Research Article

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Effects of quercetin and hepatic damage by CYC

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Protective/preventive effects of quercetin against cyclophosphamide-induced hepatic inflammation, apoptosis and fibrosis in rats

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Abstract

Background and Aim: The purpose of this study was to investigate the hepatoprotective effects of quercetin, a potent antioxidant, against hepatotoxicity caused by cyclophosphamide (CYC) in the rat liver using histopathological parameters.

Materials and Methods: Thirty female rats were divided into five groups – control, quercetin (Q), CYC, Q+CYC, and CYC+Q. At the end of the study, the liver tissues were removed and stained with routine histological hematoxylin and eosin, Periodic acid-Schiff, and Masson's trichrome. Caspase-3 (Cas-3), B-cell lymphoma protein 2-associated X (Bax), tumor necrosis factor alpha (TNF-α), and interleukin 1 beta (IL-1β) levels were investigated in immunohistochemically stained liver tissues.

Results: Histopathological examination showed that CYC caused impairment and degeneration in the structure of the hepatocyte cordon, necrosis in the periportal space, sinusoidal dilatation (p=0.000), congestion and edema (p=0.000), mononuclear cell infiltration, and increased connective tissue density (p=0.000). Cas-3, Bax, TNF-α, and IL-1β immunoreactivities were significantly higher in the CYC group (for all, p=0.000). Q administration gradually reduced histopathological structural damage and Cas-3, Bax, TNF-α (p=0.000), and IL-1β (p=0.000) intensity in the rat liver.

Conclusion: The administration of Q protected the liver tissue against CYC-induced damage, and successfully protected the liver against apoptosis, inflammation, and histopathological changes.

Keywords: Apoptosis; cyclophosphamide; IL-1β; quercetin; rat; TNF-α.

Introduction

Cyclophosphamide (N,N-bis(2-chloroethyl) tetrahydro-2H-1, 3,2ox-azaphosphorine-2-amine 2-oxid; CYC) is a synthetic alkylating agent chemically related to nitrogen mustards. It is an antineoplastic and immunosuppressive agent that has been used in the treatment of several types of cancer, including solid tumors, systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis.[1–3] Despite its wide spectrum of application, the use of CYC in the clinical setting is frequently limited due to cytotoxicity and side-effects such as nausea, vomiting, alopecia, bone marrow suppression, hepatotoxicity, nephrotoxicity, urotoxicity, cardiotoxicity, immunotoxicity, mutagenicity, teratogenicity, and carcinogenicity that have been proved in human and animal studies.[3,4]

CYC is subjected to metabolic activation by the hepatic microsomal cytochrome P450 mixed function oxidase system to produce its two metabolites, phosphoramide mustard and acrolein, responsible for the induction of oxidative stress. These produce an alkylating effect on DNA cross-links and on DNA itself, thus causing cytotoxicity.[3,5,6] Acrolein is capable of binding to reduced glutathione (GSH) and can thus lead to overproduction of reactive oxygen species (ROS), followed by oxidative stress and lipid peroxidation.[7,8] Experimental evidence has shown that CYC causes lipid peroxidation and protein oxidation in the liver, oxidative stress being implicated in CYC hepatotoxicity.[9,10] Studies have also reported that CYC-induced histological damage in the liver is associated with alterations in enzyme activities.[4,11] Oxidative stress is regulated by cells' antioxidant mechanisms and triggers apoptotic cell death.[12] Improving chemotherapy tolerance against the toxic metabolites of CYC is an urgent problem. Very great importance is therefore attached to the investigation of agents capable of reducing side-effects without impairing drugs' main therapeutic effects.[13] Researchers have recently emphasized that biological compounds with antioxidant and anti-inflammatory characteristics can help protect cells and tissues against the deleterious effects of CYC-induced free radicals.[3,14]

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) (Q) is a plant flavonoid compound and member of the polyphenolic group found in numerous fruits and vegetables and[6,15] numerous pharmacological studies have reported that it exhibits potent antioxidant, anti-angiogenic, anti-inflammatory, neuroprotective, and anti-apoptotic activities.[16–18] It has also been suggested that due to its powerful antioxidant and anti-inflammatory activities, it can prevent diseases such as diabetes, cancer, and obesity. In addition to being a potent antioxidant and freer radical scavenger, Q has been described as more powerful than Vitamins E and C and other antioxidants that prevent lipid peroxidation.[18]

The purpose of this study was to investigate the preventive and protective properties against hepatic inflammation, apoptosis, and fibrosis of Q, a potent antioxidant and CYC-induced hepatotoxicity using histopathological parameters.

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Materials and Methods

Ethical procedures and animals

Thirty-six healthy female Wistar Albino rats (age 12–16 weeks, weight 300–400 g) were obtained from the Harran University (HRU) Experimental Animals Application and Research Center (HRU-HDAM), (Sanliurfa, Turkiye) for use in the study. The study commenced following receipt of approval from the HRU animal experiments local ethical committee (HADYEK) (study protocol license no. 2022/010/08). All rats were housed under standard laboratory conditions at 22±2°C, in 50% ±10 humidity and in a 12-h light:12-h dark cycle throughout the experiment. All rats were also given standard laboratory chow and ad libitum access to water during the experiment. All animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health.

Experimental Design

The 30 female Wistar Albino rats (age 12–16 weeks, weight 300–400 g) were randomly assigned to one of five groups:

Control group (n: 6): The pure control group exposed to no procedures.

Q group (n: 6): 100 mg/kg Q was administered per day for 5 days via the oral route (p.o.).[19]

CYC group (n: 6): This group received 200 mg/kg CYC through the intraperitoneal route (i.p.) on the 1st day of the experiment, followed by 8 mg/kg per day (total 14 doses).[20] At the end the 15th day, the animals were sacrificed by exsanguination.

Q + CYC group (n: 6): Q 100 mg/kg per day was administered p.o. for 5 days, followed by a first i.p. dose of CYC of 200 mg/kg CYC and maintenance doses of CYC of 8 mg/kg per day (total 14 doses). These animals were sacrificed by exsanguination on day 20.

CYC + Q group (n: 6): 200 mg/kg CYC was administered i.p. on the 1st day of the experiment, followed by CYC 8 mg/kg per day i.p. (total of 14 doses), and then by 100 mg/kg Q administration p.o. for 5 days. These animals were sacrificed by exsanguination at the end of the experiment (day 20).

Following sacrifice by exsanguination under general anesthesia at the conclusion of the experimental period, liver tissue specimens were collected for light microscopic examinations.

Histopathological Preparation and Evaluation of the Rat Liver

Liver tissues from rats in all the study groups were fixed in 10% of neutral formaldehyde solution for histopathological examination. These were then dehydrated and rendered transparent and embedded in paraffin blocks. Sections 5 µm in thickness were then taken from the paraffin blocks using a semi-automatic rotary microtome (Thermo Shandon Finesse ME+ Microtome, Runcorn, UK) and stained with hematoxylin and eosin (H&E), Periodic acid-Schiff (PAS), and Masson's trichrome (Trichrome Masson Stain Kit-Sigma Aldrich, Code: HT15-1KT, St. Louis, USA). All findings and evaluations were recorded onto a computer using a Zeiss Axioscope II (Carl Zeiss Microscopy GmbH, Göttingen, Germany) microscope and photographed with a Zeiss Axiocam MRc camera attachment (Carl Zeiss MicroImaging GmbH, Göttingen, Germany). Hepatic degeneration/regeneration in every microscopic specimen was evaluated based on the following criteria using morphometric and semiquantitative scoring: measurement of central vein diameter; the degrees of sinusoidal dilatation, hepatocyte degeneration, inflammatory cell infiltration, vacuolization and congestion, and fibrovascular area[7] were scored: normal=0, mild=1, moderate=2, and severe=3.

Immunohistochemistry Staining

Sections 5 µm in thickness were taken from the paraffin-embedded blocks and deparaffinized. After washing, they were next washed on PBS buffer solution for 5 min. The sections were then boiled in citrate buffer (pH: 6.0), and antibody retrieval was performed. The specimens washed in PBS were next subjected to peroxidase blocking in 3% H_2O_2 solution. Tumor necrosis factor-alpha (TNF-α) (Santa Cruz Biotechnology Inc., Heidelberg, Germany, cat no. sc-52746), interleukin 1 beta (IL-1β) (Santa Cruz Biotechnology Inc., Heidelberg, Germany, cat no. sc-52012) Caspase-3 (Cas-3) (Santa Cruz Biotechnology, Inc., Heidelberg, Germany, cat no. sc-56053), and Bax (Santa Cruz Biotechnology, Inc., Heidelberg, Germany, cat no. sc-7480) antibodies diluted to 1:100 were then dropped onto the specimens and left to incubate at +4°C. The subsequent procedures were performed using secondary antibody kits (Thermo Scientific, MA, USA, cat no. TP-060-HL), and al steps were carried out in line with the manufacturer's instructions. A 3,3'-Diaminobenzidine chromogen kit was employed (Sigma-Aldrich St. Louis, USA, cat no. D3939). The specimens were counterstained with Mayer's hematoxylin, covered with Entellan, and examined under a light microscope, and microphotographs were taken.[21] Three distinct areas were randomly selected in each section for immunohistochemical analyses. TNF-α and IL-1β positivity was defined as brown color and numerical evaluations were performed. Scores defined in terms of percentage frequency were used for TNF-α, IL-1β, Cas-3, and Bax expression in the area under examination: No expression (0), mild (1), moderate (2), powerful (3), and very powerful (4) expression. Positive cell percentages were scored <5% positive expression (0), 6%–15% (1), (16%–50% (2), 51%–80% (3), and >80% (4).[22]

Statistical Analysis

All statistical analyses were performed using Statistical Package for the Social Sciences (SPPS) version 24.0 (IBM SPSS Inc., Chicago, IL, USA). Mean (±) standard deviation (SD) was employed for morphological evaluations and immunohistochemical damage scores. Kruskal–Wallis H analysis of variance was applied for multiple one-way comparisons between groups. Dual comparisons between groups exhibiting significant values were evaluated using Tamhane's T2 test. Statistical significance was set at $p<0.05$ for all tests.

Results

Histopathology and Immunohistochemical Analyses

Histopathological examinations performed based on H&E, PAS, and Masson's trichrome staining revealed normal histological structures in liver tissues from the control and Q groups. In terms of morphology, hepatocytes were polygonal in shape, formed cords around the central vein were separated by normal sinusoidal spaces (Fig. 1a, b), the hepatocytes preserved their glycogen structure (Fig. 2a, b), and the portal triad was normal in appearance (Fig. 3a, b). Hepatocytes in liver tissue from the CYC group exhibited a heterochromatic structure, an impaired cord structure, necrosis in the periportal area, central vein/sinusoidal congestion, edema and dilatation, and widespread findings of mononuclear cell infiltration among the Remark cords and in the portal area (Fig. 1c-4). Hepatocyte glycogen stores

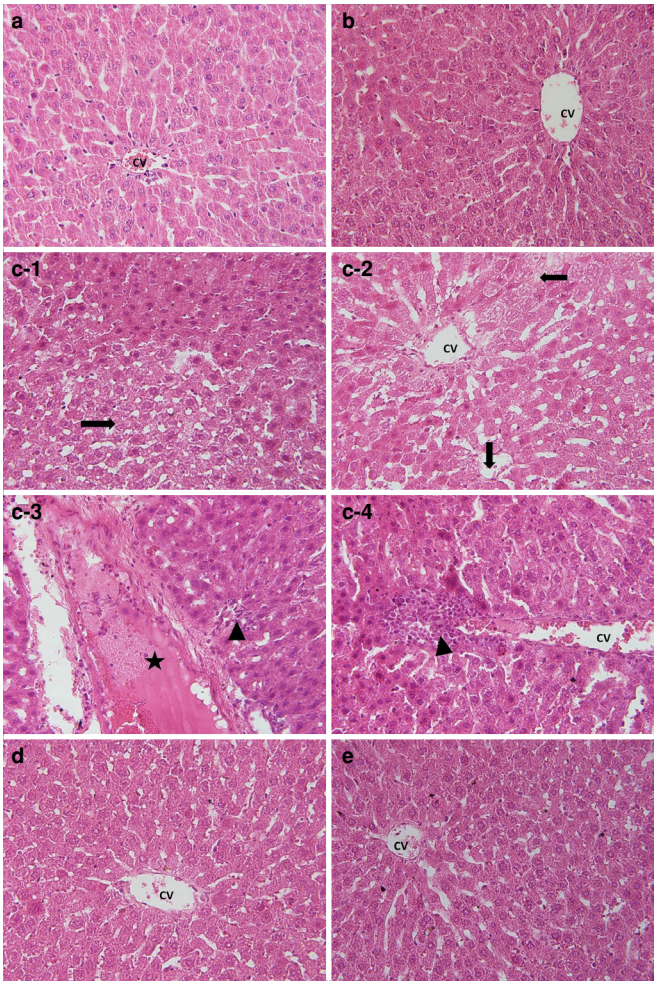

Figure 1. Photomicrographs depicting liver section of rat from different groups. **(a)** Control Group, **(b)** Q Group, **(c-1–4)** CYC Group, **(d)** Q+CYC Group, **(e)** CYC+Q Group, Separation and degeneration in the hepatocytes cordons (left arrow), leukocyte infiltration (arrowhead), necrosis (right arrow), congestion, edema (star), sinusoids dilatation (down arrow) and CV; Central ven, CYC; Cyclophosphamide, Q; Quercetin, (H&E 40x).

were depleted (Fig. 2c), and a marked increase in connective tissue was present in the portal area (Fig. 3c1-2). These histopathological damage findings decreased in the Q+CYC and CYC+Q treatment groups, although mild hepatocyte degeneration was detected in the Q+CYC group. The morphological structure of the CYC+Q group was close to that of the control group (Fig. 1d, e). The glycogen content of the hepatocytes was more pronounced compared to the CYC group, but was lower than in the control group, while the structure of the portal area was close to normal (Fig. 2d, e; Fig. 3d, e). The results of semiquantitative histopathological examination of the liver are shown in Table 1. Morphometric and semiquantitative scoring revealed significantly higher hepatocyte degeneration, vascular congestion, sinusoidal dilatation, infiltration, connective tissue density, and central vein diameter in the CYC group compared to the control group ($p<0.01$). Histological damage score findings in the Q+CYC and CYC+Q groups were significantly lower than in the CYC group ($p<0.01$). No significant difference was observed between the Q+-CYC and CYC+Q groups ($p>0.05$).

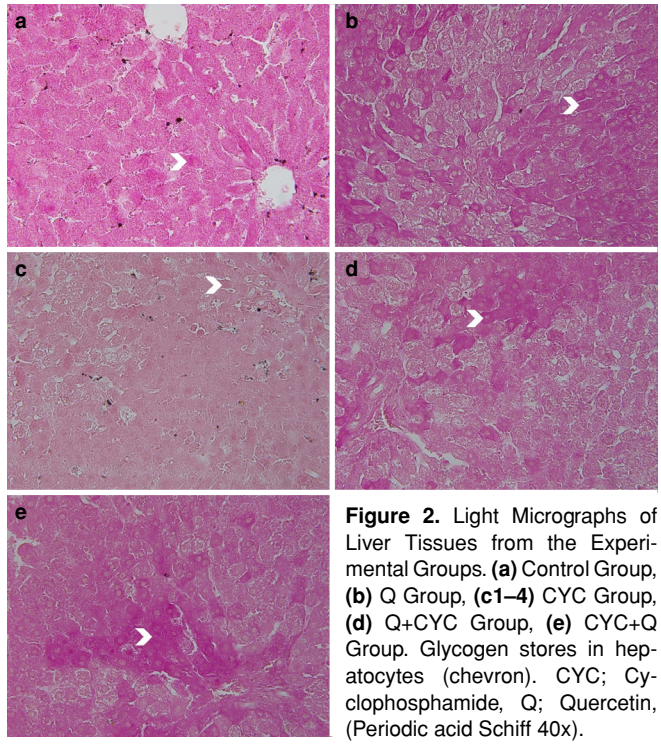

Figure 2. Light Micrographs of Liver Tissues from the Experimental Groups. **(a)** Control Group, **(b)** Q Group, **(c1–4)** CYC Group, **(d)** Q+CYC Group, **(e)** CYC+Q Group. Glycogen stores in hepatocytes (chevron). CYC; Cyclophosphamide, Q; Quercetin, (Periodic acid Schiff 40x).

Biomarkers of Inflammatory Cytokines and Apoptosis

Liver samples from the experimental groups were stained immunohistochemically to determine the intensity of the IL-1β, TNF-α, Bax, and Cas-3 antigens. The immunoreactivity scores are shown in Table 2. No IL- 1β, TNF-α, Bax, or Cas-3 immune positivity were observed in the control or Q groups ($p>0.05$) (Fig. 4a, b; Fig. 5a, b). Pro-inflammatory and apoptosis markers increased markedly in the CYC group compared to the control group (Fig. 4c; Fig. 5c) ($p<0.01$). Moreover, TNF-α, Bax, and Cas-3 intensities decreased significantly in the Q+CYC and CYC+Q group compared to the CYC group ($p<0.01$). There was no significant difference between the Q+CYC and CYC+Q groups ($p>0.05$) (Fig. 4d, e; Fig. 5d, e).

Discussion

The liver is one of the most vital organs in the body. Despite its high regenerative capacity in case of toxicity, it is defenseless in the face of severe toxicity, when severe hepatotoxic damage can develop.[23,24] Hepatotoxicity is the main reason for the US Food and Drug Administration refusing to approve drugs or withdrawing their approval.[25] The liver also plays a protective role in the pathogenesis of diseases and the detoxification of various chemicals and drugs.[26] Increased free radical production and oxidative stress can be induced during xenobiotic detoxification.[25] CYC is a cytostatic alkylating agent possessing broad antitumor activity, that is primarily metabolized in the liver to active metabolites, and that is chemically related to nitrogen mustards.[24,27,28] Studies have shown that excessive or long-term use of CYC can cause hepatotoxicity.[24,29] Due to the inescapable use of CYC in clinical treatment, improving tolerance to cytostatic chemotherapy is a matter of urgency, and it is therefore important to discover substances capable of reducing the effects of drugs without lowering their therapeutic effectiveness.[13,24] Q is a natural flavonoid widely present in several plants and vegetables. It possesses unmatched biological characteristics, including antioxidant,

Table 1. Hepatic histopathological damage scores in experimental rat groups

Groups	Hepatocyte degeneration Mean±SD	Dilatation Mean±SD	Congestion Mean±SD	Inflammatory cell infiltration Mean±SD	Fibrovascular area Mean±SD	Central vein diameter Mean±SD
Control	0.27±0.45	0.20±0.41	0.40±0.50	0.33±0.48	0.23±0.43	14.6±7.11
Q	0.23±0.43	0.27±0.45	0.20±0.41	0.43±0.50	0.40±0.50	18.24±6.74
CYC	2.40±0.50[a,b]	1.40±0.50[a,b]	1.50±0.51[a,b]	1.63±0.49[a]	1.80±0.41[a]	90.23±40.27[ab]
Q+CYC	0.60±0.50[b]	0.63±0.49[b]	0.83±0.38[b]	0.33±0.55[b]	0.50±0.51[b]	37.95±17.5[b]
CYC+Q	0.70±0.47[a,b]	0.40±0.50[b]	0.53±0.51[b]	0.20±0.41[b]	0.50±0.57[b]	27.82±14.92[b]

SD: Standard deviation; CYC: Cyclophosphamide; Q: Quercetin; a: P<0.05 compared to the control group; b: P<0.05 compared to the CYC group.

Table 2. TNF-α, IL-1β, Cas-3, and bax immunopositivity of experimental rat groups

Groups	TNF-α Mean±SD	IL-1β Mean±SD	Cas-3 Mean±SD	Bax Mean±SD
Control	0.40±0.50	0.33±0.48	0.37±0.49	0.20±0.41
Q	0.43±0.57	0.37±0.49	0.20±0.41	0.40±0.50
CYC	1.80±0.66[a]	1.57±0.57[a]	1.43±0.73[a]	1.57±0.50[a]
Q+CYC	0.80±0.71[b]	0.70±0.70[b]	0.70±0.47[b]	0.73±0.45[a,b]
CYC+Q	0.70±0.65[b]	0.60±0.56[b]	0.60±0.50[b]	0.53±0.51[b]

SD: Standard deviation; CYC: Cyclophosphamide; Q: Quercetin; a: P<0.05 compared to the control group; b: P<0.05 compared to the CYC group.

anti-inflammatory, anti-carcinogenic, and antiviral properties. It also represents the basis of potential benefits to overall health and resistance to disease, including its capacity to stimulate mitochondrial biogenesis.[30,31] The essential aim of this study was to seek to understand the therapeutic effect of Q against CYC-induced liver damage.

Cengiz et al.[1] reported that histopathological examination revealed that exposure to CYC caused shrinkage, opacity, irregularity around the hepatocyte nucleus, and dark staining due to increased eosinophilia in cytoplasm. Senthilkumar et al.[11] observed widespread edema and sinusoidal narrowing in the liver tissues of rats treated with CYC. Ayhancı et al.[32] reported parallel findings in their own histopathological analyses. Basu et al.[33] observed findings of severe hepatocellular swelling, expansion of the central vein, inflammatory cell infiltration, fatty degeneration, and vacuolization in the hepatic histology of mice treated with CYC. In agreement with previous studies,[1,24,33,34] in terms of hepatic histopathology in the present study, CYC impaired hepatocyte cell membrane integrity, causing destruction of hepatic lobules, enlargement of the central vein, and accumulation of inflammatory cells. CYC-derived toxicities are believed to be essentially associated with the induction of oxidative stress through the formation of free radicals in normal tissues and organs.[35,36] The hepatic biotransformation of CYC to phosphoramide mustard and acrolein results in a high level of free radical formation.[4,33] Acrolein inhibits P-450 by alkylating sulfhydryl groups during this process. Acrolein is essentially metabolized by the rapid modification of GSH sulfhydryl groups (GSG) and gives rise to mercapturic acid which is expelled through urine. As a result of this mechanism, acrolein is reported to compromise the antioxidant defense system by directly increasing cellular oxidative stress.[36,37] The hepatic histoarchitecture was protected through the administration of 100 mg/kg Q in the present study, and its ability to mitigate liver damage was

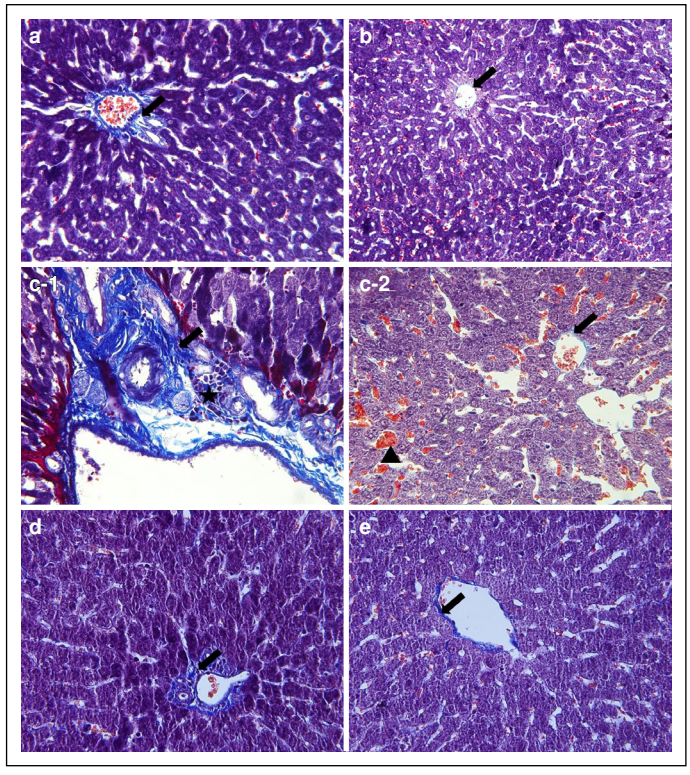

Figure 3. Light Micrographs of Liver Tissues from the Experimental Groups. **(a)** Control Group, **(b)** Q Group, **(c1–4)** CYC Group, **(d)** Q+CYC Group, **(e)** CYC+Q Group. Connective tissue density in portal area (arrow), leukocyte infiltration (arrowhead), sinusoidal dilations and congestion (star). CYC; Cyclophosphamide, Q; Quercetin, (Masson's Trichrome 40x).

more evident in the group receiving Q together with CYC. Q has been reported to possess antioxidant activity, to contain hydroxyl groups and double bonds that result in free radical scavenging, and to provide hepatoprotection.[38–40] In their study evaluating histological changes in rats exposed to lead poisoning, Liu et al.[41] reported that Q treatment significantly reduced histological changes in hepatocyte degeneration. Decreased histopathological damage has also been reported in a group given Q in methotrexate-induced liver damage scores.[42] The findings of the present study confirmed that CYC caused hepatic damage, resulting from CYC metabolites impairing the integrity of the hepatocyte membrane.[1] We concluded that the biological properties of Q may provide gradual protection of the morphological structure of the liver against the injury.

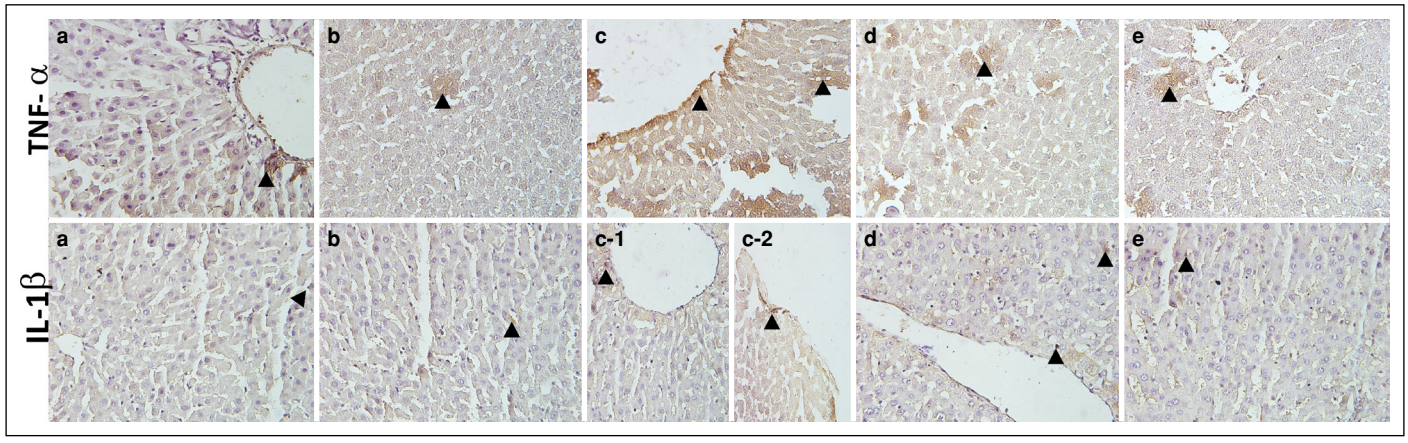

Figure 4. The immunohistochemical staining for Tumor Necrosis Factor (TNF-a) and Interleukin-1β (IL-1β) Expression in the liver samples of different study groups. **(a)** Control Group, **(b)** Q Group, **(c)** CYC Group, **(d)** Q+CYC Group, **(e)** CYC+Q Group. TNF-α and IL-1β positive immunostaining (arrowhead) (40x). CYC; Cyclophosphamide, Q; Quercetin.

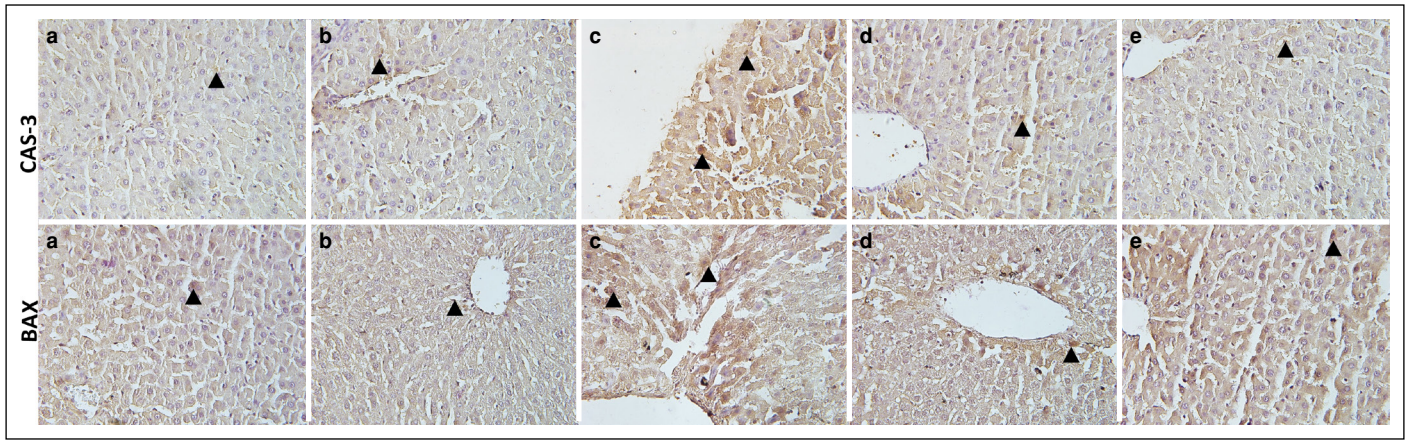

Figure 5. The immunohistochemical staining for Cas-3 and Bax Expression in the liver samples of all groups. **(a)** Control Group, **(b)** Q Group, **(c)** CYC Group, **(d)** Q+CYC Group, **(e)** CYC+Q Group. Cas-3 and Bax positive immunostaining (arrowhead) (40x). CYC; Cyclophosphamide, Q; Quercetin.

Cytokines play important roles in the development of cellular and humoral immune responses, in triggering inflammatory responses, in the regulation of hematopoiesis, in controlling cell proliferation and differentiation, and in initiating wound healing processes.[43] A wide spectrum of *in vivo* and *in vitro* studies has shown that CYC can cause an inflammatory response in various organs.[5,44] CYC has been reported to cause a tissue-wide inflammatory reaction with the upregulation of nuclear factor-κB (NF-κB) that leads to an increase in the production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1)[7,23] and to reduce anti-inflammatory IL-10 expression.[43] Shi et al.[5] reported increased levels of the pro-inflammatory cytokines TNF-a and IL-1β in liver tissue exposed to CYC, and a decrease in the levels of the anti-inflammatory cytokine IL-10. TNF-α is a pro-inflammatory cytokine primarily released by macrophages and monocytes, and several studies have emphasized significant increases in hepatic gene expression and protein following CYC application.[38,45] Another study of CYC-induced injury observed invasion by large numbers of leukocytes and necrotic areas in the parenchyma in terms of the histopathological manifestation.[23] Several studies have shown that Q reduced the secretion of pro-inflammatory cytokines such as TNF-α and IL-1β.[30,46] In terms of pro-inflammatory cytokines, the immunohistochemical findings of the present research were consistent with those of previous studies, with CYC-induced increased in TNF-a and IL-1β levels being observed. Q administration reduced the expression of TNF-a and IL-1β and may exhibit preventive/protective activity associated with an anti-inflammatory effect against CYC-related inflammation.

Another aspect of cytokines is that they indicate apoptosis in hepatic tissue through the upregulation of Cas-3 and downregulation of Bcl-2 on the apoptotic pathway.[23] The mitochondrial pathway of intracellular apoptosis is controlled by proteins from the Bcl-2 family including both hem anti-apoptotic (essentially Bcl-2) and pro-apoptotic (essentially Bax) factors. Mitochondrial external membrane integrity is preserved by Bcl-2, while membrane permeability increased by Bax releases apoptogenic factors that activate Cas-3 and Caspase-9 in the cytosol.[7,47] Cas-3, the principal driver of apoptosis, causes chromatin concentration, and protein and DNA fragmentation.[7] Studies have reported that CYC induces apoptosis in liver tissue.[37,48] Alqahtani and Mahmoud[45] investigated CYC-induced hepatocyte apoptosis with proapoptotic factors and reported an increase in Cas-3 and Bax gene and protein expression levels. The present research is consistent with previous studies, showing that CYC increased Bax and Cas-3 expression in hepatic tissue.

Research has emphasized that Q can prevent cell death by reducing Cas-3 activation. Yang et al.[49] showed that Q can prevent cell death by lowering Cas-3 activation in an ischemic brain damage model. Jia et al.[50] determined that Q restored cadmium-induced increases in Cas-3 and Bax activity and decreases in Bcl expression in granulosa cells. Those authors emphasized that Q is a powerful antioxidant with cytoprotective effects in preventing granulosa cell cytotoxicity caused by exposure to cadmium. The present study confirms these previous studies in the literature and showed that CYC increased Bax and Cas-3 expression in hepatic tissue, while the application of Q significantly reduced that apoptosis. Although this research shows that Q affects Cas-3 and Bax activation and may play an important role in the apoptotic process, further supporting studies are now needed.

Hepatic fibrosis is another outcome of hepatotoxicity. The etiology of fibrosis commences with acute hepatocyte injury under the effects of ROS. ROS, inflammation, and apoptosis begin releasing certain pro-fibrotic cytokines, such as TNF-α, that activate quiescent hepatic stellate cells and convert them into microfibroblasts.[51] The presence of a pyknotic nucleus, vacuolization and fatty changes (steatosis), and increased adipose tissue in the portal area were widely observed histological findings resulting from CYC administration in this study. However, Q reduced steatosis and adipose tissue intensity. On the basis of these findings, we think that CYC induces hepatotoxicity marked by inflammation, fibrosis, and apoptosis, while Q can protect the liver from severe fibrotic findings.

Conclusion

In conclusion, the three main players in CYC-induced hepatotoxicity, oxidative stress, apoptosis, and the cumulative impact of these lead to damage to the hepatocyte cell membrane and impairment of its histological structure. However, the application of Q exhibits protective/preventive effects against that damage through its antioxidant, anti-inflammatory, and antiapoptotic properties.

Ethics Committee Approval: The Harran University Animal Experiments Local Ethics Committee granted approval for this study (date: 29.12.2022, number: 2022/010/08).

Peer-review: Externally peer-reviewed.

Conflict of Interest: The author have no conflict of interest to declare.

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References

1. Cengiz M, Cetik Yildiz S, Demir C, Şahin İK, Teksoy Ö, Ayhanci A. Hepato-preventive and anti-apoptotic role of boric acid against liver injury induced by cyclophosphamide. J Trace Elem Med Biol 2019;53:1-7. [CrossRef]
2. Perini P, Calabrese M, Rinaldi L, Gallo P. The safety profile of cyclophosphamide in multiple sclerosis therapy. Expert Opin Drug Saf 2007;6(2):183-190. [CrossRef]
3. Zarei M, Shivanandappa T. Amelioration of cyclophosphamide-induced hepatotoxicity by the root extract of Decalepis hamiltonii in mice. Food Chem Toxicol 2013;57:179-184. [CrossRef]
4. Shokrzadeh M, Ahmadi A, Naghshvar F, Chabra A, Jafarinejhad M. Prophylactic efficacy of melatonin on cyclophosphamide-induced liver toxicity in mice. Biomed Res Int 2014;2014:470425. [CrossRef]
5. Shi L, Liu Y, Tan DH, Yan T-C, Song D-Q, Hou M-X, et al. Blueberry anthocyanins ameliorate cyclophosphamide-induced liver damage in rats by reducing inflammation and apoptosis. J Funct Foods 2014;11:71-81. [CrossRef]
6. Bao D, Wang J, Pang X, Liu H. Protective effect of quercetin against oxidative stress-induced cytotoxicity in rat pheochromocytoma (PC-12) cells. Molecules 2017;22(7):1122. [CrossRef]
7. Fouad AA, Qutub HO, Al-Melhim WN. Punicalagin alleviates hepatotoxicity in rats challenged with cyclophosphamide. Environ Toxicol Pharmacol 2016;45:158-162. [CrossRef]
8. Mohammad MK, Avila D, Zhang J, Barve S, Arteel G, McClain C, et al. Acrolein cytotoxicity in hepatocytes involves endoplasmic reticulum stress, mitochondrial dysfunction and oxidative stress. Toxicol Appl Pharmacol 2012;265(1):73-82. [CrossRef]
9. Selvakumar E, Prahalathan C, Mythili Y, Varalakshmi P. Mitigation of oxidative stress in cyclophosphamide-challenged hepatic tissue by DL-alpha-lipoic acid. Mol Cell Biochem 2005;272(1-2):179-185. [CrossRef]
10. Stankiewicz A, Skrzydlewska E, Makieła M. Effects of amifostine on liver oxidative stress caused by cyclophosphamide administration to rats. Drug Metabol Drug Interact 2002;19(2):67-82. [CrossRef]
11. Senthilkumar S, Devaki T, Manohar BM, Babu MS. Effect of squalene on cyclophosphamide-induced toxicity. Clin Chim Acta 2006;364(1-2):335-342.
12. Singh C, Prakash C, Tiwari KN, Mishra SK, Kumar V. Premna integrifolia ameliorates cyclophosphamide-induced hepatotoxicity by modulation of oxidative stress and apoptosis. Biomed Pharmacother 2018;107:634-643.
13. Molodykh OP, Sorokina IV, Vinogradova EV, Kapustina VI, Khodakov AA. Ultrastructure of the liver in response to cyclophosphamide and triterpenoids. Bull Exp Biol Med 2020;168(3):400-405. [CrossRef]
14. Jalali AS, Hasanzadeh S, Malekinejad H. Crataegus monogyna aqueous extract ameliorates cyclophosphamide-induced toxicity in rat testis: stereological evidences. Acta Med Iran 2012;50(1):1-8.
15. Waseem M, Parvez S. Neuroprotective activities of curcumin and quercetin with potential relevance to mitochondrial dysfunction induced by oxaliplatin. Protoplasma 2016;253(2):417-430. [CrossRef]
16. Wang J, Qian X, Gao Q, Lv C, Xu J, Jin H, et al. Quercetin increases the antioxidant capacity of the ovary in menopausal rats and in ovarian granulosa cell culture *in vitro*. J Ovarian Res 2018;11(1):51. [CrossRef]
17. Sekeroğlu V, Aydin B, Sekeroğlu ZA. Viscum album L. extract and quercetin reduce cyclophosphamide-induced cardiotoxicity, urotoxicity and genotoxicity in mice. Asian Pac J Cancer Prev 2011;12(11):2925-2931.
18. Smart E, Lopes F, Rice S, Nagy B, Anderson RA, Mitchell RT, et al. Chemotherapy drugs cyclophosphamide, cisplatin and doxorubicin induce germ cell loss in an *in vitro* model of the prepubertal testis. Sci Rep 2018;8(1):1773. [CrossRef]
19. Wu L, Wang C, Li J, Li S, Feng J, Liu T, et al. Hepatoprotective effect of quercetin via TRAF6/JNK pathway in acute hepatitis. Biomed Pharmacother 2017;96:1137-1146. [CrossRef]
20. Melekoglu R, Ciftci O, Eraslan S, Cetin A, Basak N. Beneficial effects of curcumin and capsaicin on cyclophosphamide-induced premature ovarian failure in a rat model. J Ovarian Res 2018;11(1):33. [CrossRef]
21. Seker U, Kaya S, Irtegun Kandemir S, Sener D, Unay Demirel O, Nergiz Y. Effects of black cumin seed oil on oxidative stress and expression of membrane-cytoskeleton linker proteins, radixin, and moesin in streptozotocin-induced diabetic rat liver. Hepatol Forum 2021;3(1):21-26. [CrossRef]
22. Kaymaz A, Ulas F, Erimsah S, Kara Oztabag C. Investigation of the effect of quercetin in an experimental oxygen-induced retinopathy model. Exp Biomed Res 2021;4(2):131-140. [CrossRef]
23. Abdelfattah-Hassan A, Shalaby SI, Khater SI, El-Shetry ES, Abd El Fadil H, Elsayed SA. Panax ginseng is superior to vitamin E as a hepatoprotector against cyclophosphamide-induced liver damage. Complement Ther Med 2019;46:95-102. [CrossRef]
24. Qian L, Yang F, Lin X, Jiang S, Zhang Y, Tang Y. Pyrroloquinoline quinone ameliorates liver injury in mice induced by cyclophosphamide. Environ Sci Pollut Res Int 2022;29(20):30383-30393. [CrossRef]
25. Fahmy SR, Amien AI, Abd-Elgleel FM, Elaskalany SM. Antihepatotoxic efficacy of Mangifera indica L. polysaccharides against cyclophosphamide in rats. Chem Biol Interact 2016;244:113-120. [CrossRef]

26. Malhi H, Gores GJ. Cellular and molecular mechanisms of liver injury. Gastroenterology 2008;134(6):1641-1654. [CrossRef]
27. Iqubal A, Iqubal MK, Sharma S, Ansari MA, Najmi AK, Ali SM, et al. Molecular mechanism involved in cyclophosphamide-induced cardiotoxicity: Old drug with a new vision. Life Sci 2019;218:112-131. [CrossRef]
28. Olayinka ET, Ore A, Ola OS, Adeyemo OA. Ameliorative effect of gallic acid on cyclophosphamide-induced oxidative injury and hepatic dysfunction in rats. Med Sci (Basel) 2015;3(3):78-92. [CrossRef]
29. Zhai J, Zhang F, Gao S, Chen L, Feng G, Yin J, et al. Schisandra chinensis extract decreases chloroacetaldehyde production in rats and attenuates cyclophosphamide toxicity in liver, kidney and brain. J Ethnopharmacol 2018;210:223-231. [CrossRef]
30. Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, et al. Quercetin, inflammation and immunity. Nutrients 2016;8(3):167. [CrossRef]
31. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: Food sources and bioavailability. Am J Clin Nutr 2004;79(5):727-747. [CrossRef]
32. Ayhancı A, Acar Ö, Şahintürk V, Güneş S, Kulcanay Şahin İ, Musmul A, et al. Selenium ameliorates cyclophosphamide-induced hepatotoxicity. Osmangazi J Med 2016;38(3):34-39. [CrossRef]
33. Basu A, Bhattacharjee A, Samanta A, Bhattacharya S. Prevention of cyclophosphamide-induced hepatotoxicity and genotoxicity: Effect of an L-cysteine based oxovanadium(IV) complex on oxidative stress and DNA damage. Environ Toxicol Pharmacol 2015;40(3):747-757. [CrossRef]
34. El-Naggar SA, Abdel-Farid IB, Germoush MO, Elgebaly HA, Alm-Eldeen AA. Efficacy of rosmarinus officinalis leaves extract against cyclophosphamide-induced hepatotoxicity. Pharm Biol 2016;54(10):2007-2016. [CrossRef]
35. Bhattacharjee A, Basu A, Ghosh P, Biswas J, Bhattacharya S. Protective effect of Selenium nanoparticle against cyclophosphamide induced hepatotoxicity and genotoxicity in Swiss albino mice. J Biomater Appl 2014;29(2):303-317. [CrossRef]
36. Ghosh P, Bhattacharjee A, Basu A, Singha Roy S, Bhattacharya S. Attenuation of cyclophosphamide-induced pulmonary toxicity in Swiss albino mice by naphthalimide-based organoselenium compound 2-(5-selenocyanatopentyl)-benzo[de]isoquinoline 1,3-dione. Pharm Biol 2015;53(4):524-532. [CrossRef]
37. Cuce G, Çetinkaya S, Koc T, Esen HH, Limandal C, Balcı T, et al. Chemoprotective effect of vitamin E in cyclophosphamide-induced hepatotoxicity in rats. Chem Biol Interact 2015;232:7-11. [CrossRef]
38. Sherif IO. The effect of natural antioxidants in cyclophosphamide-induced hepatotoxicity: Role of Nrf2/HO-1 pathway. Int Immunopharmacol 2018;61:29-36. [CrossRef]
39. Costa LG, Garrick JM, Roquè PJ, Pellacani C. Mechanisms of neuroprotection by quercetin: Counteracting oxidative stress and more. Oxid Med Cell Longev 2016;2016:2986796. [CrossRef]
40. Yoon JS, Chae MK, Lee SY, Lee EJ. Anti-inflammatory effect of quercetin in a whole orbital tissue culture of Graves' orbitopathy. Br J Ophthalmol 2012;96(8):1117-1121. [CrossRef]
41. Liu CM, Zheng YL, Lu J, Zhang ZF, Fan SH, Wu DM, et al. Quercetin protects rat liver against lead-induced oxidative stress and apoptosis. Environ Toxicol Pharmacol 2010;29(2):158-166. [CrossRef]
42. Vietrova KV, Zupanets IA, Sakharova TS. The hepatoprotective effect of the combination of glucosamine derivatives with quercetin against methotrexate-induced liver toxicity. Ceska Slov Farm 2020;69(5-6):222-229. [English]
43. Vilcek J. First demonstration of the role of TNF in the pathogenesis of disease. J Immunol 2008;181(1):5-6. [CrossRef]
44. Matar P, Rozados VR, Gervasoni SI, Scharovsky GO. Th2/Th1 switch induced by a single low dose of cyclophosphamide in a rat metastatic lymphoma model. Cancer Immunol Immunother 2002;50(11):588-596. [CrossRef]
45. Alqahtani S, Mahmoud AM. Gamma-glutamylcysteine ethyl ester protects against cyclophosphamide-induced liver injury and hematologic alterations via upregulation of pparγ and attenuation of oxidative stress, inflammation, and apoptosis. Oxid Med Cell Longev 2016;2016:4016209.
46. Porras D, Nistal E, Martínez-Flórez S, Pisonero-Vaquero S, Olcoz JL, Jover R, et al. Protective effect of quercetin on high-fat diet-induced non-alcoholic fatty liver disease in mice is mediated by modulating intestinal microbiota imbalance and related gut-liver axis activation. Free Radic Biol Med 2017;102:188-202. [CrossRef]
47. Tsamandas AC, Thomopoulos K, Zolota V, Kourelis T, Karatzas T, Ravazoula P, et al. Potential role of bcl-2 and bax mRNA and protein expression in chronic hepatitis type B and C: a clinicopathologic study. Mod Pathol 2003;16(12):1273-1288. [CrossRef]
48. Yaidikar L, Thakur S. Punicalagin attenuated cerebral ischemia-reperfusion insult via inhibition of proinflammatory cytokines, up-regulation of Bcl-2, down-regulation of Bax, and caspase-3. Mol Cell Biochem 2015;402(1-2):141-148. [CrossRef]
49. Yang R, Shen YJ, Chen M, Zhao JY, Chen SH, Zhang W, et al. Quercetin attenuates ischemia reperfusion injury by protecting the blood-brain barrier through Sirt1 in MCAO rats. J Asian Nat Prod Res 2022;24(3):278-289. [CrossRef]
50. Jia Y, Lin J, Mi Y, Zhang C. Quercetin attenuates cadmium-induced oxidative damage and apoptosis in granulosa cells from chicken ovarian follicles. Reprod Toxicol 2011;31(4):477-485. [CrossRef]
51. Iqubal A, Syed MA, Ali J, Najmi AK, Haque MM, Haque SE. Nerolidol protects the liver against cyclophosphamide-induced hepatic inflammation, apoptosis, and fibrosis via modulation of Nrf2, NF-κB p65, and caspase-3 signaling molecules in Swiss albino mice. Biofactors 2020;46(6):963-973. [CrossRef]