http://dergipark.org.tr/trkjnat
Trakya University Journal of Natural Sciences, 25(1): 81-90, 2024
ISSN 2528-9691
DOI: 10.23902/trkjnat.1410800

Research Article

Effects of gossypin on acetaminophen-induced hepatotoxicity in mice

Irfan Cinar[1*], Muhammed Yayla[2], Erdem Toktay[3], Damla Binnetoğlu[2]

[1] Kastamonu University, Faculty of Medicine, Department of Pharmacology, 3700 Kastamonu, TÜRKİYE
[2] Kafkas University, Faculty of Medicine, Department of Pharmacology, Kars, TÜRKİYE
[3] Kafkas University, Faculty of Medicine, Department of Histology and Embryology, Kars, TÜRKİYE

Cite this article as:

Cinar I., Yayla M., Toktay E. & Binnetoğlu D. 2024. Effects of gossypin on acetaminophen-induced hepatotoxicity in mice. *Trakya Univ J Nat Sci*, 25(1): 81-90, DOI: 10.23902/trkjnat.1410800

Received: 27 December 2023, Accepted: 15 March 2024, Online First: 28 March 2024, Published: 15 April 2024

Edited by:
Yeşim Sağ Açıkel

****Corresponding Author:***
Irfan Cinar
atairfan.nar@gmail.com

ORCID iDs of the authors:
IC. 0000-0002-9826-2556
MY. 0000-0002-0659-3084
ET. 0000-0002-0715-2707
DB. 0000-0002-7041-7253

Key words:
Gossypin
Acetaminophen
CYP2E1
AST
NF-kB
Anti-oxidative

Abstract: Liver injury from paracetamol (acetaminophen) (APAP) is common worldwide. To prevent intoxication with a drug with high poisoning, treatment can be made possible with an easily accessible and harmless substance. This study aimed to investigate the hepatoprotective effects of Gossypin (GOS) in mice exposed to an overdose of APAP -the possible mechanism of action. Specifically, serum [alanine aminotransferase (ALT), aspartate transaminase (AST), and hepatic biochemical parameters (glutathione (GSH), malondialdehyde (MDA) and superoxide dismutase (SOD)] were evaluated. Protein and mRNA levels of inflammatory, apoptotic, and cytochrome factors, including TNF-α, IL-1β, IL-6, NF-kB, and CYP2E1, were analyzed using real-time PCR. Pretreatment with GOS significantly reduced APAP-induced hepatic injury via oxidative stress. Along with potent antioxidant activity, GOS promoted APAP hepatic detoxification by regulating AST, ALT, GSH, MDA, and SOD activities and mRNA levels of the cytochrome CYP2E1 gene. The anti-inflammatory activity of GOS increases its production. TNF-α, IL-1β and IL-6, through possible NF-kB blockade, are also responsible for its hepatoprotective effect. Taken together, GOS has the potential to be developed as a preventive agent to be administered to patients suffering from APAP overdose.

Özet: Parasetamole (Asetaminofen) (APAP) bağlı karaciğer hasarı dünya çapında yaygındır. Zehirlenme oranı yüksek bir ilaçla zehirlenmeyi önlemek için kolay ulaşılabilir ve zararsız bir maddeyle tedavi mümkün kılınabilir. Bu çalışma, aşırı dozda APAP'a maruz kalan farelerde Gossypin (GOS) hepatoprotektif etkilerini (olası etki mekanizması) araştırmayı amaçladı. Spesifik olarak serum [alanin aminotransferaz (ALT), aspartat transaminaz (AST) ve hepatik biyokimyasal parametreler (glutatyon (GSH), malondialdehit (MDA) ve süperoksit dismutaz (SOD)] değerlendirildi. Daha sonra inflamatuar, apoptotik protein ve mRNA seviyeleri değerlendirildi ve TNF-a, IL-1β, IL-6, NF-kB ve CYP2E1 dahil olmak üzere sitokrom faktörleri, gerçek zamanlı PCR kullanılarak analiz edildi. GOS ile ön tedavi, oksidatif stres ve sentrilobüler yoluyla APAP'ın neden olduğu karaciğer hasarını önemli ölçüde azalttı. GOS, güçlü antioksidan aktivitesinin yanı sıra, sitokrom CYP2E1 geninin AST, ALT, GSH, MDA ve SOD aktivitelerini ve mRNA seviyelerini düzenleyerek APAP hepatik detoksifikasyonunu destekledi. GOS'in anti-inflamatuar aktivitesi, üretimini artırır. Olası NF-kB blokajı yoluyla TNF-α, IL-1β ve IL-6 da hepatoprotektif etkisinden sorumludur. Birlikte ele alındığında GOS, APAP doz aşımından muzdarip olan hastalara uygulanacak önleyici bir ajan olarak geliştirilme potansiyeline sahiptir.

Introduction

Acetaminophen (APAP), an N-acetylated p-aminophenol derivative, is one of the most widely used antipyretic analgesic drugs in the world due to its effectiveness and safety at therapeutic doses in pregnant women and children. APAP is available in more than 300 drug preparations in Türkiye. Overdose of APAP can cause serious liver damage (Holubek *et al.* 2006). Fifty percent of cases of APAP toxicity occur in patients who regularly consume pain relievers for acute or chronic pain management, while the remaining fifty percent result from suicide attempts facilitated by the ease of access to these medications (Holubek *et al.* 2006). While the absorption of APAP occurs in the intestine, its metabolism takes place in the liver. Approximately 85-90% of it undergoes phase II conjugation, leading to the formation of harmless sulfated and glucuronide metabolites (McGill & Jaeschke 2013). The remaining 10-15% of APAP is

converted to a toxic, highly reactive intermediate, N-acetyl-p-benzoquinone imine (NAPQI), via hepatic cytochrome P-450 (CYP) (Bunchorntavakul & Reddy 2013). NAPQI is usually detoxified by glutathione (GSH), but the glucuronidation and sulfation pathways become saturated at supratherapeutic doses of APAP. Large NAPQI (produced via the CYP2E1 pathway) depletes the hepatic pool of GSH, increasing APAP-protein bindings thereby causing oxidative stress, mitochondrial damage, and centrilobular necrosis (Raffa *et al.* 2014). In recent studies, exacerbation of APAP-induced liver injury has been associated with a systematic inflammatory response (Palabiyik *et al.* 2016, Rotundo & Pyrsopoulos 2020). Recent APAP toxicity studies showed that oxidative stress and molecular pathways secreted by necrotic hepatocytes cause the activation of local hepatic macrophages, Kupffer cells and neutrophils. In addition, activated hepatic macrophages secrete proinflammatory cytokines such as TNF-α, IL-6, and IL-1β. It has been reported that liver damage is increased even more (Karakus *et al.* 2013, Krenkel *et al.* 2014).

Although n-acetylcysteine (NAC) is used clinically as an antidote to APAP poisoning, as a substrate for glutathione synthesis, unfortunately, the effectiveness of NAC decreases as the time between overdose and treatment increases (Krenkel *et al.* 2014). In addition, redness, rash-itching, angioedema, bronchospasm, nausea-vomiting, hypotension, tachycardia, and respiratory distress can be seen in the treatment of patients.

One thousand seven hundred forty-nine cases of acetaminophen intoxication in Australia were reviewed retrospectively. Treatment with intravenous (IV) NAC was started in 399 instances; anaphylactic reaction was encountered in 37, some very serious, and five patients died (Bunchorntavakul & Reddy 2013). In a study investigating the protective effect of stiripentol in acetaminophen intoxication in rats, the mortality rate was 38% in the group administered NAC after acetaminophen intoxication (Bunchorntavakul & Reddy 2013). Although NAC is still the first choice in the toxicity treatment of acetaminophen, alternative treatment options that can be used in acetaminophen intoxication are being investigated due to possible side effects and mortality. Herbal medicines and natural products are of great importance in developing new drugs, since they target multiple disease mechanisms and effectively control the progression of the disease. Therefore, significant interest is shown in the discovery of therapeutically and prophylactically effective natural compounds (Ferrucci *et al.* 2010).

Gossypin (GOS), isolated from *Hibiscus vitifolius L.* (3,5,8,3,4-pentahydroxy-7-o-glucosyl flavone 8-glucosyl), is an effective flavonoid with anticancer, antioxidant, anti-inflammatory and analgesic properties (Babu *et al.* 2003, Cinar *et al.* 2019, Cinar 2021). It also activates antioxidant defense enzymes, providing protection against oxidative stress (Gautam & Flora 2010).

In this study, when the damage caused by APAP toxicity and the associated inflammatory development, apoptosis and pathophysiology are combined, it comes to mind that GOS may be an important agent in preventing APAP-related damage.

In the light of all this information, the aim of our study is to reveal the possible protective role of GOS in hepatotoxicity.

Materials and Methods

Animals

All procedures and protocols of this study were carried out in accordance with the guidelines for national care and research of experimental animals. 42 male Balb/C mice weighing 25-30 grams were used in the study.

Experimental Design

The groups were designed as follows: Control, APAP (300 mg/kg) (n=6), Healthy+GOS (40 mg/kg) (n=6), APAP (300 mg/kg)+GOS (5 mg/kg) (n=6), APAP (300 mg/kg)+GOS (10 mg/kg) (n=6), APAP (300 mg/kg)+GOS (20 mg/kg) (n=6) and APAP (300 mg/kg)+NAC (140 mg/kg) (n=6) (Cinar *et al.* 2022).

Hepatotoxicity Model

GOS (Cat. no: 652-78-8, Sigma Aldrich, MO, USA) was dissolved in a mixture of 0.1% Dimethyl sulfoxide (DMSO) and at doses of 5, 10, 20 mg/kg/day and NAC (Asist 200 mg capsules) was obtained from Husnu Arsan Drugs, İstanbul/Türkiye. NAC dissolved in saline was administered to 40 animals by oral gavage at a dose of 140 mg/kg/day for seven days. The control group was given saline by the gavage method. Paracetamol (APAP) (A5000) (dissolved in saline) was purchased from Sigma-Aldrich (St Louis, Missouri, USA) and was administered orally by gavage to 40 animals only on the 7th day, at 300 mg/kg (Palabiyik *et al.* 2016).

On the 8th day, 30 mg/kg thiopental sodium was given to all mice were anesthetized. After the blood was collected, the liver tissues were removed, some were treated with liquid nitrogen for biochemistry and molecular analysis and then stored at -80°C for examination, the other part was held in formaldehyde for histopathological studies.

Biochemical assay of oxidative stress parameters

AST and ALT levels of the collected sera were measured with an auto-analyzer (AU 5800, BECHMAN-COULTER diagnostic system, California, USA). Each liver tissue sample was stored at -80°C for biochemical and molecular analyses; SOD activity and GSH and MDA levels were measured from supernatants as described in previous studies (Ugan *et al.* 2018, Cadirci *et al.* 2019).

Molecular assay of gene expressions

RNA isolation and cDNA synthesis

Tissues total RNA was isolated from homogenized liver tissues with the RNeasy Mini Kit (Qiagen). cDNA

extraction was performed as specified in previous studies, following the instructions of the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, California, USA) (Dincer *et al.* 2022).

Relative analysis of gene expressions (Real-Time Quantitative PCR)

Expression analysis of TNF-α (5'AGCCAGGAGGGAGAACAGA'3, R 5'CAGTGAGTGAAAGGGACAGAAC'3), IL-1β (5' AGCCAGGAGGGAGAACAGA'3, R5' CAGTGAGTGAAAGGGACAGAAC'3), IL-6 (5' CTATGATAGCAAAGCCCCGAATG'3, R5' TCCTCCCCTCCCGTCACA'3), NF-κB (5' CTATGATAGCAAAGCCCCGAATG'3, R5'TCCTCCCCTCCCGTCACA'3), and CYP2E1 (5' TTTCCCTAAGTATCCTCCGTGACT'3, R 5' GCTGGCCTTTGGTCTTTTTG'3) as target genes was run with StepOne Plus Real-Time PCR System technology (Applied Biosystems) for each cDNA sample synthesized from mice liver RNA. The expression analysis of β-actin was used as an endogenous reference gene. Quantitative real-time PCR was achieved by the One-Step TaqMan Gene Expression Assays Probe-based technology (Primer Design Ltd., Southampton, UK) as previously described (Cinar *et al.* 2022). The data obtained were expressed as fold-change in expression using the 2-ΔΔCt method compared to the control group (Livak & Schmittgen 2001).

Histological procedure

Liver tissues were designed according to the procedure in the direction of relevant literature (Toktay *et al.* 2020, Cinar *et al.* 2022).

Statistical analysis

IBM SPSS v.25 was used in the statistical analysis of the results. One-way ANOVA followed by Duncan multiple range test (DMRT) was used. Data with $p < 0.05$ were considered significant. All data were presented as mean ± SE. Different letters (a,b,c,d,e,f) in the graphs indicate statistical differences between groups according to Duncan's multiple range test ($p < 0.05$). There is no statistically significant difference between the groups which have the same letters ($p > 0.05$).

Results

Biochemical Results

APAP significantly increased serum AST and ALT activities compared to the control group ($p < 0.05$). This shows APAP's hepatotoxicity and our model's good fit (Fig. 1d, e and Table 1). The AST and ALT values of the groups receiving pretreatment with GOS decreased dose-dependent, indicating that GOS prevents APAP toxicity.

GOS inhibited APAP-induced oxidative stress in the liver

In the control group, GOS did not cause a significant change in SOD activity or GSH and MDA levels (Fig. 1a, b, c and Table 1). On the contrary, APAP treatment decreased GSH and SOD activities compared to the activities in the control group but increased MDA levels. These values were adjusted in the control groups and peaked at the GOS (20 mg/kg) dose ($p < 0.05$).

Liver tissue mRNA expression results

TNF-α, IL-6, IL-1β, NF-kB, and CYP2E1 mRNA expression results are given in Fig. 2 and Table 2. Levels of cytokines were significantly higher in the APAP group than in the control group. CYP2E1 was considerably lower in the APAP group than in the control group. TNF-α, IL-6, IL-1β, NF-kB, and CYP2E1 mRNA expression levels showed a dose-dependent normalization in the APAP+GOS 10 and APAP+GOS 20 groups ($p < 0.05$) compared to the APAP group. No significant difference was observed between CYP2E1 mRNA expression levels and the APAP+NAC in the APAP+GOS 20 group. These results implied that GOS accelerated the non-toxic metabolism of APAP and suppressed the generation of toxic metabolite.

Table 1. Effects of GOS on the APAP-induced hepatic biochemical changes.

Biochemicals / Groups	SOD (U/mg protein)	MDA (nmol/mg protein)	GSH (umol/g protein)	AST (U/L)	ALT (U/L)
CONTROL	188.8±0.92 ^{e,f}	2.382±0.56 ^{a}	31.09±0.68 ^{e,f}	90.26±1.01 ^{a}	28.25±0.24 ^{a}
GOS 40	180.9±1.12 ^{e}	2.675±0.45 ^{b}	29.73±0.79 ^{e}	90.18±0.92 ^{a}	30.64±0.52 ^{a}
APAP+NAC	170.6±0.65 ^{d,e}	3.254±0.27 ^{b,c}	28.60±1.03 ^{e}	115.2±0.78 ^{a,b}	80.83±0.48 ^{c}
APAP	99.94±1.14 ^{a}	8.864±1.14 ^{f}	14.85±0.89 ^{a}	527.5±1.85 ^{f}	347.3±1.65 ^{f}
APAP+ GOS 5	129.8±0.85 ^{b}	7.709±1.07 ^{e}	18.66±0.78 ^{b}	436.5±1.73 ^{e}	295.4±1.05 ^{e}
APAP+ GOS 10	143.8±0.74 ^{c}	6.403±1.13 ^{d}	23.05±0.70 ^{c}	363.9±1.52 ^{d}	187.4±1.11 ^{d}
APAP+ GOS 20	159.3±0.28 ^{d}	4.141±0.95 ^{c}	26.64±61 ^{d}	224.1±0.91 ^{c}	98.72±0.87 ^{c}

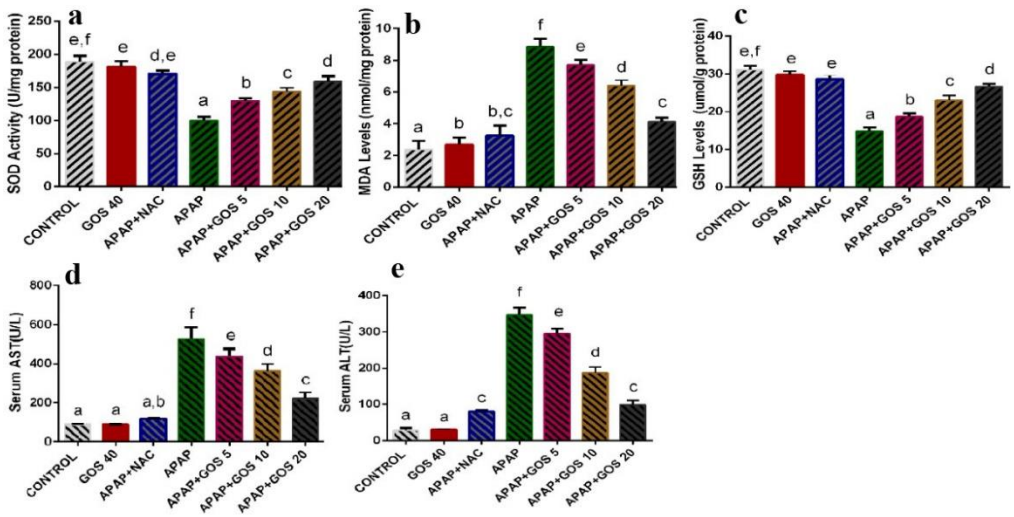

Fig. 1. Effects of GOS on the APAP-induced hepatic biochemical changes. **a.** Hepatic activity of SOD, **b.** hepatic level of MDA, **c.** hepatic level of GSH, **d.** serum level of AST, **e.** serum level of ALT. Different letters (a,b,c,d,e,f) indicate statistical differences between groups according to Duncan's multiple range test ($p < 0.05$). There is no statistically significant difference between the groups which have the same letters ($p > 0.05$).

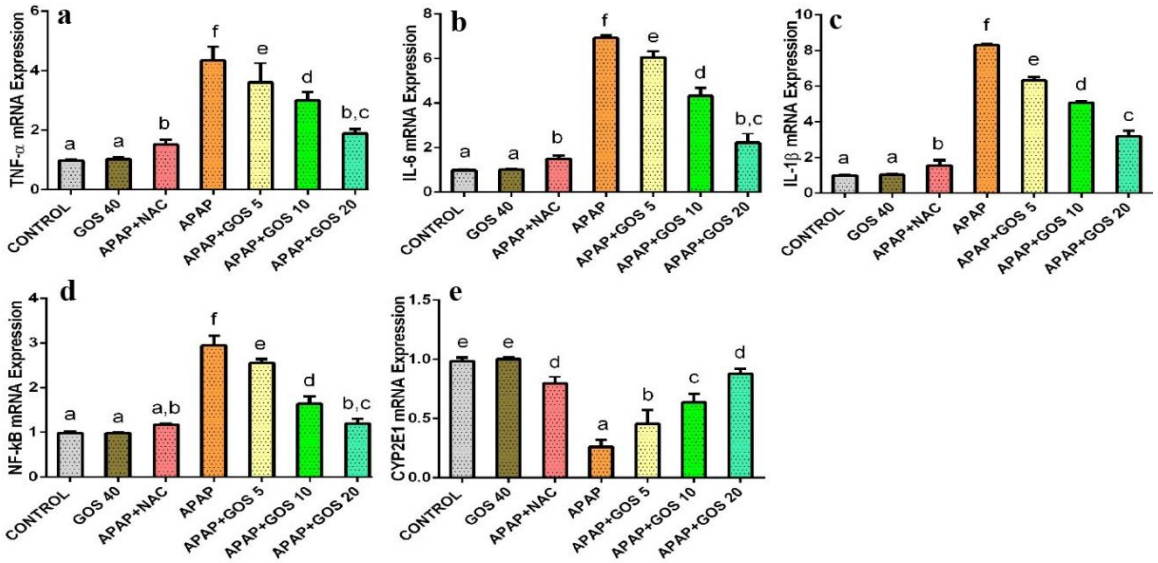

Fig. 2. Effects of GOS on the APAP-induced hepatic mRNA levels changes. The hepatic mRNA levels of **a.** TNF-α, **b.** IL-6, **c.** IL-1β, **d.** NF-kB, **e.** CYP2E1 expression normalized against β-actin. Different letters (a,b,c,d,e,f) indicate statistical differences between groups according to Duncan's multiple range test ($p < 0.05$. There is no statistically significant difference between the groups which have the same letters ($p > 0.05$).

Table 2. Effects of GOS on the APAP-induced hepatic mRNA levels changes.

mRNA / Levels Groups	TNF-α	IL-6	IL-1β	NF-kB	CYP2E1
CONTROL	0.986±0.22 ^{a}	0.998±0.12 ^{a}	1.001±0.15 ^{a}	0.987±0.17 ^{a}	1.012±0.13 ^{e}
GOS 40	1.029±0.26 ^{a}	1.011±0.16 ^{a}	1.034±0.11 ^{a}	0.985±0.12 ^{a}	1.003±0.11 ^{e}
APAP+NAC	1.524±0.42 ^{b}	1.499±0.20 ^{b}	1.557±0.26 ^{b}	1.176±0.31 ^{a,b}	0.797±0.10 ^{d}
APAP	4.345±0.48 ^{f}	6.923±0.45 ^{f}	8.309±0.74 ^{f}	2.946±0.28 ^{f}	0.261±0.26 ^{a}
APAP+ GOS 5	3.614±0.39 ^{e}	6.059±0.52 ^{e}	6.328±0.65 ^{e}	2.554±0.29 ^{e}	0.453±0.54 ^{b}
APAP+ GOS 10	3.002±0.34 ^{b}	4.314±0.60 ^{d}	5.076±0.34 ^{d}	1.637±0.18 ^{d}	0.637±0.51 ^{c}
APAP+ GOS 20	1.891±0.24 ^{d,c}	2.220±0.21 ^{b,c}	3.191±0.32 ^{c}	1.201±0.12 ^{b,c}	0.878±0.43 ^{d}

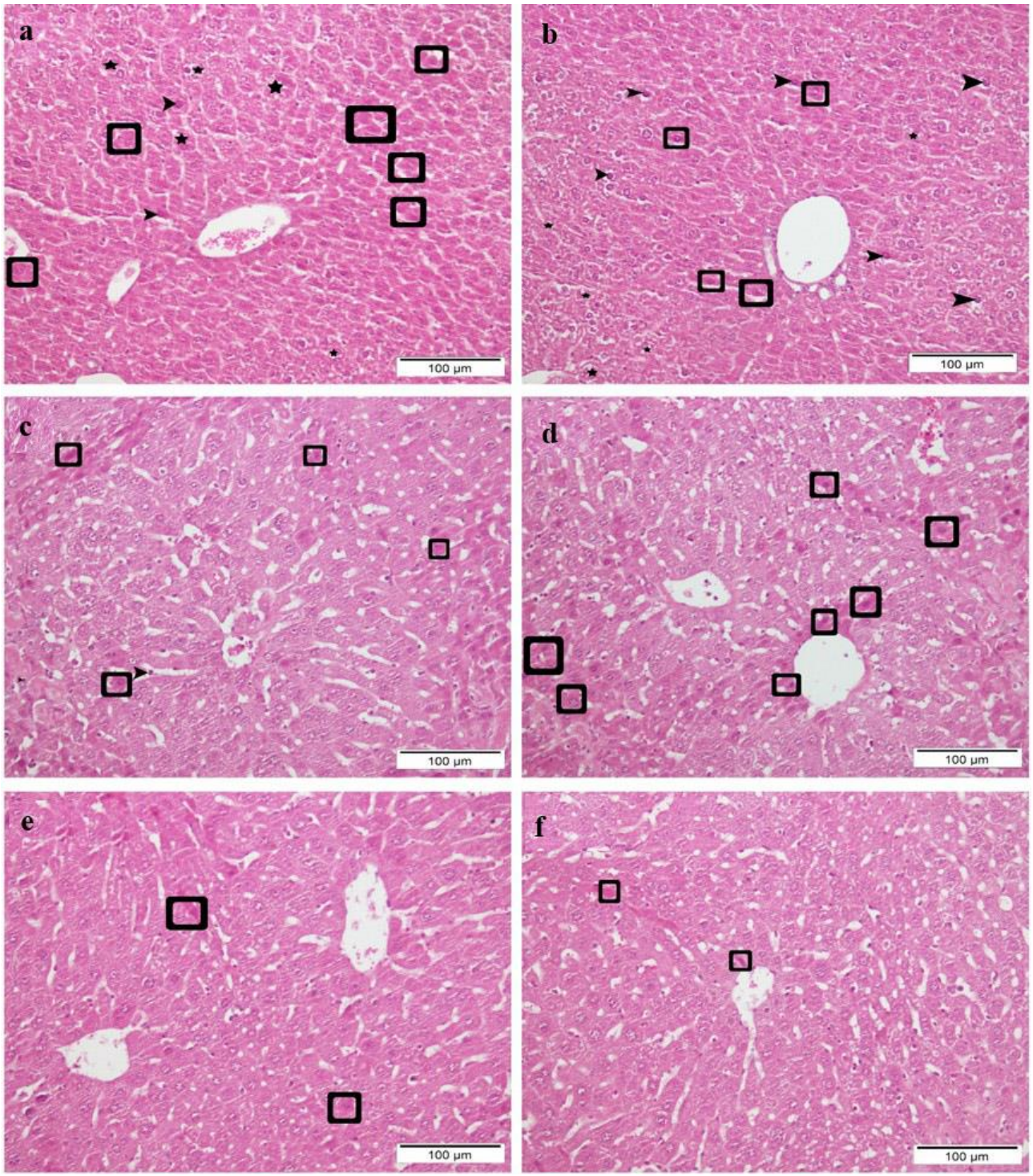

Fig 3. **a-b.** Histopathological findings of APAP+GOS5, **c-d.** APAP +GOS10, **e-f.** APAP +GOS20 groups. Asterisk: Necrotic Cell, Arrowhead: Picnotic Cell and Square: Eosinophilic Cell.

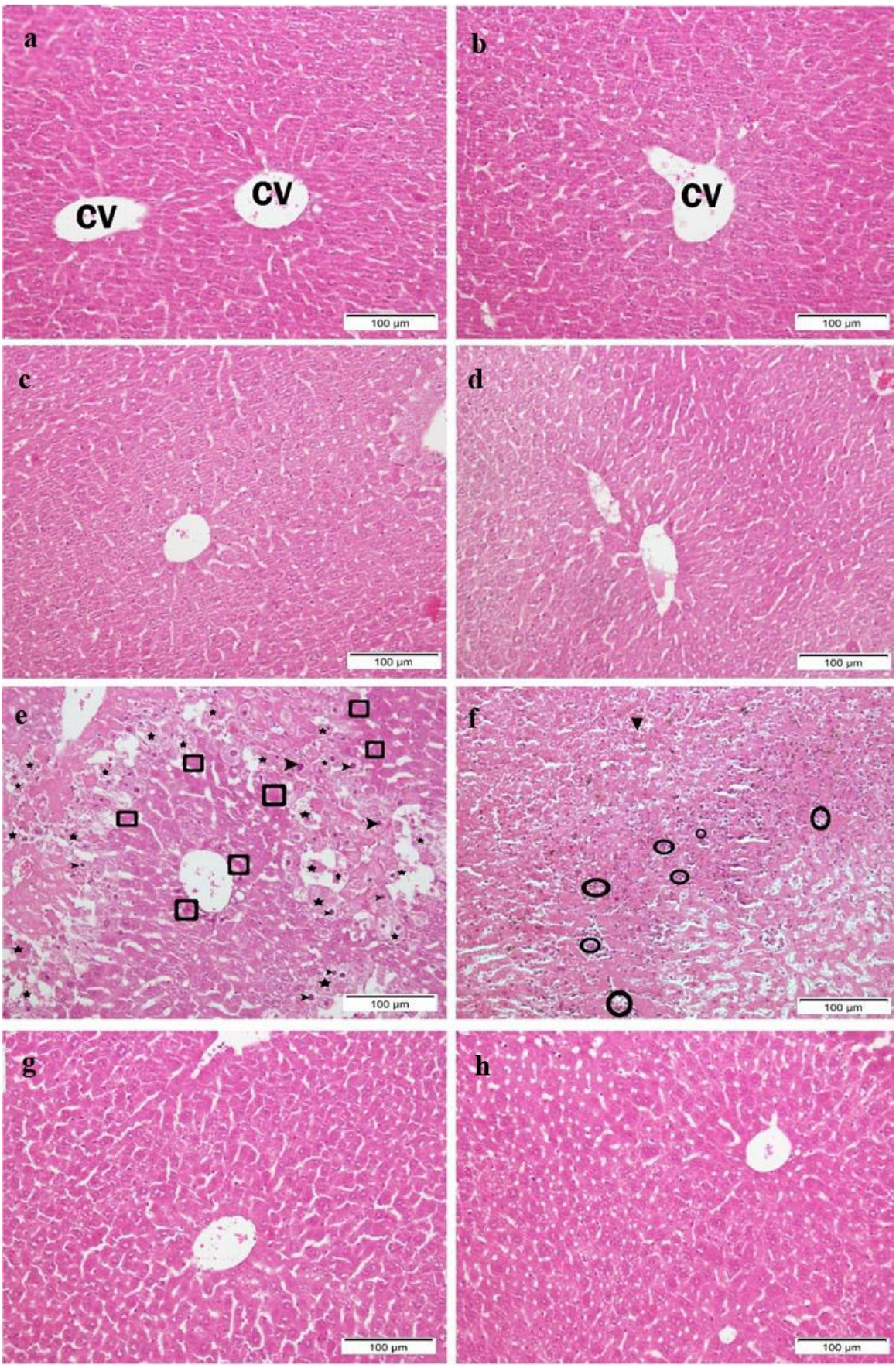

Fig. 4. **a-b.** Histopathological findings of the healthy, **c-d.** GOS20, **e-f.** APAP, **g-h.** APAP +NAC groups; CV: Central Vein, Star: Necrotic Cell, Arrowhead: Picnotic Cell, Square: Eosinophilic Cell, Round: Inflammatory areas, Triangle: Congestion.

Histopathology of Liver Tissues

According to the histopathological examination, regularly ordered hepatocyte cords in normal appearance were observed around the central vein in the healthy group. Sinusoidal capillary areas were within the normal range. No pathological appearance was found in this group (Fig. 3a, b). A histological appearance similar to the healthy group was observed in the GOS 20 group. No pathological finding was found (Fig. 3c, d). In the APAP group, normal-appearing cells were observed around the central vein, while cells with necrotic and pycnotic nuclei were observed near the portal area. In some regions, inflammatory cell areas and signs of hemorrhage and congestion were observed. It was noted that some cells stained relatively more eosinophilically (Fig. 3e, f). A histological appearance similar to the healthy group was observed in the APAP +NAC group. No pathological finding was found in APAP+GOS10 (Fig. 4c, d). Results obtained in the APAP group were not observed in this group (Fig. 4g, h). In the APAP +GOS5 group, cells with necrotic and pycnotic nuclei were observed near the portal area. Cells with eosinophilic staining were seen in liver tissue. These findings were relatively low compared to the APAP group. In the APAP +GOS10 group, only locally eosinophilic stained cells were seen, and the general appearance was similar to the healthy group. In the APAP +GOS20 group, cells with eosinophilic staining were rarely seen. Similar to the healthy group and had a histological appearance identical to the APAP +NAC groups. Histopathological findings were scored semiquantitatively, considering pathology mists such as inflammation, necrotic, pycnotic, and eosinophilic cells. Accordingly, it was shown as absent (-), mild (+), moderate (++), and severe (+++) (Table 1).

Table 3. Effects of GOS on the APAP-induced hepatic histopathology scores

GROUPS	Inflammation	Necrotic cell	Pycnotic cell	Eosinophilic cell
Healthy	-	-	-	-
GOS 40	-	-	-	-
APAP	+++	+++	++	+++
APAP+NAC	-	-	-	-/+
APAP+GOS5	-	+	+	++
APAP+GOS10	-	-/+	-/+	+
APAP+GOS20	-	-	-	-/+

Discussion

The liver is the most important organ playing the essential role to metabolize drugs, hormones, and nutrients. It also plays an active role in the detoxification of toxic substances, especially high doses of drugs. In other words, the liver can transform drugs into non-toxic and less harmful substances. Biotransformation may cause drug toxicity in some cases. This may be an advantage in experimental studies. The most commonly used drug to induce a liver injury pattern is APAP.

Recent studies have made intense efforts to discover hepatoprotective agents from traditional drugs and natural compounds with similar potency and less side effects, as well as various pharmacological activities (Madrigal-Santillan *et al.* 2014). In the present study, we investigated whether GOS, a natural flavonoid, could ameliorate APAP-induced hepatotoxicity. The results investigated The results investigated liver toxicity caused by acute acetaminophen administration, and APAP-induced hepatotoxicity can be reduced by treatment with GOS.

The most frequently evaluated parameters in liver damage are serum ALT and AST levels (Ferah *et al.* 2013, Liang *et al.* 2015). Pretreatment with GOS before the toxic APAP dose increased ALT and AST levels in the plasma due to liver toxicity induced by APAP, showing that APAP causes liver dysfunction, in line with previous studies (Golestan Jahromi *et al.* 2010, Matic *et al.* 2021). Treatment of mice with GOS or NAC reduced the level of AST and ALT and hence reduced liver toxicity. More importantly, GOS lowered ALT as much as NAC (Fig. 1e). NAC could not normalize AST and ALT values (Fig. 1d, e).

As the main mechanism in APAP toxicity, inhibition of CYP enzymes, depletion of glutathione stores and inhibition of glucuronidation are considered as the formation of high levels of the toxic APAP metabolite (Bromer & Black 2003, Yapar *et al.* 2007). However, when there is GSH deficiency in the liver, inadequate detoxification of NAPQI will lead to intracellular structural damage and subsequent centrilobular necrosis. The early toxicity phase is similar to mitochondrial dysfunction, while the late stage includes mitochondrial collapse, oncotic necrosis, and cell death *in vivo* (Aycan *et al.* 2014). As expected, hepatic GSH levels of mice decreased significantly after APAP administration in our study. Here, GOS application evaluates the toxic effect of APAP. We showed that it reduces NAPQI accumulation and normalizes the decreased GSH level.

It was stated, in previous studies, that lipid peroxidation was associated with APAP-induced toxicity, and MDA was shown as the most important marker of MDA lipid peroxidation (Li *et al.* 2013). APAP significantly increased MDA levels in our study, while GOS pretreatment normalized the increase. This indicated that GOS was a potent inhibitor of lipid peroxidation. It is the anti-oxidative defense system that plays a vital role in defending against oxidative damage. The first line of this system is SOD, which catalyzes the conversion of superoxide anion radicals into hydrogen peroxides, which are then converted into oxygen and water (Wu *et al.* 2010). Our study clearly shows that it impairs the antioxidant defense system due to a significant decrease in SOD activity in the APAP group. This effect was markedly reversed by GOS pretreatment.

The critical triggers of the inflammatory response in APAP toxicity are ROS and cell debris. This leads to a significant and acute accumulation of neutrophils responsible for inflammatory pathogenesis (Larsen & Wendon 2014). APAP promotes the release of proinflammatory cytokines that occur early in the pathogenic inflammation process and lead to the severity of liver damage. In line with previous studies, we found that proinflammatory cytokines, including IL-6, IL-1β, and TNF-α, are upregulated following APAP overdose (Dong *et al.* 2014, Song *et al.* 2014). Previous studies have shown that GOS down-regulates proinflammatory cytokines (Katary & Salahuddin 2017, Cinar *et al.* 2019, Dincer *et al.* 2022). In our study, GOS suppressed hepatic mRNA expressions of IL-6, IL-1b, and TNF-a. This may be attributed in part to the protective effect of GOS on APAP-induced liver injury by inhibition of inflammatory cytokines. We further investigated its anti-inflammatory activity.

NF-kB regulates the expression of iNOS and proinflammatory cytokines at the transcriptional level and acts as their primary transcriptional regulator (Gilroy *et al.* 2004). Under normal conditions, NF-κB dimmers bind to inhibitory IκB proteins and remain in the cytoplasm in a latent form as a transcription factor. After stimulation, IκB is phosphorylated, triggering its dissemination and proteasomal degradation, releasing NF-κB for translocation to the nucleus where it is involved in the inflammatory response, including cytotoxic factors (iNOS) and proinflammatory cytokines such as TNF-α, IL-1β, and IL-6, and stimulates transcription of domain-specific target genes (La Rosa *et al.* 1994, Kim & Lee 2015). Remarkably, our results showed that GOS significantly suppressed APAP-induced NF-κB activation.

The histopathology of APAP-induced hepatotoxicity is well-defined and associated with hepatic obstruction and necrosis. Moreover, the histological study of APAP-induced tissue damage in the liver confirmed previous findings that overdose causes conspicuous centrilobular necrosis in the first (Roomi *et al.* 2008). We also observed confluent necrosis in liver tissues in the APAP group. GOS and/or NAC administration ameliorated this damage to the liver. These histopathological data also supported the biochemical and molecular liver findings related to GOS administration.

Conclusion

Our results confirm that GOS exerts a protective effect against APAP toxicity in-vivo. The protective mechanism of GOS against damage caused by APAP is probably demonstrated by its strong antioxidative property and strengthening of the anti-oxidative system and anti-inflammatory mechanism. However, more comprehensive studies are needed to fully elucidate the mechanism and molecular pathways. Taken together, GOS has the potential to be developed as a preventive agent to be administered to patients suffering from APAP overdose.

Ethics Committee Approval: Ethics committee approval was received for this study from the Kafkas University Local Animal Care Committee by the number KAU-HADYEK/2019-071.

Data Sharing Statement: Data available on reasonable request.

Author Contributions: Concept: I.C., Design: I.C., M.Y., Execution: I.C., Material supplying: I.C., M.Y., E.T., Data acquisition: I.C., E.T., D.B., Data analysis/interpretation: I.C., M.Y., E.T., D.B., Writing: I.C., Critical review: I.C., M.Y.

Conflict of Interest: The authors have no conflicts of interest to declare.

Funding: The study was supported by the Science and Technology Research Projects of Kastamonu University with project number KÜ-BAP01/2021-6.

References

1. Aycan, I.O., Tufek, A., Tokgoz, O., Evliyaoglu, O., Firat, U., Kavak, G.O., Turgut, H. & Yuksel, M.U. 2014. Thymoquinone treatment against acetaminophen-induced hepatotoxicity in rats. *International journal of surgery*, 12(3): 213-218. https://doi.org/10.1016/j.ijsu.2013.12.013
2. Babu, B.H., Jayram, H.N., Nair, M.G., Ajaikumar, K.B. & Padikkala, J. 2003. Free radical scavenging, antitumor and anticarcinogenic activity of gossypin. *Journal of experimental & clinical cancer research: CR*, 22(4): 581-589. https://pubmed.ncbi.nlm.nih.gov/15053300/
3. Bromer, M.Q. & Black, M. 2003. Acetaminophen hepatotoxicity. *Clinics in liver disease*, 7(2): 351-367. https://doi.org/10.1016/S1089-3261(03)00025-4
4. Bunchorntavakul, C. & Reddy, K.R. 2013. Acetaminophen-related hepatotoxicity. *Clinics in* liver *disease*, 17(4): 587-607. https://doi.org/10.1016/j.cld.2013.07.005
5. Cadirci, E., Ugan, R.A., Dincer, B., Gundogdu, B., Cinar, I., Akpinar, E. & Halici, Z. 2019. Urotensin receptors as a new target for CLP induced septic lung injury in mice. *Naunyn-Schmiedeberg's archives of pharmacology*, 392(2): 135-145. https://doi.org/10.1007/s00210-018-1571-8
6. Cinar, I. 2021. Apoptosis-Inducing Activity and Antiproliferative Effect of Gossypin on PC-3 Prostate Cancer Cells. *Anti-cancer agents in medicinal chemistry*, 21(4): 445-450. https://doi.org/10.2174/1871520620666200721103422
7. Cinar, I., Sirin, B., Aydin, P., Toktay, E., Cadirci, E., Halici, I. & Halici, Z. 2019. Ameliorative effect of gossypin against acute lung injury in experimental sepsis model of rats. *Life sciences*, 221: 327-334. https://doi.org/10.1016/j.lfs.2019.02.039
8. Cinar, I., Yayla, M., Tavaci, T., Toktay, E., Ugan, R.A., Bayram, P. & Halici, H. 2022. *In Vivo* and *In Vitro* Cardioprotective Effect of Gossypin Against Isoproterenol-Induced Myocardial Infarction Injury. *Cardiovascular*

toxicology, 22(1): 52-62. https://doi.org/.1007/s12012-021-09698-3

9. Dincer, B., Cinar, I., Yayla, M. & Toktay, E. 2022. Evaluation of the protective effects of gossypin for ischemia/reperfusion injury in ovary tissue. *The journal of obstetrics and gynaecology research*, 48(3): 748-756. https://doi.org/10.1111/jog.15127

10. Dong, D., Xu, L., Han, X., Qi, Y., Xu, Y., Yin, L., Liu, K. & Peng, J. 2014. Effects of the total saponins from Rosa laevigata Michx fruit against acetaminophen-induced liver damage in mice via induction of autophagy and suppression of inflammation and apoptosis. *Molecules*, 19(6): 7189-7206. https://doi.org/10.3390/molecules19067189

11. Ferah, I., Halici, Z., Bayir, Y., Demirci, E., Unal, B. & Cadirci, E. 2013. The role of infliximab on paracetamol-induced hepatotoxicity in rats. *Immunopharmacology and immunotoxicology*, 35(3): 373-381. https://doi.org/10.3109/08923973.2013.775589

12. Ferrucci, L.M., Bell, B.P., Dhotre, K.B., Manos, M.M., Terrault, N.A., Zaman, A., Murphy, R.C., Vanness, G.R., Thomas, A.R., Bialek, S.R., Desai, M.M. & Sofair, A.N. 2010. Complementary and alternative medicine use in chronic liver disease patients. *Journal of clinical gastroenterology*, 44(2): e40-45. https://doi.org/10.1097/MCG.0b013e3181b766ed

13. Gautam, P. & Flora, S.J. 2010. Oral supplementation of gossypin during lead exposure protects alteration in heme synthesis pathway and brain oxidative stress in rats. *Nutrition*, 26(5): 563-570. https://doi.org/10.1016/j.nut.2009.06.008

14. Gilroy, D.W., Lawrence, T., Perretti, M. & Rossi, A.G. 2004. Inflammatory resolution: new opportunities for drug discovery. *Nature reviews. Drug discovery*, 3(5): 401-416. https://doi.org/10.1038/nrd1383

15. Golestan Jahromi, M., Nabavizadeh, F., Vahedian, J., Nahrevanian, H., Dehpour, A.R. & Zare-Mehrjardi, A. 2010. Protective effect of ghrelin on acetaminophen-induced liver injury in rat. *Peptides*, 31(11): 2114-2117. https://doi.org/10.1016/j.peptides.2010.08.009

16. Holubek, W.J., Kalman, S. & Hoffman, R.S. 2006. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. *Hepatology*, 43(4): 880; author reply 882. https://doi.org/10.1002/hep.21106

17. Karakus, E., Halici, Z., Albayrak, A., Polat, B., Bayir, Y., Kiki, I., Cadirci, E., Topcu, A. & Aksak, S. 2013. Agomelatine: an antidepressant with new potent hepatoprotective effects on paracetamol-induced liver damage in rats. *Human & experimental toxicology*, 32(8): 846-857. https://doi.org/10.1177/0960327112472994

18. Katary, M. & Salahuddin, A. 2017. Ameliorative effect of gossypin against gentamicin-induced nephrotoxicity in rats. *Life sciences*, 176: 75-81. https://doi.org/10.1016/j.lfs.2017.03.009

19. Kim, M.S. & Lee, D.Y. 2015. Insulin-like growth factor binding protein-3 enhances etoposide-induced cell growth inhibition by suppressing the NF-kappaB activity in gastric cancer cells. *Molecular and cellular biochemistry*, 403(1-2): 107-113. https://doi.org/10.3978/j.issn.2304-3881.2014.11.01

20. Krenkel, O., Mossanen, J.C. & Tacke, F. 2014. Immune mechanisms in acetaminophen-induced acute liver failure. *Hepatobiliary surgery and nutrition*, 3(6): 331-343. https://doi.org/10.3978/j.issn.2304-3881.2014.11.01

21. La Rosa, F.A., Pierce, J.W. & Sonenshein, G.E. 1994. Differential regulation of the c-myc oncogene promoter by the NF-kappa B rel family of transcription factors. *Molecular and cellular biology*, 14(2): 1039-1044. https://doi.org/10.1128/mcb.14.2.1039-1044.1994

22. Larsen, F.S. & Wendon, J. 2014. Understanding paracetamol-induced liver failure. *Intensive care medicine*, 40(6): 888-890. https://doi: 10.1007/s00134-014-3293-9

23. Li, G., Chen, J.B., Wang, C., Xu, Z., Nie, H., Qin, X.Y., Chen, X.M. & Gong, Q. 2013. Curcumin protects against acetaminophen-induced apoptosis in hepatic injury. *World journal of gastroenterology*, 19(42): 7440-7446. https://doi.org/10.3748/wjg.v19.i42.7440

24. Liang, B., Guo, X.L., Jin, J., Ma, Y.C. & Feng, Z.Q. 2015. Glycyrrhizic acid inhibits apoptosis and fibrosis in carbon-tetrachloride-induced rat liver injury. *World journal of gastroenterology*, 21(17): 5271-5280. https://doi.org/10.3748/wjg.v21.i17.5271

25. Livak, K.J. & Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*, 25(4): 402-408. https://doi.org/10.1006/meth.2001.1262

26. Madrigal-Santillan, E., Madrigal-Bujaidar, E., Alvarez-Gonzalez, I., Sumaya-Martinez, M.T., Gutierrez-Salinas, J., Bautista, M., Morales-Gonzalez, A., Garcia-Luna y Gonzalez-Rubio, M., Aguilar-Faisal, J.L. & Morales-Gonzalez, J.A. 2014. Review of natural products with hepatoprotective effects. *World journal of gastroenterology*, 20(40): 14787-14804. https://doi.org/10.3748/wjg.v20.i40.14787

27. Matic, M.M., Paunovic, M.G., Milosevic, M.D., Ognjanovic, B.I. & Saicic, Z.S. 2021. Hematoprotective effects and antioxidant properties of beta-glucan and vitamin C against acetaminophen-induced toxicity: an experimental study in rats. *Drug and chemical toxicology*, 44(3): 302-309. https://doi.org/10.1080/01480545.2019.1587451

28. McGill, M.R. & Jaeschke, H. 2013. Metabolism and disposition of acetaminophen: recent advances in relation to hepatotoxicity and diagnosis. *Pharmaceutical research*, 30(9): 2174-2187. https://doi.org/10.1007/s11095-013-1007-6

29. Palabiyik, S.S., Karakus, E., Akpinar, E., Halici, Z., Bayir, Y., Yayla, M. & Kose, D. 2016. The Role of Urotensin Receptors in the Paracetamol-Induced Hepatotoxicity Model in Mice: Ameliorative Potential of Urotensin II Antagonist. *Basic & clinical pharmacology & toxicology*, 118(2): 150-159. https://doi.org/10.1111/bcpt.12447

30. Raffa, R.B., Pergolizzi, J.V., Jr., Taylor, R., Jr., Decker, J.F. & Patrick, J.T. 2014. Acetaminophen (paracetamol) oral absorption and clinical influences. *Pain practice: the official journal of World Institute of Pain*, 14(7): 668-677. https://doi.org/10.1111/papr.12130

31. Roomi, M.W., Kalinovsky, T., Ivanov, V., Rath, M. & Niedzwiecki, A. 2008. A nutrient mixture prevents acet-

aminophen hepatic and renal toxicity in ICR mice. *Human & experimental toxicology*, 27(3): 223-230. https://doi.org/10.1177/0960327108090276

32. Rotundo, L. & Pyrsopoulos, N. 2020. Liver injury induced by paracetamol and challenges associated with intentional and unintentional use. *World journal of hepatology*, 12(4): 125-136. https://doi.org/10.4254/wjh.v12.i4.125

33. Song, E., Fu, J., Xia, X., Su, C. & Song, Y. 2014. Bazhen decoction protects against acetaminophen induced acute liver injury by inhibiting oxidative stress, inflammation and apoptosis in mice. *PloS one*, 9(9): e107405. https://doi.org/10.1371/journal.pone.0107405

34. Toktay, E., Selli, J., Gurbuz, M.A., Tastan, T.B., Ugan, R.A., Un, H. & Halici, Z. 2020. Effects of soy isoflavonoids (genistein and daidzein) on endometrial receptivity. *Iranian journal of basic medical sciences*, 23(12): 1603-1609. https://doi.org/10.22038/ijbms.2020.48294.11089

35. Ugan, R.A., Cadirci, E., Halici, Z., Toktay, E. & Cinar, I. 2018. The role of urotensin-II and its receptors in sepsis-induced lung injury under diabetic conditions. *European journal of pharmacology*, 818: 457-469. https://doi.org/10.1016/j.ejphar.2017.11.011

36. Wu, Y.L., Jiang, Y.Z., Jin, X.J., Lian, L.H., Piao, J.Y., Wan, Y., Jin, H.R., Joon Lee, J. & Nan, J.X. 2010. Acanthoic acid, a diterpene in Acanthopanax koreanum, protects acetaminophen-induced hepatic toxicity in mice. *Phytomedicine: international journal of phytotherapy and phytopharmacology*, 17(6): 475-479. https://doi.org/10.1016/j.phymed.2009.07.011

37. Yapar, K., Kart, A., Karapehlivan, M., Atakisi, O., Tunca, R., Erginsoy, S. & Citil, M. 2007. Hepatoprotective effect of L-carnitine against acute acetaminophen toxicity in mice. *Experimental and toxicologic pathology: official journal of the Gesellschaft fur Toxikologische Pathologie*, 59(2): 121-128. https://doi.org/10.1016/j.etp.2007.02.009