

Research Article



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The effect of krill oil on Wnt/ β -catenin signaling pathway in acetaminophen-induced acute liver injury in mice

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Abstract

Objectives: This study investigated the effect of krill oil (KO) on liver damage caused by acetaminophen (APAP).

Methods: In the present study, the control and APAP groups were given distilled water by gavage for 14 days. In addition, the KO and APAP+KO groups were given 500 mg/kg krill oil by gavage for 14 days. At the end of 14 days, 0.9 % sodium chloride solution (saline solution) administration was applied intraperitoneally to the control and KO groups. Meanwhile, 220 mg/kg acetaminophen was administered to the APAP and APAP+KO groups. While some biochemical

parameters in plasma were examined, some oxidative stress parameters in plasma and liver tissue were evaluated. Apoptotic and inflammatory responses of some primer sequences determined by quantitative Real-Time PCR (qPCR) in liver tissue. After histopathological examination of liver tissue, immunohistochemical analysis was performed with Wnt inhibitory factor-1 (Wif-1), beta-catenin (β -Catenin), and 8-hydroxy-2'-deoxyguanosine (8-OHdG).

Results: The Wif-1 positivity in hepatocytes increased significantly in the APAP group (5.29 ± 0.71) compared to the control (1.14 ± 0.51), and KO (2.14 ± 0.55) groups ($p < 0.001$). The 8-OHdG positivity in hepatocytes increased significantly in the APAP group (19.57 ± 0.58) compared to the control (0.43 ± 0.20), KO (3.57 ± 0.48), and APAP+KO (4.00 ± 2.53) groups ($p < 0.001$).

Conclusions: As a result, krill oil could be used as a nutritional supplement to protect the liver against acetaminophen-induced liver injury.

Keywords: acetaminophen; acute liver injury; krill oil; oxidative stress; qPCR.

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Introduction

Acetaminophen is one of the most preferred drugs as an antipyretic and analgesic agent worldwide [1]. Acetaminophen, also known as paracetamol, can cause acute liver damage due to hepatotoxicity due to a conscious or unconscious overdose [2]. In recent years, scientific studies have been carried out on protective substances such as herbal agents against liver damage caused by acetaminophen (paracetamol) [3].

Krill (*Euphausia Superba*), the tiny marine crustacean, is an essential source of nutrients, which include unsaturated fatty acids (omega-3), vitamins (A and E), minerals, astaxanthin, phospholipids and flavonoids such as eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). The krill oil obtained from this small crustacean can be beneficial in

preventing and treating asthma, hypertension, cardiovascular and inflammatory diseases [4, 5].

The liver constitutes several damages related to oxidative stress according to dosage and duration of exposure [6]. In particular, repetitive and high toxic doses excess regenerative capacity and, unfortunately, develop hepatic injuries [7, 8]. In the regeneration process, some growth factors, cytokines, and signaling have a potential role in hepatocyte growth, proliferation, and self-renewal. One of these pathways is the Wnt/ β -catenin pathway [9]. Oxidative stress is the deterioration of the reactive oxygen species (ROS)/antioxidant balance due to the increase and/or accumulation of ROS in cells. Due to this increase in ROS, oxidative damage in DNA, proteins and fats and as a result physiological and structural changes in cells occur. In particular, 8-OHdG can be used to determine the oxidative damage of DNA [10].

It may be necessary to use protective agents to help curb side effects due to overdose paracetamol use (patients who will use drugs with obligatory antipyretic and analgesic effects). For this reason, the protective effect of krill oil, which is used as a nutritional supplement, against liver damage caused by acetaminophen (paracetamol) has been investigated.

Materials and methods

Animal experimental protocol

The study was started after the approval of the Kırıkkale University Animal Experiments Ethics Committee (2020-41). 40 Balb/C mice (male, 30 ± 5 g, 6–8 weeks old) were used in the study. The mice were divided into four groups, with 10 mice in each. In the present study, the control and APAP groups were given distilled water by gavage for 14 days. In addition, the KO and APAP+KO groups were given 500 mg/kg krill oil (Krilom[®], Tabilaç, Türkiye) by gavage for 14 days [11]. At the end of 14 days, the control and KO groups were applied intraperitoneally to saline solution. Likewise, at the end of 14 days, the APAP and APAP+KO groups were applied intraperitoneally to 220 mg/kg acetaminophen (Partemol[®], Vem, Turkey). 12 hours after the last intraperitoneal administration, the mice blood (lithium heparin tube) was drawn from *vena cava caudalis* anaesthesia [90 mg/kg ketamine (Vetaketam[®], Vetagro, Poland), 10 mg/kg xylazine (Xylazimbio 2 %[®], Bioveta, Czech Republic), i.p.] after blood collection, and liver tissue was taken [12].

Biochemical analysis

Blood samples taken from the mice under anaesthesia were centrifuged (1,600 g, 4 °C, 10 min), and their plasma was separated. Plasma aspartate transaminase (AST) and alanine aminotransferase (ALT) enzyme activities, glucose (GLU), total cholesterol (TCHOL), and total protein (TP) levels were determined by a colorimetric method in a spectrophotometer device (Shimadzu, Japan) with the protocol of commercial kits (19205, 19210, 19200, 19160, and 19265 M kits catalogue numbers, respectively, Diaclinica, Turkey).

The liver tissue was cleaned off the blood and similar residues with distilled water, washed with cold saline solution, and dried with gauze. Before analysis, phosphate buffer (1/10, pH; 7.4) was added to liver tissue (approximately 0.5 g, wet tissue) and divided into small pieces. These pieces were first homogenized (10 s) on ice, kept for 30 s, and then homogenized again using a homogenizer (an ultrasonic, 10 s, five repetitions). Then, these homogenate tubes were centrifuged (10,000 g, 10 min) to obtain supernatants.

Using the method of Buege and Aust [13], malondialdehyde (MDA) levels in tissue supernatant and plasma were determined using a spectrophotometer (Shimadzu, Japan) at a wavelength of 536 nm. This method is based on the principle that thiobarbituric acid and MDA react to form a pink-coloured compound. Using the method of Erel [14], total antioxidant status (TAS) levels in tissue supernatant and plasma were determined in a microplate reader (Thermo Scientific[™] Multiskan, UK) using commercial test kits (RL0017, Rel Assay Diagnostics, Turkey). This method is based on reducing the coloured 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical to a colourless reduced form with the antioxidants in the blood and liver tissue samples.

Quantitative real-time PCR

RNA samples were isolated from liver tissue samples using the total RNA isolation kit (74106, Qiagen, Germany). Total RNA samples were measured at 260 and 280 nm using a nanodrop plate reader (Thermo Scientific Multiskan Go, USA), and concentration and purity values were determined by A_{260}/A_{280} ratio. The primer sequences are given in Table 1. cDNA samples were synthesized using a kit (#K1682, Thermo Scientific, USA). Each was quantified to 250 ng and used for amplifying the investigated genes using qPCR reactions as previously described [15].

Histopathological examinations

The liver tissues from mice were treated with graded alcohol, xylol series, and blocked-in paraffin (Leica, Germany). Sections (5 μ m) obtained from paraffin blocks were stained according to the hematoxylin-eosin (H&E) method [20]. These sections were evaluated under the microscope (Olympus, Japan), and images were taken.

Table 1: Primer sequences were used in the qPCR analyses.

Gene	Forward (F)/ Reverse (R)	Primer sequence (5'–3')	References
<i>Bcl-2</i>	F	CCTGTGGATGACTGAGTACCTG	[16]
	R	AGCCAGGAGAAATCAAACAGAGG	
<i>Caspase-3</i>	F	CCTCAGAGAGACATTCATGG	[17]
	R	GCAGTAGTCGCCTCTGAAGA	
<i>COX-1</i>	F	CACTGGTGGATGCCTTCTCT	[18]
	R	CCGTACAGCTCTCTCAACTC	
<i>COX-2</i>	F	CCCTGAAGCCGTACACATCA	[18]
	R	TGTCAGTGTAGAGGGCTTTCAATT	
<i>GAPDH</i>	F	CTGGGATGGAAATTGTGAGG	[19]
	R	TGGCCTCCAAGGAGTAAGAA	

Bcl-2, B-cell lymphoma-2; COX-1, cyclooxygenase 1; COX-2, cyclooxygenase 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Immunoperoxidase detection

Streptavidin-biotin complex peroxidase (Strept ABC-P) method was followed on salinized slides according to manual instruction in the peroxidase detection system (Novocastra RE7120-CE, Leica, Germany). Wif-1 (sc373780, Santa Cruz, USA), β -Catenin (sc7963, Santa Cruz, USA) and 8-OHdG (ab62623, Abcam, UK) were used as primary antibodies. Then, primary antibodies were dripped onto slides and incubated at 37 °C overnight. Phosphate-buffered saline was used in the negative control instead of primary antibodies. As a positive control, cross-reaction was shown using mice tissue. Diaminobenzidine was used as chromogen, and Gill's hematoxylin was used for the counterstain. All sections were covered with coverslips and a non-aqueous mounting medium (107961, Entellan, Merck, Germany).

Lesion scoring and statistical analysis

Data were given as arithmetic mean $\pm$ standard error (SE). Statistical analyses were performed using the SPSS program (PASW Statistics for Windows, ver. 18.0. Chicago, USA). For biochemical findings, One-Way ANOVA was used as the groups showed parametric distribution, and the Duncan test (post hoc) was used to control the significance of the difference between groups ($p < 0.05$).

Since the immunohistochemical findings did not show the parametric distribution in the groups, the Kruskal Wallis test and Mann-Whitney U test (Bonferroni adjustment; $p < 0.0083$) were used to control the significance of the difference between groups. Statistical analyses of qPCR results and graphs were performed using GraphPad Prism (GraphPad Software ver. 8.0, San Diego, CA, USA). Differences in the expression values were evaluated by the One-Way ANOVA and Tukey's tests. Tukey's tests (post hoc) were used to control the significance of the difference between groups ($p < 0.05$). The histopathological findings were scored semi-quantitatively from negative to strong counting % calculation from 10 High Power Fields (HPFs). (–): 0–10 %/10 HPFs, (+): mild 10–30 %/10 HPFs, (++) moderate 30–70 %/10 HPFs, (+++): strong >70 %/10 HPFs.

Results

Biochemical parameters

The plasma ALT, AST, GLU, TCHOL and TP results of the study groups are given in Table 2. The ALT enzyme activity of the APAP group was found to be significantly higher than the control, KO, APAP+KO groups ($p < 0.01$). Although the APAP group's AST, GLU, TCHOL, and TP parameters were higher than those of the control, KO, APAP+KO groups, no statistical significance was found ($p > 0.05$).

Oxidative stress parameters

The plasma and liver tissue oxidative stress parameters of the study groups are given in Table 3. There was no statistical difference in plasma MDA levels among the control, KO, APAP, and APAP+KO groups ($p > 0.05$). Although the TAS

Table 2: Plasma biochemical parameter levels of the study groups.

Groups	ALT, U/L	AST, U/L	GLU, mg/dL	TCHOL, mg/dL	TP, mg/dL
Control	8.32 $\pm$ 0.43 ^a	43.16 $\pm$ 2.21	259.70 $\pm$ 7.61	194.01 $\pm$ 7.50	4.39 $\pm$ 0.17
KO	8.27 $\pm$ 0.87 ^a	42.46 $\pm$ 1.73	236.88 $\pm$ 10.85	188.71 $\pm$ 12.73	4.06 $\pm$ 0.13
APAP	13.31 $\pm$ 1.36 ^b	47.65 $\pm$ 1.23	272.08 $\pm$ 9.84	202.58 $\pm$ 7.62	4.58 $\pm$ 0.14
APAP+KO	9.06 $\pm$ 1.52 ^a	42.86 $\pm$ 3.07	258.11 $\pm$ 6.19	196.31 $\pm$ 3.64	4.14 $\pm$ 0.19

^{a,b}The difference between values (mean $\pm$ SE) with different letters in the same column was statistically significant (^{a,b} $p < 0.01$). KO group, krill oil group; APAP group, acetaminophen group; APAP+KO, acetaminophen + krill oil group; ALT, alanine aminotransferase; AST, aspartate transaminase; GLU, glucose; TCHOL, total cholesterol; TP, total protein.

(plasma) level was higher in the KO group compared to the other groups, it was not statistically significant ($p > 0.05$). However, there was a statistically significant increase in MDA levels (liver tissue) in the KO, APAP, and APAP+KO groups compared to the control group ($p < 0.001$). In addition, there was an increase in liver tissue TAS levels in the KO, APAP, and APAP+KO groups compared to the control group ($p < 0.01$). Although there was no statistical difference, the liver tissue TAS level of the KO group was numerically higher than the APAP and APAP+KO groups ($p > 0.05$).

qPCR

Decreased Bcl-2, increased caspase-3 mRNA expression values in APAP-applied mice and raised COX-1 mRNA levels in KO-applied animals were determined by group means

Table 3: The liver tissue and plasma oxidative stress parameters of the control and experimental groups.

Groups	Liver tissue		Plasma	
	MDA, μ mol/g wet tissue	TAS, μ mol/g wet tissue	MDA, μ mol/L	TAS, mmol/L
Control	3.21 $\pm$ 0.32 ^b	0.59 $\pm$ 0.10 ^d	1.23 $\pm$ 0.03	0.53 $\pm$ 0.11
KO	5.40 $\pm$ 0.27 ^a	2.82 $\pm$ 0.72 ^c	1.23 $\pm$ 0.02	0.86 $\pm$ 0.14
APAP	6.87 $\pm$ 0.74 ^a	1.98 $\pm$ 0.27 ^c	1.26 $\pm$ 0.03	0.44 $\pm$ 0.11
APAP+KO	6.41 $\pm$ 0.61 ^a	2.59 $\pm$ 0.48 ^c	1.20 $\pm$ 0.06	0.43 $\pm$ 0.12

^{a,b}The difference between values (mean $\pm$ SE) with different letters in the same column was statistically significant (^{c,d} $p < 0.01$, ^{a,b} $p < 0.001$). KO group: krill oil group; APAP group, acetaminophen group; APAP+KO, acetaminophen + krill oil group; MDA, malondialdehyde; TAS, total antioxidant status.

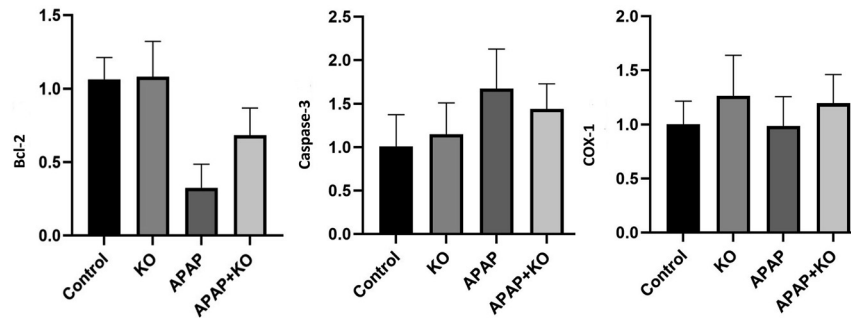


Figure 1: qPCR analysis results of the control, KO, APAP and APAP+KO groups. mRNA levels were normalized to the housekeeping GAPDH. Data are presented as the mean $\pm$ standard error (SE). There is no statistically significant difference between the groups ($p > 0.05$). KO group, krill oil group; APAP group, acetaminophen group; APAP+KO, acetaminophen + krill oil group; Bcl-2, B-cell lymphoma-2; COX-1, cyclooxygenase 1.

(Figure 1). However, all these alterations did not reveal a statistically significant level ($p > 0.05$). In addition, we could not detect COX-2 expression in studied liver tissues.

Histopathological findings

The histopathological findings of the study groups are given in Supplementary Table 1. In the APAP group, degenerative and necrotic results were highly elevated. Hepatocyte cytoplasm contained clear and nonstained vacuoles with a rough

edge. In many fields, hepatocyte nuclei were lost. Some of the nuclei were pyknotic and/or lytic in appearance. Such areas were observed at the periphery of necrotic areas, which had lost the liver microarchitecture. Kupffer cell activations were especially dense in such microscopic fields. Many portal and central veins, as well as sinusoids, were enlarged with erythrocytes. In some portal areas and sinusoids, inflammatory cells, including lymphocytes and macrophages, were encountered. In the APAP+KO group, the aforementioned degeneration and necrosis were not distributed at every lobule. In some hepatocytes founding paracentral

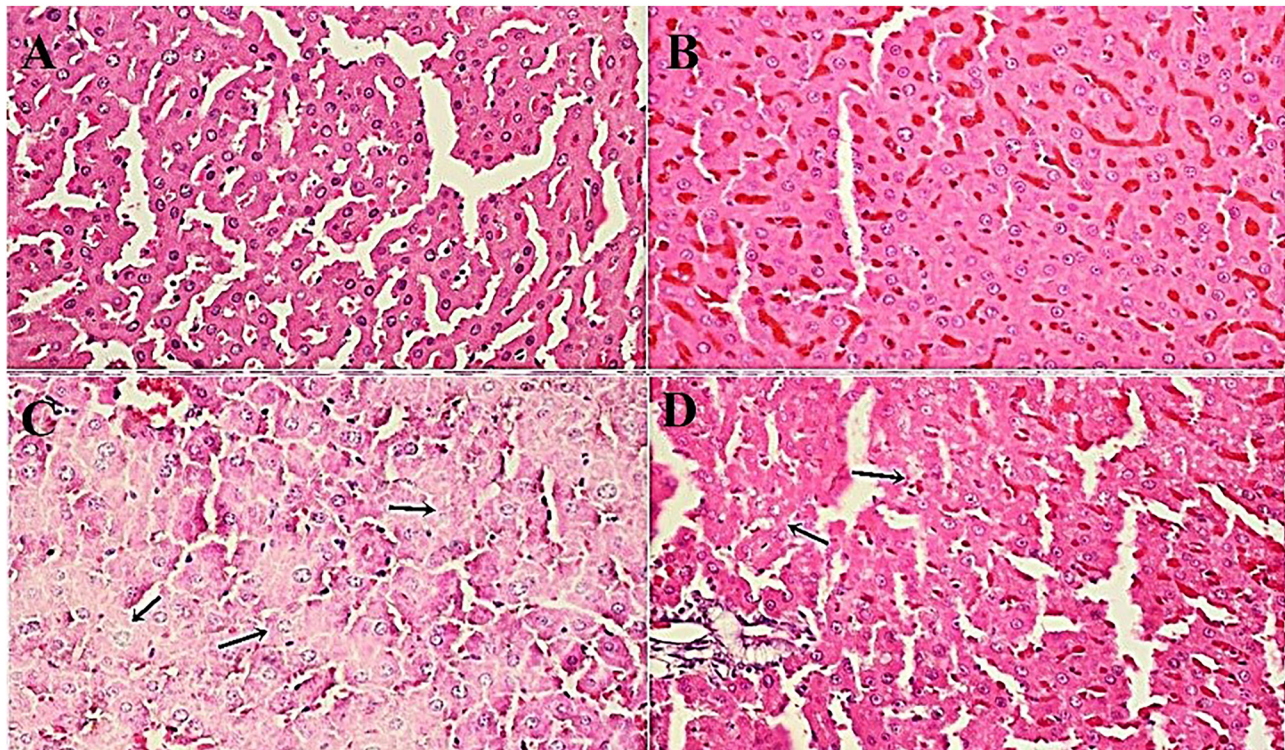


Figure 2: The histopathological images of the liver tissue of experimental groups. Degeneration in hepatocytes (arrows), (A) control group, (B) KO group, (C) APAP group, (D) APAP+KO group, $\times 400$ magnification, H&E staining. KO group, krill oil group; APAP group, acetaminophen group; APAP+KO, acetaminophen + krill oil group.

regions showed cellular alterations. Kupffer cell activations were lower in this group when compared to the previous (APAP) group. Degeneration and necrosis were distinctively low in the KO group, and Kupffer cell activation was lower than in the previous group. A few central veins were hyperemic in some fields. In the control group, degeneration was distinctively low. The histopathological images of the liver tissue of the study groups were given in Figure 2.

Presence of immunodetection

The liver tissue immunohistochemical findings of the study groups are given in Supplementary Table 2. The positives were seen as light to dark brown according to the strength of the antigenic reaction. Control group findings regarding Wnt-inhibiting factor, or Wif, were slightly positive in hepatocytes. In Kupffer cells, there were no signals. β -catenin reactions

were found slightly. 8-OHdG expressions were found in a few hepatocytes. When compared to control group positivity by these immunomarkers, Wif positivity was slightly increased in some hepatocytes locating paracentral fields of the hepatic lobes of the KO group ($p>0.05$). However, APAP group wif positivity were increased significantly in hepatocytes ($p<0.001$). It was more evaded in hepatocytes of the APAP group during whole lobes when compared to hepatocytes in the KO group. In contrast to this finding, APAP-KO findings were not as evident as in that of the APAP group. The positivity was observed in some hepatocytes of hepatic lobes. The positivity was denser in the paracentral fields of the lobes. On the other hand, β -catenin positivity was founded parallel to Wif positivity in the control group and APAP-KO group even though the positive cell numbers were increased in the KO group in terms of β -catenin immunomarker ($p>0.05$). In the APAP group, β -catenin was decreased in contrast to previous groups ($p>0.05$). 8-OHdG positivity was found in a couple of

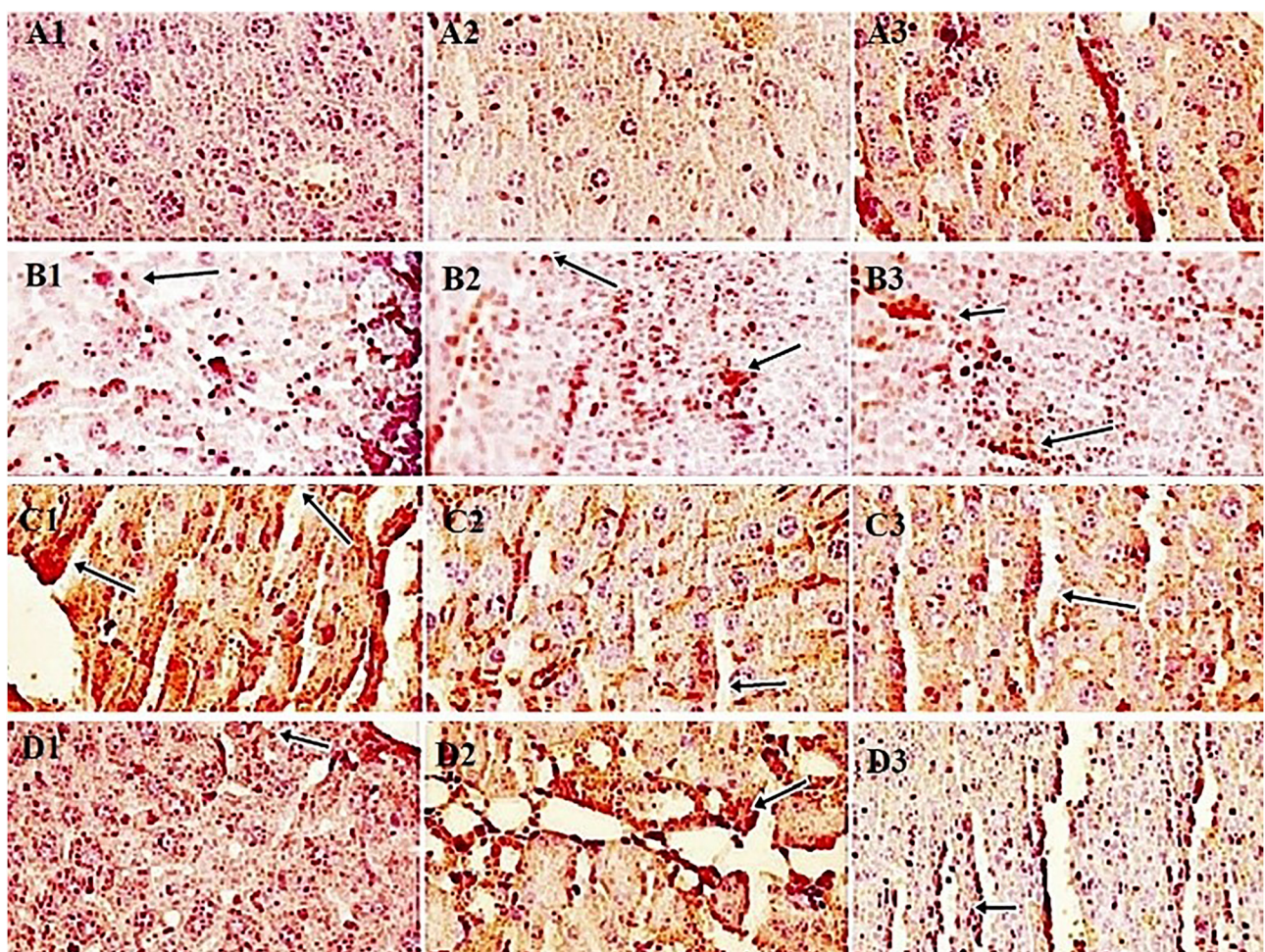


Figure 3: The immunohistochemical images of the liver tissue of experimental groups. Positivities in hepatocytes (arrows), (A) control group, (B) KO group, (C) APAP group, (D) APAP+KO group, 1: 8-OHdG, 2: β -catenin, 3: Wif, $\times 400$ magnification, strept ABC-P staining. KO group, krill oil group; APAP group, acetaminophen group; APAP+KO, acetaminophen + krill oil group; Wif, Wnt inhibitory factor-1; β -catenin, beta-catenin; 8-OHdG, 8-Hydroxy-2'-deoxyguanosine.

cells of control group animals. KO and APAP-KO group findings showed similar reaction numbers with previous immunomarkers in hepatocytes. However, APAP group 8-OHdG positivity was increased significantly in hepatocytes ($p < 0.001$). The immunohistochemical images of the liver tissue of the study groups are given in Figure 3.

Discussion

Side effects of some drugs in treating various diseases can cause serious problems [21]. The use of common and frequently used drugs, such as paracetamol can especially cause different side effects, such as acute liver failure [22]. Therefore, many researchers are investigating the protective effects of drugs to minimize drug-induced side (toxic) effects [12, 23]. Recently, nutritional supplements that are known to have a protective effect against most diseases are commonly used [24].

Many studies have confirmed the relationship of paracetamol-induced acute liver injury (hepatotoxicity) with changes in ALT and AST enzyme activities. They reported an increase in ALT and AST enzyme activity levels due to liver function damage [12, 23]. Similarly, ALT and AST enzyme activity increased after APAP administration in the APAP group compared to the control group.

Here we also aimed to determine the alterations in mRNA expressions of apoptotic and inflammatory genes due to APAP and KO applications in the liver of mice. We determined that while Bcl-2 level decreased in the APAP-administered group, Bcl-2 and COX-1 levels increased in the KO-administered group. However, these alterations did not reach statistically significant levels ($p > 0.05$). In a study investigating the structural effects of APAP on the liver and kidney, Guo et al. [25] reported a reduction in Bcl2 levels in APAP-damaged liver. Additionally, KO application was found to increase Bcl-2 expression in a study investigating the mechanisms of KO application in an A β 25-35-induced Alzheimer's disease mice model [10]. The authors suggested that KO might be a potential agent for preventing and treating Alzheimer's disease. Caspase-3 is an eventual executioner protease in apoptotic cell death [26], and consistent with our results caspase-3 levels were also reported to increase after APAP application in primary cultured mice hepatocytes [27].

Malondialdehyde is a product of lipid peroxidation and causes cellular deterioration as a result of disruption of the structural function of the cellular membrane [28]. Many studies have reported a relationship between oxidative stress and paracetamol-induced hepatotoxicity and an increase in MDA level, indicating lipid peroxidation and liver

function deterioration [29, 30]. The study found that the increase in MDA level caused by paracetamol was reduced by dose krill oil. Kim et al. [10] investigated the effects of krill oil against amyloid β -induced Alzheimer's disease in mice. And they reported that krill oil (500 mg/kg) decreased the MDA level in brain tissue compared to the control group. In the study, it was found that the MDA level increased in the KO (500 mg/kg) group compared to the control group. The reason why the presented study is different from the aforementioned study [10] is thought to be due to the tissue difference. Gokkaya et al. [31], investigated the effects of resveratrol and avocado oil against paracetamol-induced hepatotoxicity in rats. They found that resveratrol and resveratrol+avocado increased the TAS level that paracetamol decreased the liver tissue. Similarly, the present study found that krill oil increased the TAS level while paracetamol decreased in liver tissue. Krill oil contains the powerful natural antioxidant astaxanthin. Studies with astaxanthin have revealed many positive health effects, such as suppression of some types of cancer, protection against free radicals, and prevention of cardiovascular diseases [32, 33]. The study is compatible with the studies above and shows the antioxidant properties of krill oil on liver tissue. The hepatocytes select death under higher oxidative stress, such as higher acetaminophen administration, because they cannot resist or tolerate these catabolites. Thus, degeneration and necrosis are frequently observed due to such kinds of hepatotoxicities [7, 34]. It is reported by Al-Doaiss [35] that hepatocytes of APAP-treated rats showed effects such as degeneration and necrosis. In our study, we found that hepatocytes in the APAP group showed more degenerative and necrotic findings. In many fields, hepatocytes were lost in some areas. Such findings were fewer in the KO and APAP-KO groups. In particular, the decreasing levels of these alternative changes in the last two groups were found to be significant. On one side, the number of Kupffer cell activations was more increased in fields including liver injury in APAP-KO groups. Thus, we believe that there is a capacity for higher compensation for cellular alteration under APAP toxicity.

In the APAP-KO group, both cellular injuries and kupffer cell activation were found to be as low as those of the KO group. On the other side, many portal and central veins, as well as sinusoids, were enlarged with erythrocytes in the APAP group. Blood vessel & sinusoid congestion is related to findings reported by Al-Doaiss [35].

One possible factor has been showing activation of the Wnt/ β -catenin pathway in hepatocyte proliferation [9]. In this signaling system, β -catenin is mentioned as a pioneer in the way of providing both regulations of cytochrome P450 and phosphorylation of S45/Thr41 and S33/37/Thr41 sites after

overdose acetaminophen administration. The experimental studies noted that the early activation of β -catenin, depending on the dose of APAP (overdose APAP in a day) promotes liver regeneration by promoting the G1/S phase cell cycle transition [36–38]. So, it is proven that the early selective activation of pioneer β -catenin has produced a proliferation of these cells. This positive stimulation initializes in the frequent pericentral region of hepatic lobes.

As a result of the study, the lack of reactive metabolite formation, Wnt/ β -catenin, and also β -catenin activation may affect regeneration in the presence of an APAP overdose [36]. Our study also found higher regeneration, especially in the periphery of the central area of hepatic lobes. These positive stimuli were denser in APAP-KO and KO groups when compared to the overdose APAP group. Regeneration signaling system markers, Wnt and β -catenin, and their levels were found at balance in APAP-KO and KO groups. From another point of view, excessive formation of ROS causes peroxidation in cell membranes, DNA damage, and protein oxidation [39]. As a result of ROS accumulation in these kinds of cells, hydroxyl radicals are over-produced in the nucleus, and this situation can easily modify DNA structure. The 8-OHdG is the most abundant of the oxidized bases and is utilized for showing DNA oxidation [40]. In our study, 8-OHdG, which is known as this oxidative stress marker of DNA, was found at lower levels in the APAP-KO and KO groups. In contrast to this, pioneer β -catenin signals were found lower in the overdose of the APAP group than in others. When compared to previous immunomarkers, this elevated 8-OHdG positivity was discussed as a prominent sign in the way of degeneration and necrosis in the APAP group. However, other group findings were significantly decreased when compared to the APAP group. Another concrete result in the way of support of krill oil success is Wnt and β -catenin signals. In our study, Wnt inhibition expressions were elevated in the APAP group; however, the expressions were decreased in other groups, including krill oil, apart from controls. β -catenin signals were getting by this Wnt inhibition levels in krill oil, including groups. Hence, we believe that krill oil additive groups provide low oxidative stress for cells, which will help ensure their survival. The results of this study show that krill oil can even trigger cellular regeneration by blocking cellular debris.

In conclusion, krill oil may increase antioxidant status (i.e. TAS) in the microenvironment against paracetamol toxicity. Because cellular catabolism has been stopped by krill oil, cellular functions such as enzymes and molecular pathways have run within normal limits. In particular, Wnt- β -catenin signaling interactions in preserving cellular homeostasis bears a key point after krill oil administration. For this

reason, krill oil can be used as a nutritional supplement to protect against liver damage caused by paracetamol (as a result of any health problem). In addition, due to the protective effect of krill oil against liver damage, new studies can be conducted at different doses.

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Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The study was started after the approval of the Kırıkkale University Animal Experiments Ethics Committee (2020-41).

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