GÜSBD 2023; 12(2): 556 - 566 | **Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi** | **Araştırma Makalesi**
GUJHS 2023; 12(2): 556 - 566 | Gümüşhane University Journal of Health Sciences | Original Article

Effects of Quercetin on Cypermethrin-Induced Stomach Injury: The Role of Oxidative Stress, Inflammation, and Apoptosis

Kuersetin'in Sipermetrin Kaynaklı Mide Hasarı Üzerine Etkileri: Oksidatif Stres, Enflamasyon ve Apoptozun Rolü

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ABSTRACT

This study was conducted to investigate the effects of quercetin (QUE) on cypermethrin (CYP) induced gastrotoxicity in rats.

35 Sprague-Dawley rats were randomly divided into five groups, 7 in each group. In the study, 25 and 50 mg/kg QUE were administered orally 30 min after 25 mg/kg cypermethrin was administered to rats for 28 days. Oxidative stress, inflammation, ER stress, apoptosis and autophagy markers were biochemically analyzed in gastric tissues. Additionally, histological analysis was performed for microscopic evaluation of gastric tissue.

The results revealed that QUE prevented tissue damage by reducing CYP-induced lipid peroxidation (MDA) and increasing GSH, SOD, CAT and GPx activities. It also showed anti-inflammatory effect by suppressing inflammatory markers such as NF-κB, IL-1β, TNF-α, iNOS and COX-2. QUE administration down-regulated CYP-induced increased PERK, ATF6, Caspase-3 and Beclin-1 markers. In addition, administration of QUE ameliorated the pathological tissue damage in gastric tissue due to CYP. The data of this study show that Que suppresses CYP-induced gastric toxicity by reducing oxidative stress, inflammation, ER stress, apoptosis a autophagy.

Keywords: Cypermethrin, Gastrotoxicity, Oxidative Stress, Quercetin, Rat

ÖZ

Bu çalışma, ratlarda sipermetrin (CYP) kaynaklı gastrotoksisite üzerine kuersetin'in (QUE) etkilerini araştırmak için yapıldı.

35 adet Sprague Dawley ratı, her grupta 7 tane olacak şekilde rastgele beş gruba ayrıldı. Çalışmada, ratlara 28 gün boyunca 25 mg/kg sipermetrin uygulandıktan 30 dakika sonra oral olarak 25 ve 50 mg/kg QUE verildi. Mide dokularında oksidatif stres, inflamasyon, ER stres, apoptoz ve otofaji belirteçleri biyokimyasal olarak analiz edildi. Ayrıca mide dokusu mikroskobik değerlendirme için histolojik analiz yapıldı.

Sonuçlar, QUE'nin CYP kaynaklı lipit peroksidasyonunu (MDA) düşürerek ve GSH, SOD, KAT ve GPx aktivitelerini artırarak doku hasarını önlediğini ortaya koydu. Ayrıca NF-κB, IL-1β, TNF-α, iNOS ve COX-2 gibi inflamatuar belirteçleri baskılayarak anti-inflamatuar etki gösterdi. CYP kaynaklı artan PERK, ATF6, Kaspaz-3 ve Beklin-1 belirteçlerini QUE uygulanması aşağı regüle etti. Ek olarak, CYP'ye bağlı olarak oluşan mide dokusundaki patolojik doku hasarını QUE verilmesi iyileştirdi. Bu çalışmanın verileri Que nin oksidatif stresi, enflamasyonu, ER stresi, apoptozu ve otofajiyi iyileştirerek CYP kaynaklı mide toksisitesini baskıladığını göstermektedir.

Anahtar Kelimeler: Gastrotoksisite, Kuersetin, Oksidatif Stres, Rat, Sipermetrin

Ethical approval for the study was obtained from Atatürk University Animal Experiments Ethics Committe (Approval No: 2022–11/232).

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Geliş Tarihi / Received: 28.12.2022
Kabul Tarihi/Accepted: 15.06.2023

INTRODUCTION

Pesticides are critical to agriculture, forestry, the control of insects and pests. Pesticides have recently become the subject of intense research due to their important effects and properties.[1] Although it has contributions, especially in modern agriculture, long-term exposure and incorrect application can have bad results. The World Health Organization (WHO) statement revealed that more than 18.2 per 100,000 suffer from acute pesticide poisoning yearly.[2]

The use of cypermethrin (CYP), a type II synthetic pyrethroid from pesticides, has increased a lot and is included in the list of moderate pests in the statement published by WHO.[3] Uncontrolled and widespread use of these chemicals causes both to mix with water resources and penetrate agricultural products. CYP also harms aquatic organisms and is included in the food chain. The fact that CYP has entered the food chain is the main source of toxicity in the living things that consume these foods.[4] The fact that CYP can be rapidly absorbed from the gastrointestinal tract, by the placenta and simply by skin contact accelerates this toxicity.[5] Due to its lipophilic structure, CYP accumulates in body fat, skin, liver, kidneys, adrenal glands, ovaries and brain, and its effects on important organs such as the central nervous system, male reproductive system, liver and kidney have been demonstrated.[6, 7] Although these toxic effects of CYP in different tissues and organs have been reported, there are no studies on the effect and damage mechanism on the stomach, and this issue is still not sufficiently clarified. Among the causes of toxic effects caused by CYP, ROS production, mitochondrial dysfunction, nucleic acid damage in the cell, protein damage, lipid and cell membrane damage are at the forefront.[8, 9] Previous studies have revealed that cypermethrin causes oxidative stress, endoplasmic reticulum stress, autophagy, inflammatory and apoptotic effects, directly or indirectly.[10, 11]

The use of natural medicinal products is increasing to relieve the side effects of many toxic conditions and diseases. Natural compounds are widely used as antioxidants, anti-apoptotic and anti-inflammatory.[12,13] Quercetin (QUE,3,3',4',5,7 pentahydroxy-flavone) is one of the most effective antioxidants of the flavonoid family. It is found in cabbage, strawberries, broccoli, onions, cherries, red grape and tea. The anti-inflammatory and anti-apoptotic properties of this agent have been demonstrated before. It has also been reported to modulate ER stress-mediated by calcium dynamic dysregulation.[14-16]

Considering all this information, although the toxic effect of CYP on many organs has been demonstrated by experimental studies, its effect on the stomach is not fully known. Therefore, this study investigated the possible protective effects of QUE on CYP-induced gastric injury.

MATERIAL AND METHOD

Chemicals

QUE (CAS No: 849061–97–8), CYP (CAS No: 52315–07–8) and other chemicals used in the study are of analytical purity and were purchased from Sigma-Aldrich (St. Louis, MO).

Experimental Animals

35 male rats weighing 200-250 g, obtained from Atatürk University experimental animals center, were housed in appropriate laboratory conditions (12 hours night / 12 hours day and 24 °C temperature). During the experiment, the experimental animals were given sufficient (ad libitum) water and pellet feed. Thirty five male rats were randomly divided into five groups, 7 in each group. Animals were divided into 5 groups as control, QUE, CYP, CYP + QUE 25 and CYP + QUE 50. The doses of CYP and Que given were adjusted according to a previous study.[10]

The groups were designed as follows:

Control Group: Physiological saline was given orally for 28 days.

QUE Group: Rats in this group were given 50 mg/kg QUE orally for 28 days.

CYP Group: Rats were given 25 mg/kg CYP dissolved in corn oil for 28 days.

CYP + QUE 25 Group: Rats in this group were given 25 mg/kg QUE 30 min after 25 mg/kg CYP dissolved in corn oil was administered for 28 days.

CYP + QUE 50 Group: For gastric toxicity, 25 mg/kg CYP dissolved in corn oil was administered for 28 days and 50 mg/kg QUE was given 30 min later.

Animals were decapitated under mild sevoflurane anesthesia 24 h after QUE was administered. Gastric tissue and blood serum were stored for biochemical and histological analyses.

Biochemical Analysis

To make biochemical measurements, gastric tissues stored at -80 °C were powdered in nitrogen and diluted with 1.15% KCl. Then, MDA, GSH levels and SOD, CAT, GPx activities were measured from the supernatants of the centrifuged tissues, respectively. Total protein analysis was then calculated.[17-22]

RT-PCR Analysis

Total RNA isolation was performed from the stomach tissues of animals using hybridol reagent (HibriGen) according to the manufacturer's instructions. The concentrations of the RNAs obtained were measured in the NanoDrop device and the RNA concentrations were equalized according to the results obtained. In the next step, cDNAs were synthesized from total RNAs. For this, a cDNA synthesis kit (BIO-RAD, USA) was used and the process was carried out with the instructions given by the manufacturer. In the last step, the reaction was started by preparing cDNAs and the primers of the genes whose sequences are presented in Table 1, and a mixture with iTaq Universal SYBR® Green Supermix. The reaction was carried out in the ROTOR-GENE Q (Qiagen, Germany) instrument at the temperature cycles provided by the manufacturer. β-actin was used as the internal control. At the end of the period, the relative mRNA transcript levels of the genes were calculated using the CT values obtained from the device and the $2^{-deltadelatCT}$ method.[23]

Table 1. Primary Sequences

Gene	Sequences (5'-3')	Length (bp)	Accession no
NF-κB	F: AGTCCCGCCCCTTCTAAAAC R: CAATGGCCTCTGTGTAGCCC	106	NM_001276711.1
IL-1β	F: ATGGCAACTGTCCCTGAACT R: AGTGACACTGCCTTCCTGAA	197	NM_031512.2
TNF-α	F: CTCGAGTGACAAGCCCGTAG R: ATCTGCTGGTACCACCAGTT	139	NM_012675.3
iNOS	F: AGATCAATGCAGCTGTGCTC R:GGCTCGATCTGGTAGTAGTAGA	235	NM_012611.3
COX-2	F: AGGTTCTTCTGAGGAGAGAG R: CTCCACCGATGACCTGATAT	240	NM_017232.3
Caspase-3	F: ACTGGAATGTCAGCTCGCAA R: GCAGTAGTCGCCTCTGAAGA	270	NM_012922.2
Beclin-1	F: TCTCGTCAAGGCGTCACTTC R: CCATTCTTTAGGCCCCGACG	198	NM_053739.2
ATF-6	F: TCAACTCAGCACGTTCCTGA R: GACCAGTGACAGGCTTCTCT	130	NM_001107196.1
PERK	F: GATGCCGAGAATCATGGGAA R: AGATTCGAGAAGGGACTCCA	198	NM_031599.2
β-Actin	F: CAGCCTTCCTTCTTGGGTATG R: AGCTCAGTAACAGTCCGCCT	360	NM_031144.3

Histopathological Analysis

Stomach tissues obtained from rats were fixed in 10% formaldehyde for 24 h. It was then washed overnight in tap water to undergo the routine histological follow-up procedure. Then, dehydration was performed by passing it through graded alcohol sericin. After clearing with xylol and infiltration with paraffin, the blocks were prepared. Sections of 5 µm thickness were taken from the blocks by microton. Sections taken were stained with Hematoxylin-Eosin (H&E). The stained sections were examined and photographed using a Binocular Olympus Cx43 microscope.

Statistical Analysis

All values were expressed as mean ± SD. One-way analysis of variance (ANOVA) and the Tukey test was used to determine the difference and significance levels between the groups. (versiyon 20.0; SPSS, Chicago, IL). $p < 0.05$ was considered a significant difference.

Ethical Approval

The study was approved by Atatürk University Ethics Committee (11/232/2022).

RESULTS AND DISCUSSION

Evaluation of Oxidative Stress Parameters

The parameters used to show the oxidative stress level are shown in figure 1. MDA levels were measured to show lipid peroxidation. It was observed that there was a significant increase in MDA level in the CYP-given group compared to the control group ($p<0.05$). However, administration of QUE with CYP decreased the MDA level. It is also among the findings that QUE 50 mg/kg dose is more effective than QUE 25 mg/kg dose ($p<0.05$). However, it was determined that SOD, CAT, GPx activities were significantly decreased in the CYP applied group compared to the control group, and the activities of these enzymes were increased with the administration of QUE with CYP ($p < 0.05$). It was observed that GSH level, which is a non-enzymatic antioxidant, also decreased with CYP application, QUE supplementation increased GSH levels to levels close to the control group, and QUE 50 mg/kg dose was more effective than QUE 25 mg/kg dose ($p<0.05$).

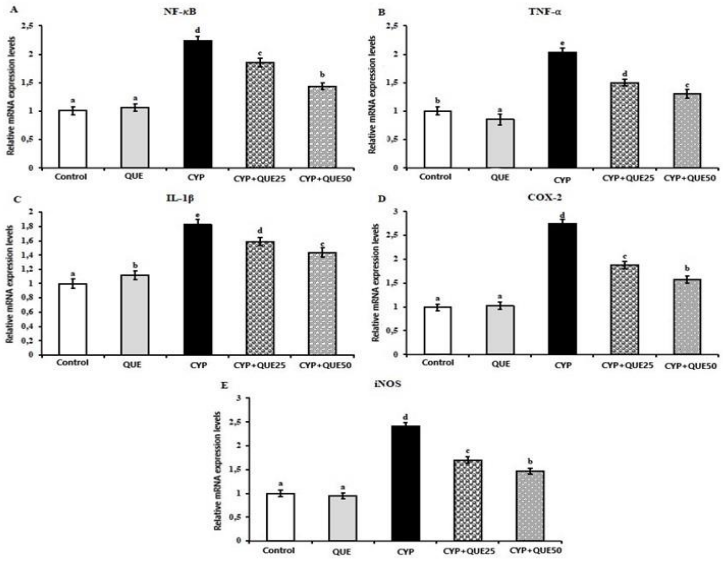

Figure 1. Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT), Glutathione (GSH) and Glutathione Peroxidase (GPX) Results of All Experimental Groups are Presented. a,b,c,d: There is a Statistically Significant Difference Between the Pillars Where the Same Symbols are Found in All Grafics. (p < 0.05).

Evaluation of Inflammation Parameters

NF-κB, IL-1β, TNF-α, iNOS and COX-2 mRNA transcript levels of factors and cytokines involved in inflammation were measured and given in figure 2. The levels of these markers showed a remarkable increase in the CYP-treated group compared to the control ($p<0.05$). NF-κB, TNF-α, IL-1β, iNOS and COX-2 mRNA expiration levels of the groups that were administered QUE with CYP were significantly decreased compared to the expression levels of the groups that were administered CYP. It was observed that QUE 50 mg/kg dose was more effective, especially with CYP ($p<0.05$).

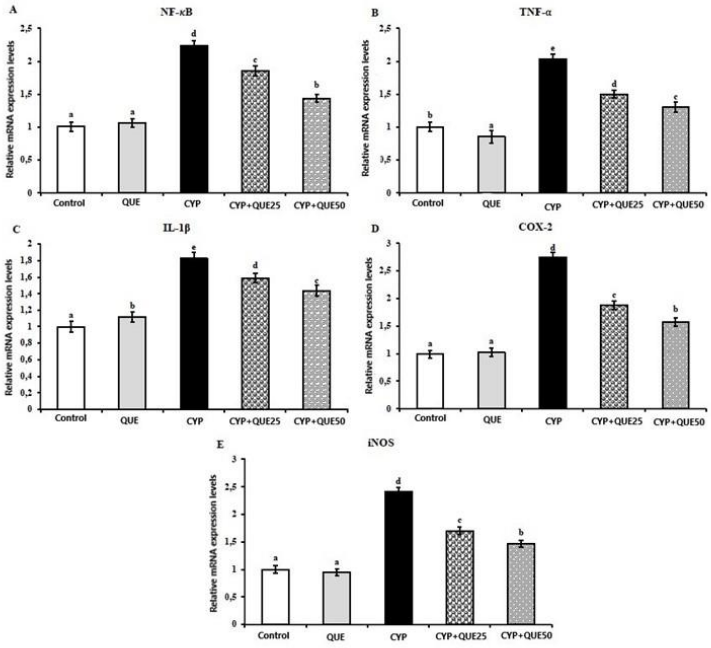

Figure 2. Effects of CYP and QUE Administrations on NF-κB, TNF-α, IL-1β, iNOS and COX-2 Activities in Stomach Tissue of Rats. NF-κB: Nuclear factor kappa-B, TNF-α: Tumor Necrosis Factor Alpha, IL-1β: Interleukin-1 beta, iNOS: Inducible Nitric Oxide Synthase, COX-2: Cyclooxygenase-2. a,b,c,d: There is a Statistically Significant Difference Between the Pillars Where the Same Symbols are Found in All Grafics. (p <0 .05).

Evaluation of ER Stress Results

The mRNA transcript levels of markers indicating ER stress are shown in figure 3. Our findings show that CYP exposure causes ER stress by increasing PERK and ATF6 mRNA transcript levels. It was determined that PERK and ATF6 levels in the groups given QUE and CYP together showed a significant decrease compared to the group in which CYP was administered ($p<0.05$). It was determined that 50 mg/kg dose of QUE with CYP down-regulated ATF6 level compared to 25 mg/kg dose of QUE ($p<0.05$).

Evaluation of Apoptosis and Autophagy Results

The mRNA transcript levels of Caspase-3 and Beclin-1 markers, which show apoptosis and autophagy in gastric tissue, are shown in figure 3. We observed that the expression of both Caspase-3 and Beclin-1 markers in the CYP-treated group was significantly increased compared with the control. However, co-administration of CYP and QUE decreased Caspase-3 and Beclin-1 levels depending on the dose increase.

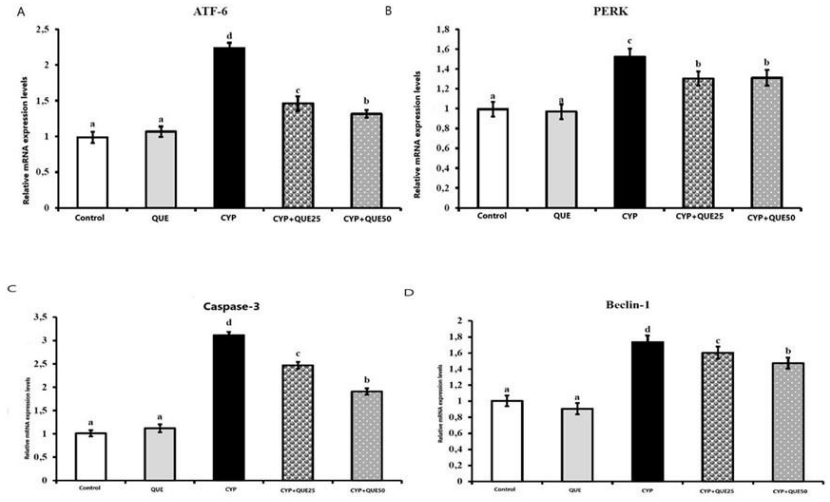

Figure 3. Effects of CYP and QUE Administrations on ATF-6, PERK, Caspase-3 and Beclin -1 Activities in Stomach Tissue of Rats. a,b,c,d: There is a Statistically Significant Difference Between the Pillars Where the Same Symbols are Found in All Grafics. (p < 0.05).

Evaluation of Histopathological Results

When the gastric tissues of the control group were examined under the light microscope, it was observed that the mucosa, submucosa, muscularis and serosa layers were in normal morphology. Additionally, the glands showed were normal morphology in the lamina propria, and the architectural structure of the parietal and principal cells showed a smooth appearance. (Fig. 4A). Rats treated with QUE alone had normal stomach morphology (Figure 4B). Shedding was observed in the surface epithelial cells of rat stomach tissues exposed to CYP toxicity. Dilatation of the gastric glands and lymphocyte cell infiltration in the submucosa were observed. Additionally, hemorrhagic areas were detected in the submucosal area (Figure 4.C). We observed that the pathological deteriorations in the stomach were reversed and they had a morphology close to the control with the application of QUE with CYP. Inflammatory cell infiltration and hemorrhagic areas were decreased in the gastric tissue of these groups. (Fig. 4.D,E). The results show that the concomitant administration of CYP and 50 mg/kg OUE has a more modulating effect against the pathological effects in the stomach induced by CYP. The careless use and worldwide spread of CYP, one of the pyrethroid insecticides, exceeds its main target and causes acute and chronic toxicity in many living species, including humans.[24,25] Studies conducted to investigate the mechanism of these effects have shown that CYP causes damage in different tissues by causing oxidative stress, inflammation and apoptosis. [6, 7, 26, 27]

However, the mechanism of action on gastric tissue is unclear. Considering this information, this study was conducted to investigate the potential protective effect of CYP on gastric damage caused by oxidative stress, inflammation, ER stress, apoptosis and autophagy. Studies in the literature suggest that ROS-mediated oxidative stress is under the toxic effect of pesticides.[26,27]

Oxidative stress occurs because of the disruption of the balance between the ROS and antioxidant systems. In studies, the degree of oxidative stress damage caused by CYP has been reported by looking at the accumulation of lipid peroxidation.[28,29]

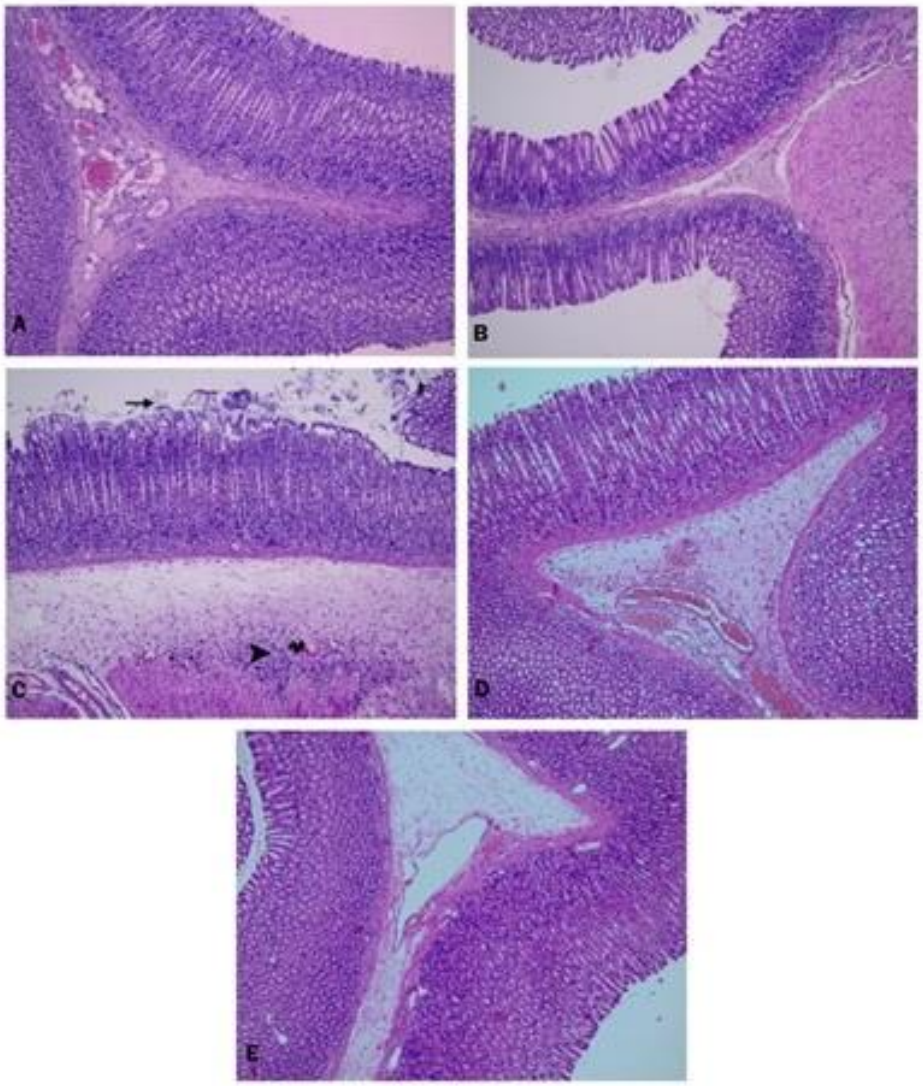

Figure 4. Photomicrographs of Histological Changes in Stomachs Tissue. (H&E Staining) A. Control Group, B. QUE (Quercetin) Group, C. CYP (Cypermethrin) Group; Arrow: Mucosal Epithelial Shedding, Star: Gastric Gland Damage, Arrowhead: İnflammatory Cell İnfiltration, Curved Arrow: Vessel, D. CYP +QUE 25 (Quercetin+ Cypermethrin 25) Group, E. CYP +QUE 50 (Quercetin+ Cypermethrin 50) Group

In a previous study by Ghorzi et al., it was reported that CYP increases MDA level and this marker can induce oxidative stress.[29] In this study, MDA, a product of lipid peroxidation, increased significantly in rats exposed to CYP and accelerated the formation of oxidative damage in the gastric tissue. The reason for the increase in lipid peroxidation is thought to be due to the lipophilic nature of CYP, which allows it to easily pass through the double lipid layer in the cell membrane and reduces membrane fluidity. Additionally, in our study, it was determined that lipid peroxidation decreased with the application of QUE after CYP application, and it was concluded that QUE slowed down peroxidation by showing a chain-breaking effect on lipid peroxidation. The most important defence mechanism against oxidative stress damage in the body is the antioxidant system. This antioxidant system includes enzymatic and non-enzymatic structures. SOD, CAT, and GPx are in the class of enzymatic antioxidants, while GSH is a nonenzymatic antioxidant. This antioxidant system, whose most

important effect is to scavenge free radicals or convert them into more harmless compounds, is the most important defence system in protecting organs and tissues against various chemical agents.[30, 31] In this study, it was observed that CYP exposure decreased the amount of SOD, CAT, GPx enzymes and GSH levels. While SOD, the first enzyme in the antioxidant defence system, converts superoxide radicals to H_2O_2, CAT catalyses the decomposition of H_2O_2 to H_2O.[32] The decrease in the SOD activity is manifested by its inability to scavenge superoxide radicals. GSH, one of the non-enzymatic antioxidants, plays a role in cellular defence by scavenging ROS and detoxifying xenobiotics. Additionally, GSH acts as a substrate for glutathione peroxidase. GPx is the peroxidase involved in the GSH-dependent detoxification of hydroperoxide.[7] In our study results, GPx decrease after CYP can be attributed to GSH depletion. This inference was also supported by previous studies showing that the reduction of GSH in rats exposed to pesticides would is related to the reduction of GSH-dependent enzymes.[7,10] In addition to these results, in the available data, it was thought that the application of QUE with CYP increased the antioxidant enzyme level and GSH amount to a level close to control, and this was due to the antioxidant effect of QEU. Additionally, it has been reported that the effect of QUE increases the antioxidant defence system by inhibiting the enzymes involved in the formation of ROS.[10] Ahmed et al. showed the antioxidant property of QUE in a similar study and reported that QUE protects the tissue from damage due to its antioxidant, anti-inflammatory and anti-apoptotic effects.[33]

Another effect of pesticides is that they trigger the inflammation process. Studies have associated the toxic effects of pesticides with activating proinflammatory pathways and stress signals of ROS.[10, 26] One of these signals, the NF-κB transcription factor, takes part in important physiological processes such as inflammatory responses, cell proliferation and cell death in the body. Additionally, it is known that NF-κB regulates the activation of pro-inflammatories such as TNF-α, IL-1β and iNOS, and COX-2, which is involved in the inflammatory response.[10, 34] Therefore, NF-kB examination is important in terms of treatment. In our study, NF-κB, TNF-α, IL-1β, iNOS and COX-2 mRNA transcript levels increased because of CYP application. In a previous study, it was revealed that CYP application activates NF-kB. It showed this effect by increasing proinflammatory cytokines such as IL-1β and TNF-α through the phosphorylation of IKKβ.[10, 35] In this study, NF-κB signals and TNF-α, IL-1β, iNOS and COX-2 mRNA transcript levels were decreased by the administration of QUE after CYP. With these results, it is thought that QUE functions as an immune regulator and modulates inflammation by interrupting the NF-kB pathway against inflammation caused by CYP.

The ER is an organelle that is critical to the biosynthesis and folding of proteins and in the balance of ca. Oxidative stress triggered by pesticides, inflammatory stimulation and mitochondrial dysfunction can cause ER stress. To detect ER stress, RNA-activated protein kinase (PKR)-like ER kinase (PERK) and activating transcription factor-6 (ATF-6) found in the ER membranes are special stress sensors.[10, 36] According to the results of our study, it was observed that oral administration of CYP in gastric tissue up-regulated ATF-6 and PERK genes and triggered ER stress. However, the administration of QUE suppressed the expression of these genes. Yardım et al. in their study, they found that QUE reduced Er stress, similar to our studies.[37]

Apoptotic factors activate the caspase cascade. One of the most important consequences of increased oxidative stress and prolonged ER stress conditions after CYP is cell apoptosis. Apoptosis is a common form of death in the cell, triggered by various stimuli such as cytokines, hormones, toxic attacks and viruses. The stimuli effective in the apoptosis process cause mitochondrial membrane deporilization and membrane permeability

deterioration. Apoptotic factors activate the caspase cascade.[36, 38, 39] Kandemir et al. reported that Caspase-3 is critical to the development of the apoptosis process and is a key step in this process.[36] In the presented study, it was determined that oral administration of CYP increased Caspase-3 activity, and administration of QUE together with CYP decreased the expression of these genes and reduced apoptosis. An in vitro study by Ileriturk et al. revealed that QUE reduces cell death and thus has a protective effect on mitochondrial membrane permeability and stability.[10]

Autophagy is the catabolic process involved in the lysosomal elimination of impaired organelles and misfolded proteins in the cell. autophagy; oxidative stress can increase significantly in conditions such as inflammation. Beclin 1, one of the important markers showing autophagy, has been studied in CYP studies before.[10,36] In this study, it was determined that CYP increased Beclin 1, and it was thought that autophagy caused this situation. We observed that the Beclin 1 level decreased with the administration of QUE after CYP. This proves that QUE can have an antiautophagic effect. In a previous study, it was reported that natural compounds reduce autophagy after CYP.[10]

Important organs in the body, such as the nervous and reproductive system, liver and kidney, are sensitive to pesticides. [6,7,40] In the gastrointestinal tract, it is among the organs sensitive to pesticides in the body. In this study, it was seen that our histopathological findings were compatible with our biochemical findings. According to your data, degenerative changes were detected in the gastric tissue after CYP administration. Particularly epithelial cells were lost in places. Additionally, dilatations were detected in the gastric glands. Hemorrhagic areas and lymphocyte infiltration were observed in the submucosa layer of the gastric. Similar findings were found in previous in vitro pesticide study models.[35, 40]

These results suggested that there may be tissue damage due to ROS accumulation under the pathological findings in the gastric tissue. When we examined the gastric tissue in the groups given CYP and QUE, surface epithelial cells and gastric glands were seen in normal morphology. Additionally, it was observed that lymphocyte infiltration in the submucosal area was decreased.

CONCLUSION AND RECOMENDATION

As a result, a negative effect of CYP administration on the gastric tissue was observed. This effect; evidenced by increased lipid peroxidation and proinflammatory cytokines, decreased antioxidants, ER stress and resulting cell death and degenerative changes in tissue. This study revealed that QUE has a ameliorate effect by reducing oxidative damage, inflammation, ER stress, and degenerative changes in the damaged gastric tissue. Our study is important in terms of providing a new perspective on protection from the toxic effects of pesticides, as well as supporting the use of QUE, which has antioxidant, anti-inflammatory and anti-apoptotic effects, as a support for treatments, it will probably be used in the clinic in the future

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