III	0.66±0.02^{a}	3.75±0.41^{b}	84.04±8.71^{ab}	46.30±6.50^{ab}	22.47±3.75^{a}
Gr. IV	0.63±0.07^{a}	3.94±0.19^{b}	91.66±8.21^{ab}	51.52±8.82^{ab}	26.85±3.61^{a}
Gr. V	0.61±0.03^{a}	4.51±0.41^{ab}	106.22±12.31^{a}	51.12±6.96^{ab}	25.96±2.20^{a}
Gr. VI	0.61±0.04^{a}	4.40±0.72^{ab}	106.41±6.04^{a}	65.47±14.62^{a}	28.44±5.06^{a}

[The mean ± standard deviation is used to express the data. A statistically significant difference (P <0.05) between groups is indicated by different superscripts (a, b and c) in the same column. Gr. I: Control; corn oil; Gr. II: cypermethrin 25 mg/kg/day; Gr. III & IV: cypermethrin plus chrysin 50 and 100 mg/kg/day, respectively; Gr. VI & VI: chrysin 50 & 100 mg/kg/day, respectively]

properties. The -OH groups at the C5 and C7 atoms in the structure of chrysin are related to its potential to scavenge free oxygen radicals[23]. Compared to the control group, oxidative stress parameters (MDA, NO, GSH, SOD, CAT and GSH-Px), some biochemical variable (AST and ALT) and inflammation-proinflammation markers (IL-1β, IL-6, TNF- α) were observed in the chrysin alone groups. The fact that no significant change was observed in all parameters shows that chrysin applied at two dose levels (50 and 100 mg/kg body wt.) for the specified period did not cause a negative effect on the biological systems of rats. According to these findings, it was understood that chrysin does not pose an individual risk in terms of lipid peroxidation or oxidative stress, as it does not change the antioxidant defense system at the specified doses and duration. In previous studies conducted on rats and other experimental animals, it was determined also that it did not cause significant changes in oxidative stress parameters, biochemical parameters and inflammation parameters in blood/tissue[13,24,25]. In this respect, the study data overlaps with previous studies.

Cypermethrin

Due to its lipophilic structure, cypermethrin easily passes through the cell lipid bilayer and is found in higher concentrations in tissues such as adipose tissue, kidneys, liver, ovaries, adrenal glands and skin[1,3]. Pesticide accumulation in tissues has been associated with reactive oxygen species (ROS) production and oxidative stress[10]. As part of the metabolic processes, living cells produce ROS. High levels of ROS and reactive nitrogen species (RNS) lead to harmful interactions with cell components such as lipids, proteins, and DNA. Excessive formation of hydroxyl radicals and peroxynitrite causes lipid peroxidation, which damages cell membranes and lipoproteins. MDA, a lipid peroxidation marker, is one of the secondary oxidation products of lipid peroxidation. NO, another lipid peroxidation marker and RNS, interacts with superoxide (O_2) radical to form peroxynitrite ($ONOO^-$), which is responsible for its toxic effect[3]. In cells, glutathione is present in reduced (GSH) and oxidized (GSSG) forms. GSH is a critical component of metabolic defensive processes such as free radical quenching, hydroperoxide reduction, and xenobiotic detoxification. The GSH-dependent antioxidant system is comprised of GSH and functionally related enzymes such as GST, GSH-Px and GR. GSH-Px reduces hydroperoxides and H_2O_2, whereas GSH is oxidized to GSSG. Then, GR regenerates GSH from GSSG. The study revealed decreased GSHPx activity and GSH levels in all tissues of rats exposed to cypermethrin. The reduction in GSH contents encourages oxidative stress and lipid peroxidation[26]. Other researchers have verified the same findings[9,12,14]. The increase observed in MDA and NO (liver, kidney, heart, testicle, lung and plasma) levels in the blood/tissues of the group administered cypermethrin alone and, on the other hand, the decrease observed in GSH levels (liver, brain, heart, testicle, lung and plasma) indicate that cypermethrin has formed radicals at high levels that the physiological system cannot compensate. Ali *et al*.[27] suggested that the increase in the MDA level of the liver in rats may cause membrane lipid peroxidation by easily penetrating the cell membrane as a result of the lipophilic nature of cypermethrin. In addition, they reported that cypermethrin administration increased NO levels in rats by increasing inducible NOS (iNOS) and total NOS (T-NOS) concentrations[27]. Arafa *et al*.[28] showed that exposure to cypermethrin (14.5 mg/kg body wt.) causes a decrease in membrane fluidity and therefore increases lipid peroxidation, which results in an increase in MDA levels in lung tissue in rats. Similar to our study, Tekeli[12] found that

blood, liver, kidney, and heart tissue GSH levels decreased in rat exposed to cypermethrin (25 mg/kg). He stated that GSH is depleted during the detoxification of reactive toxic metabolites of cypermethrin[12].

Antioxidant enzymes are proteins that catalyze the conversion of ROS and their byproducts into stable, harmless compounds. They are the most significant defence against oxidative stress-induced cell damage. Superoxide radicals are scavenged by SOD, while hydrogen peroxide radicals are neutralized by CAT and GSH-Px[3]. When compared to the control group, the significant decreases observed in blood/tissue GSH-Px (liver, kidney, brain, heart, testicle, lung and erythrocyte), CAT (liver, brain, heart, testicle, lung, erythrocyte) and SOD (liver) activities, which are antioxidant enzymes, indicate that antioxidant enzyme changes against cypermethrin at the blood/tissue level are not similar, that is, the response of the antioxidant enzyme system to cypermethrin is different in each tissue, but when evaluated in general terms, increase MDA and NO level and the decrease in GSH level reveal that the antioxidant enzymes examined are not fully sufficient to convert the free radicals caused/generated by cypermethrin or its methabolites into less toxic or non-toxic metabolites. The considerable decrease in CAT activity in all tissues except kidneys observed in this study can be attributed to the excessive amount of H_2O_2 produced due to oxidative stress caused by cypermethrin. The decreased SOD activity in the erythrocytes, liver, and kidneys of the cypermethrin-exposed rats may be the result of its enhanced degradation and decreased production as a result of the increased oxidative stress. On the other word, the decline in SOD activities can be associated with the use of superoxide radicals during conversion to H_2O_2. Due to GSH's role as an antioxidant in inhibiting free radical reactions in the tissues, it is possible that the decrease in GSH-Px activity seen in the study was caused by GSH depletion. It may also be associated with the breakdown of high levels of H_2O_2. Gebicka & Krych-Madej[29] reported that superoxide radicals can inhibit CAT activity and increased H_2O_2 resulting from CAT inhibition can inhibit SOD activity. In animals exposed to cypermethrin, similar results have been observed[9,10,12,30]. Afolabi *et al.*[10] reported that the decrease in liver and kidney SOD activity in rats may have resulted from the depletion of SOD activity due to the accumulation of superoxide radicals and H_2O_2. In addition, the researchers stated that the decrease in CAT activity means that more H_2O_2 is converted to hydroxyl radicals via a Fenton-mediated reaction[10]. The enzymes AST and ALT indicate liver damage when they are found in high concentrations in the serum[31]. A considerable increase in serum AST and ALT activity, markers of liver damage, is seen in the current study when cypermethrin is administered. The potential release of these enzymes from the cytoplasm into the bloodstream after cellular damage and plasma membrane disruption may be the reason for this. The current study's findings are in agreement with those that have been reported in previous findings of a similar nature[9,14,27].

Cytokines can be categorized as either pro- or anti-inflammatory based on their function[32]. Pro-inflammatory cytokines including IL-1β, IL-6 and TNF-α promote inflammation, whereas anti-inflammatory cytokines including IL-10 suppress it. IL-10 prevents macrophages from producing IL-1β and IL-6[33]. Through the activation of redox-sensitive transcription factors like NF-κB, oxidative stress, which can be caused by toxins, is known to activate inflammatory responses. A variety of pro-inflammatory genes, including cytokines, are induced by NF-κB[34]. Our analysis revealed that cypermethrin elevates the levels of IL-1β, IL-6 and TNF-α in serum. The elevation in IL-1β, IL-6 and TNF-α levels in cypermethrin exposed rats suggests that cypermethrin exposure causes inflammation. Thus, in our investigation, the elevated serum levels of IL-1β, IL-6, and TNF-α may be explained by NF-kB activation caused by cypermethrin-induced oxidative stress[10,27]. Among the studies on the subject, Ashafaq *et al.*[35] found an increase in the levels of IL-6, IL-1β and TNF-α in rat to which they administered 50 mg/kg/ca cypermethrin compared to the control. Ileriturk *et al.*[11] administered cypermethrin to rats at a dose of 25 mg/kg body wt. for 28 days and observed that it increased the levels of IL 6, IL-1β, NF-κB, TNF-α and iNOS. Our study is similar to previous studies in terms of the results obtained.

Cypermethrin and Chrysin

Compared to the control group, it is understood that the statistical differences in MDA (liver, brain, heart, testicle, lung), NO (brain), GSH (brain, erythrocyte) levels in blood/tissues in the group administered chrysin at a dose of 50 mg/kg body wt./day along with cypermethrin, and in the levels of MDA (heart, testicle, lung) and GSH (erythrocyte) in blood/tissues in the group administered chrysin at a dose of 100 mg/kg

body wt./day, indicate that given for the same period of time and in the specified doses of chrysin was not fully effective against the exposure to cypermethrin, but it reduced the oxidative stress caused by cypermethrin. Accordingly, decreased MDA and NO levels are evidence that chrysin significantly inhibits lipid membrane damage. Chrysin increases GSH synthesis, suppresses free radical generation by inhibiting CYP4502E1, alcohol dehydrogenase and xanthine oxidase activity, binds the formed free radicals, converts the produced free radicals into less effective-ineffective metabolites by inducing antioxidant enzyme activation, inhibits lipid peroxidation and also has an anti-inflammatory effect[5]. Chrysin may be acting through one or more of these mechanisms, which is, by directly binding to cypermethrin, reducing the level of cypermethrin entering the systemic circulation, chelating the free radicals, and inhibiting the above-mentioned enzymes and limiting high free radical production. Chrysin can improve all parameters examined through one or more of the mechanisms listed above. In addition, limits the formation of free radicals caused also by cypermethrin with its anti-inflammatory effect. These findings support the findings of numerous other researchers. Tekeli *et al*.[36] revealed that chrysin (50 mg/kg) in rats exposed to propetamphos significantly reduced MDA and NO levels in the liver, kidney, brain, heart, and testis. Researchers reported that free radicals produced by propetamphos administration damage the lipid components of the cell membrane, and chrysin prevents lipid peroxidation by scavenging free radicals. Fatemi *et al*.[37] demonstrated that chrysin reduced MDA and NO levels in the liver of rats exposed to sodium arsenite. Treatment with chrysin, which also significantly increased levels of depleted antioxidant enzymes, prevented cellular GSH content decreases caused by cypermethrin in the liver, heart, testis, and lung tissue. Also noted by Ijaz *et al*.[24] and Tekeli *et al*.[25] was the increase in GSH storage as a result of chrysin applications. Ijaz *et al*.[24] observed that chrysin (50 mg/kg) increased renal GSH levels against arsenic-induced kidney damage in rats. Chrysin (50 mg/kg) increased the levels of GSH in the liver, kidney, brain, testis, lung, heart, and erythrocytes in a study by Tekeli *et al*.[25] that investigated the toxicity induced by emamectin benzoate.

Again, compared to the control, significant changes were observed only in GSH-Px (liver, heart) activity in the blood/tissues in the group administered chrysin at a dose of 50 mg/kg body wt., and that the antioxidant enzyme activities examined in the high dose chrysin applied group were close to the control group, indicating that chrysin supports the antioxidant enzyme defense system. Among the anti-inflammatory parameters, significant changes in the levels of IL-1β and TNF-α continued only in the group administered chrysin at a dose of 50 mg/kg body wt.. The primary mechanism underlying this effect of chrysin may be the binding of free radicals that cause oxidative stress and lipid peroxidation. Another mechanism includes the inhibition of essentially xanthine oxidase, which is responsible for producing free radicals[5]. As mentioned before, this effect regarding the change in antioxidant enzyme activities may have been accompanied by one or more of the mechanisms listed above. The current study was similar to previous research[13,37]. In this investigation, as in the previous studies, we also have demonstrated that administering chrysin to rats receiving cypermethrin decreased these hepatic enzyme aberrations (ALT). This suggests that chrysin maintains the structural integrity of membranes[25,36,37]. Our results are in line with Fatemi *et al*.[37], Ijaz *et al*.[24], Tekeli *et al*.[36] and Koc *et al*.[37], who observed that chrysin improves the activity of GSH-Px. Fatemi *et al*.[37] stated that chrysin increased GSH-Px activity in liver damage caused by sodium arsenite in rats. In rats with kidney damage induced by arsenic, Ijaz *et al*.[24] demonstrated that chrysin (50 mg/kg) improved GSH-Px activity.

Chrysin prevents NF-KB activation, which results in anti-inflammatory effects. Furthermore, by decreasing the inflammatory response in macrophages and monocytes, chrysin has the ability to suppress pro-inflammatory cytokines[38]. In the current investigation, chrysin therapy improved proinflammatory cytokine levels, including TNF-α, IL-1β and IL-6, in cypermethrin-induced rats in a dose-dependent manner. According to these findings, chrysin inhibits the pro-inflammatory cytokines, which may have anti-inflammatory effects. Koç *et al*.[13] applied chrysin to rats in sepsis with liposaccharide for 10 days at a dose of 50-100 mg/kg body wt., increasing interleukin-1 beta (IL-1β), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α) was found to reduce interleukin-6 (IL-6) levels. Akaras *et al*.[39] reported that chrysin, given at a dose of 25-50 mg/kg body wt. for 7 days in cadmium poisoned rats, showed an anti-inflammatory effect by

reducing the high levels of NF-κB, IL-1β, IL-6 and TNF-α in Cd-induced lung tissue. Tuncer *et al*.[40] applied chrysin at a dose of 25-50 mg/kg body wt. for 7 days in cadmium toxicity in rats and found a decrease in testis NF-κB, TNF-α, IL-1β, IL-6 levels compared to the cadmium-administered group. Ye *et al*.[41] administered chrysin to rats orally at doses of 25 and 50 mg/kg body wt./day for 35 days against cyclo-phosphamide, and observed a significant decrease in the increased levels of cardiac tumor TNF-α, IL1 β and IL-6. All these results were consistent with the study data.

Conclusion

The results obtained showed that cypermethrin 25 mg/kg/day for 10 days, showed lipid peroxidation/ oxidative stress parameters (MDA, NO, CAT, SOD, GSH-Px and GSH) in blood, brain, heart, kidney, liver, lung and testis caused significant changes (increase/decrease) in serum biochemical parameters (AST and ALT) and inflammation parameters (TNF-α, IL-1β and IL-6) compared to the control group. This study has demonstrated that oral administration of cypermethrin can make rats more susceptible to oxidative stress, weaken their antioxidant defences, and increase inflammation. In the groups where chrysin was applied at two dose levels, the values approached the control group values. As a result of its anti-inflammatory and free radical scavenging properties, chrysin treatment, however, demonstrated attenuation in all parameters associated with these cypermethrin-induced toxicities. This suggests that chrysin could be a good candidate as a food additive or supplement to provide protection against the adverse effects of cypermethrin exposure. In cases of poisoning, chrysin may be preferred along with other drugs used in basic treatment, both to accelerate the therapeutic response of the drugs (supportive treatment) and to limit their negative effects. On the other hand, it was determined that chrysin given alone in the specified doses and period did not have a negative effect on the parameters examined. However, further research is still needed to fully comprehend its exact mechanisms of action and the possibility of using it to alleviate pesticide toxicities.

Ethical statement

All the animal experiment protocols were done based on the guidelines of the Erciyes University Local Ethics Committee for Animal Research.

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Conflict of Interest

Authors declare no competing interests.

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