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RESEARCH

Protective effects of jervine purified from *Veratrum album* on paracetamol-induced liver toxicity in rats

Sıçanlarda parasetamolle indüklenen karaciğer toksisitesi üzerine *Veratrum album* bitkisinden saflaştırılan jervinin koruyucu etkisi

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Abstract

Purpose: Since paracetamol toxicity is a very common type of poisoning, we planned to investigate whether Jervine has an effect on paracetamol toxicity by utilizing its anti-inflammatory effect.
Materials and Methods: In our study, 42 Sprague Dawley rats of 8 weeks of age were used. Seven groups were formed with 6 animals in each group. At the 24th hour of the study, all groups underwent laparotomy under anesthesia, and liver dissection was performed. Hematoxylin and Eosin (H&E) staining was performed to evaluate liver histopathology. SOD, CAT, GSH, and MDA levels were analyzed biochemically.
Results: Histopathological, while liver tissues were normal in the control group, we observed degeneration areas, inflammation, and hemorrhage in the paracetamol group. Jervine reduced the severity of paracetamol toxicity and prevented liver damage. Jervine significantly increased SOD levels. Paracetamol administration significantly decreased CAT levels. Paracetamol significantly decreased GSH levels compared to the control group.
Conclusion: Jervine reduced the adverse effects of paracetamol toxicity on liver tissue, such as degeneration, inflammation, and hemorrhage. Jervine increased antioxidant activity and reduced the harmful effects of NAPQI, the toxic metabolite of paracetamol, on liver tissue.

Keywords:. Anti-inflammatory, jervine, paracetamol, *veratrum album*

Özet

Amaç: Parasetamol toksisitesi de çok yaygın bir zehirlenme tipi olduğundan dolayı bizde jervinin antiinflamatuvar etkisinden faydalanarak parasetamol toksisitesi üzerinde etkisinin olup olmadığını araştırmayı planladık.
Gereç ve Yöntem: Çalışmamızda 42 adet Sprague Dawley cinsi 8 haftalık sıçan kullanıldı. Her grupta 6 dişi hayvan olacak şekilde 7 grup oluşturuldu. Çalışmanın 24. saatinde tüm gruplara anestezi altında laparotomi uygulandı ve karaciğer diseksiyonu yapıldı. Karaciğer histopatolojisini değerlendirmek için Hematoksilen ve Eozin (H&E) boyama yapıldı. Biyokimyasal olarak SOD, CAT, GSH ve MDA seviyelerine bakıldı.
Bulgular: Histopatolojik olarak kontrol grubunda karaciğer dokuları normal iken, parasetamol grubunda dejenerasyon alanları, enflamasyon ve hemoraji gözlemledik. Jervin parasetamol toksisitesinin şiddetini azaltmış ve karaciğer hasarını önlemiştir. Jervin SOD seviyelerini önemli ölçüde artırmıştır. Parasetamol uygulaması CAT seviyelerini önemli ölçüde azaltmıştır. Parasetamol, kontrol grubuna kıyasla GSH seviyelerini önemli ölçüde azaltmıştır.
Sonuç: Çalışmamız, Jervinin parasetamol toksisitesinin karaciğer dokusu üzerindeki dejenerasyon, inflamasyon ve hemoraji gibi olumsuz etkilerini azalttığını ortaya koymuştur. Jervin antioksidan aktiviteyi arttırmış ve parasetamolün toksik metaboliti olan NAPQI'nin karaciğer dokusu üzerindeki zararlı etkilerini azaltmıştı

Anahtar kelimeler: Antiinflamatuar, jervin, parasetamol, *veratrum album*.

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INTRODUCTION

Liver drugs play an important functional role in the metabolism and detoxification of many micro and macromolecules. It is the largest organ that helps remove compounds formed as a result of detoxification from the body. For these reasons, it is the target organ of injuries caused by chemicals. Paracetamol is an analgesic drug which is used worldwide[1,2]. Paracetamol is considered safe in proper use, but due to its easy availability and accessibility, paracetamol toxicity is one of the most common overdoses reported to poison centers[3]. Necrosis that develops after overdose of paracetamol is the main mechanism of hepatocyte loss. Moreover, paracetamol overdose is the main cause of fulminant liver failure and one of the most common reasons for emergency liver transplantation. For this reason, accumulated metabolites resulting from paracetamol overdose in transplanted patients may also cause regional liver necrosis[4]. Following paracetamol use, hepatocytes produce a reactive oxidative species (ROS), N-acetyl-p-benzoquinone imine (NAPQI). With its short half-life, NAPQI only damages the cells in which it is created[5]. Excessive amounts of NAPQI cause decreased mitochondrial respiration in hepatocytes, increased oxidative stress, and mitochondrial dysfunction as a result of depletion of ATP stores[6]. Therefore, paracetamol overdose is primarily targeted to the liver. Detoxification of NAPQI is mediated by glutathione. The paracetamol toxicity pathogenesis involves activating cytochrome p450 isoforms and depletion of intracellular glutathione[7]. Glutathione depletion can lead to mitochondrial dysfunction, increased oxidative stress, lipid peroxidation, DNA fragmentation, hepatocellular necrosis, and severe liver damage [1]. Liver failure occurring after liver damage causes cerebral edema or sepsis in the early stages, and later causes multiple organ failure and death[6].

N-acetylcysteine (NAC) has been used clinically since the 1970s. The first use of NAC was in patients with fibrosis and in the treatment of paracetamol overdose. NAC is also used to correct decreased GSH levels in the alcoholic liver disease and to reduce liver diseases associated with oxidative stress[8].

In various parts of the world, plants have been used in medicine for a long time for both protection and treatment purposes. Nowadays, plants are used in large quantities as medicine in line with the health needs of the world population[1]. The Veratrum genus is an essential member of the Melanthiaceae family. Veratrum species contain flavonoid and stilbenoid components with different activities and more than 200 alkaloids. Among these, steroid alkaloids have significant pharmacological effects. *Veratrum album* is a poisonous veratrum species in the Turkish flora, and one of its characteristic steroid alkaloids is jervine.

Jervine is a powerful antioxidant that also has anti-inflammatory effects[9]. Jervine, which has anti-inflammatory and antioxidant effects, has been reported to increase the activities of GSH and glutathione-dependent enzymes, which are powerful antioxidants in infected tissues[9,10]. Jervine increases the activity of glutathione-dependent enzymes, which are potent antioxidants in infected tissues10. Jervine is one of the steroidal alkaloids isolated from Veratrum album[11] (Figure 1). It has anticancer and antitumor properties[12,13]. N-acetylcysteine (NAC) has been used clinically since the 1970s. The first use of NAC was in patients with fibrosis and the treatment of paracetamol overdose. NAC is also used to correct decreased GSH levels in alcoholic liver disease and to reduce liver diseases associated with oxidative stress[8]. In this study, we investigated the effects of Jervine obtained from Veratrum album on paracetamol toxicity in rat liver tissue by taking advantage of the benefits it provides to the antioxidant mechanism.

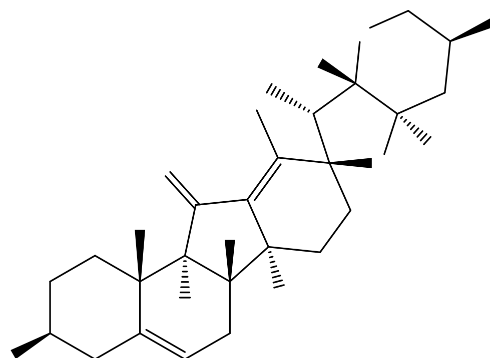
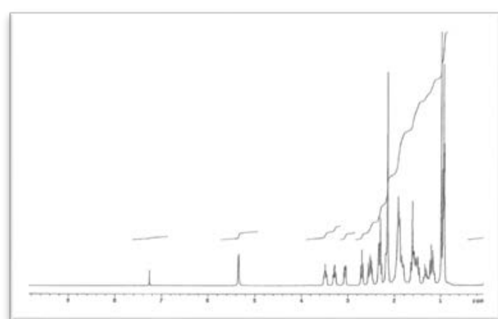


Figure 1. Structure of Jervine

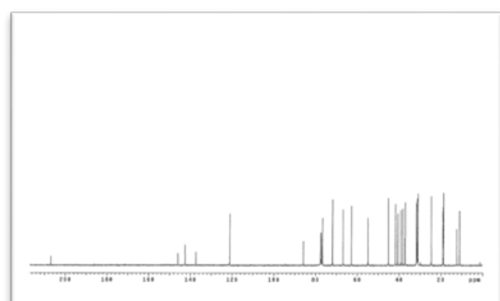
MATERIALS AND METHODS

Plant material

Veratrum album evolved in the Black Sea region. After allowing them to dry in the shade away from the sun, plant roots were ground.



^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of Jervine



^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of Jervine

Extraction and isolation

Jervine was separated from the *Veratrum album*'s origins using the same methods as earlier studies[14]. The plant sample (900 g) was macerated five times with acetone (2.5 L x 24 hours) and filtered. The filtrates were pooled, and the solvents were separated on an evaporator to obtain 12.76 g of acetone extract (1.4%). The acetone extraction (12.76 g) was divided into fractions by silica gel column chromatography (CC) (47x4 cm, 150 g, 70-230 mesh; ethyl acetate: methanol 1:0, 9:1, 8:2, 6:4, 4:6). Fractions (40 ml each) were assayed by thin layer chromatography (TLC) and fractions yielding the same stain on TLC were combined. The main component was isolated in fractions 69-81 (1.1 g). The chemical structure of the compound was confirmed to be Jervine by 1D 1H and 13 C NMR spectroscopic methods. NMR spectra were published in our previous study[14]. It was determined by High Performance Liquid Chromatography (HPLC) that Jervine was 97% pure.1H-NMR (CDCl 3): 5.35 (d, J=4.8, H6; 3.44–3.55 (m, H3); 3.29 (dt, J=10.3, 4.0, H23); 3.06 (dd, J=12.4, 4.0, H a 26; 2.65 (t, J=9.2, H22); 2.31 (t, J=12.5, H b 26); 2.15(s, H18); 0.98 (s, H19); 0.94 (d,J=6.0, H21); 0.93 (d, J=6.2, H27). 13 C-NMR (CDCl 3): 207.0 (C11); 145.9 (C12); 142.6 (C5); 137.4 (C13); 121.1 (C6); 85.8 (C17);76.6 (C23); 71.8 (C3); 66.8 (C22); 62.8 (C9); 54.8 (C26); 45.1 (C20); 41.7 (C4);40.6 (C14); 39.1 (C24); 38.2 (C8); 37.3 (C10); 37.0 (C1); 31.7 (C25); 31.4 (C16);31.2 (C2); 30.9 (C7); 24.6 (C15); 18.9 (C27); 18.7 (C19); 12.3 (C18); 10.9 (C21)

Animal grouping and drug application

42 adolescent male rats (average age 6 weeks) weighing 180–200 g were used in the investigation. Animals were fed (ad libitum), housed, and cared for under conventional lab settings of 12 hours of light and 12 hours of darkness at constant temperature and humidity. With decision 2021-129, the Kafkas University Local Ethics Committee for Animal Experiments consented to this study.

The animals were euthanized after the drug administration. Half of the liver was stored at -80 °C for biochemical analysis, and the other half was kept in 10% formalin for histological analysis

Table-1 Experimental Groups.

Name	Descriptions	Abbreviations	n
Group 1	Paracetamol (2 g/kg)	P	6
Group 2	N-acetylcysteine (140) mg/kg	NAC	6
Group 3	Paracetamol (2 g/kg) + N-acetylsisteine	P+NAC	6
Group 4	Paracetamol (2 g/kg) + Jervine 400 mg/kg	P+J-HD	6
Group 5	Paracetamol (2 g/kg) + Jervine 200 mg/kg	P+J-LD	6
Group 6	Jervine (400 mg/kg)	J-HD	6
Group 7	Control	CNT	6

Histological analysis

Following removal, the liver was preserved in a 10% formalin solution for 72 hours. Tissue tracing was carried out following fixation in accordance with the literature[15]. After tissue tracing from each paraffin block, 5μm thick sections were taken serially, and Hematoxylin-eosin (H&E) staining was performed for histopathological evaluations. Photos were shot with the Cellsense Software on an Olympus BX43 microscope.

Biochemical analysis

All tissues were homogenized in phosphate buffers (50 mM, pH 7.4) after being cooled on ice. After tissue homogenates were centrifuged at 4000 rpm for 10 min at 4°C (Eppendorf tubes), the supernatants were collected for the analysis of biochemical parameters in microcentrifuges. Serum samples were stored at -80°C before analysis. On the day of the analysis, the samples were defrosted progressively (at -20 C, +4 C, and then +25 C, respectively), and all assays were carried out immediately. All the spectrophotometric assays were carried out using a microplate reader.

Determination of superoxide dismutase (SOD) enzyme activity measurement

The technique is predicated on the idea that when nitroblue tetrazolium (NBT) is present in the sample, the superoxide dismutase enzyme suppresses free radicals during the reduction of free oxygen radicals created by the enzymatic process. Using spectrophotometry, the color shift brought about by the reaction will be measured at 560 nm [16].

Determination of catalase (CAT) enzyme activity measurement

To determine the catalysis activity in the homogenate supernatant, the method was used in the previous study [17]. The reaction will be determined by measuring at 405 nm in a spectrophotometer. Standards will be prepared from pure catalase enzyme at different concentrations (between 940 - 14.7 U/mL), a standard graph will be drawn, and the catalase activity in the samples will be calculated according to the equation of this curve. Results will be expressed as U/mL for tissue samples.

Total GSH measurement

The total GSH was measured using the Sedlak and Lindsay method [18]. 15 Absorbance was measured at 412 nm following a 30-minute incubation period at 37 °C. As standards, reduced glutathione concentrations of 2 mM, 1 mM, 0.5 mM, 0.250 mM, 0.125 mM, 0.0625 mM, and 0.035 mM were employed. The total amount of GSH was calculated by using the absorbance values from the standard measurements and their graph equivalents.

Determination of lipid peroxidation (MDA)

Based on a 60-minute incubation period at 95 °C, the absorbance of the pink complex produced by thiobarbituric acid and MDA is measured spectrophotometrically at a wavelength of 532 nm [19]. A stock standard solution was prepared from 1.1.3.3 tetraethoxypropane at a concentration of 200 μmol/L. Standard solutions at different concentrations were obtained by serial dilution from the stock standard. These solutions were used as standards, and the results were given in micromolar

Statistical analysis

GraphPad Prism (v8.0.1, GraphPad Software Inc., San Diego, CA, USA) was used to perform statistical analyses. Kruskal-Wallis test was used for the analysis of SOD, MDA, and CAT levels, and Dunn's test was used for multiple comparisons. One-way ANOVA and Tukey post hoc test were applied for GSH analyses that showed normal distribution.

RESULTS

While the liver tissues were normal in the control group, we observed degeneration areas, inflammation, and hemorrhage in the paracetamol group. No histopathologic findings were observed in the NAC group. In the group treated with paracetamol + NAC, inflammation areas were rarely observed. In the paracetamol + J-HD group and paracetamol + J-LD groups, jervine reduced the severity of paracetamol toxicity and prevented liver damage. In these groups, histopathologic findings were similar to the control group. In liver tissue, no histopathological findings were detected in the J-HD group.

When compared with the paracetamol-administered group, NAC and high-dose jervine significantly increased SOD levels. However, between these groups and the control group, no significant difference was found in terms of SOD levels.

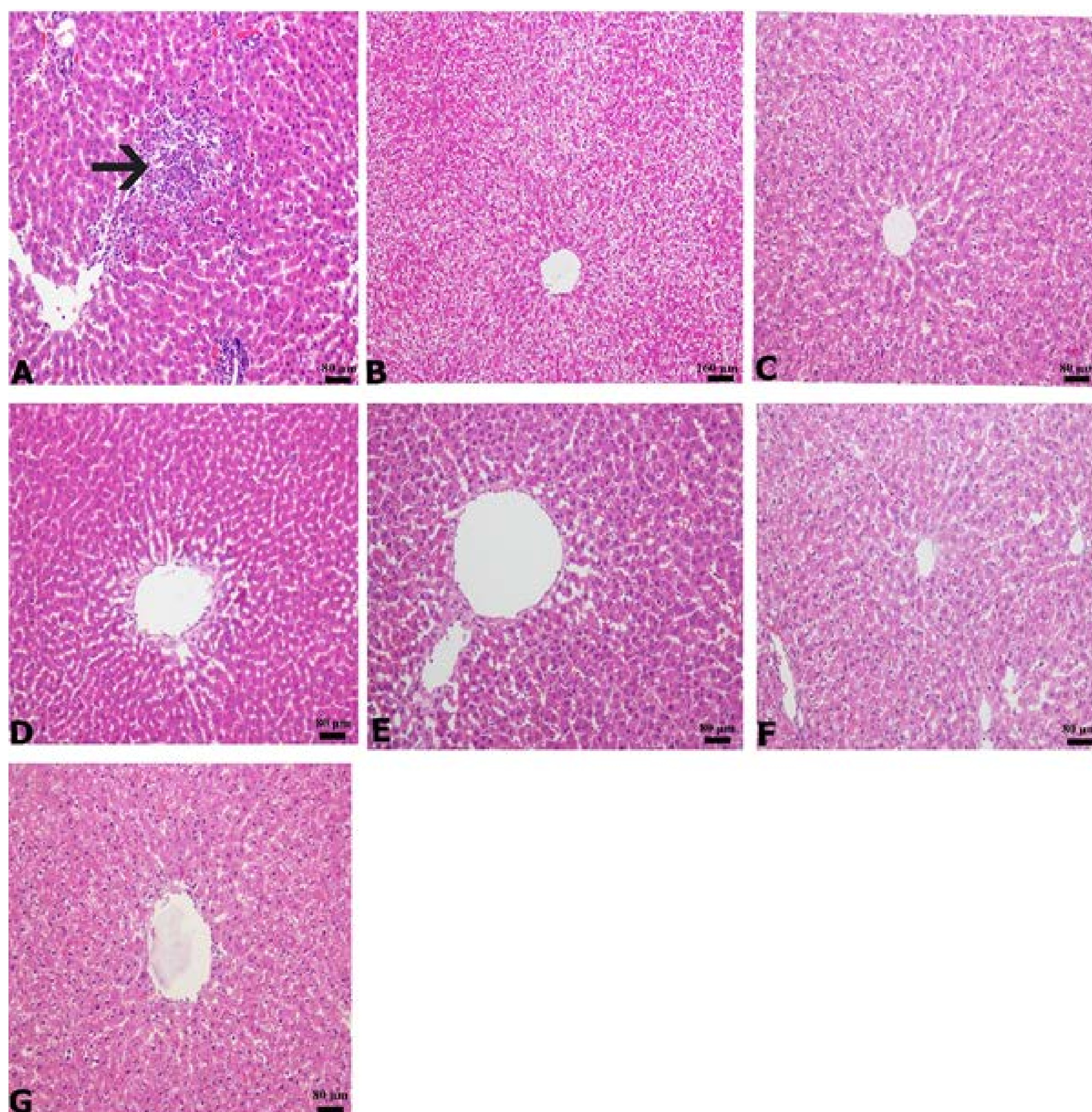


Figure 1. Hematoxylin-Eosin (H&E) staining photomicrographs of all experimental groups A: P, arrow points to the inflammation area (20x-80µm), B: NAC (10x-160µm), C: PCM+NAC (20x-80µm), D: PCM+J-HD (20x-80µm), E: PCM+J-LD (20x-80µm), F: J-HD (20x-80µm), G: CNT (20x-80µm).

Paracetamol administration significantly reduced catalase levels. This effect of paracetamol was partially blocked by a combination of paracetamol with NAC, J-HD, and J-LD, respectively.

Paracetamol significantly decreased GSH levels compared to the control group. In the positive control group, GSH levels increased to control levels when NAC was given following paracetamol. Jervine significantly increased GSH levels when administered after paracetamol at low and high doses. The difference between P + NAC group and P + J group was not statistically significant.

The highest MDA levels among the groups were observed in paracetamol-treated animals. NAC decreased this effect of paracetamol. However, when combined with paracetamol, J-HD and J-LD did not significantly alter the paracetamol effect.

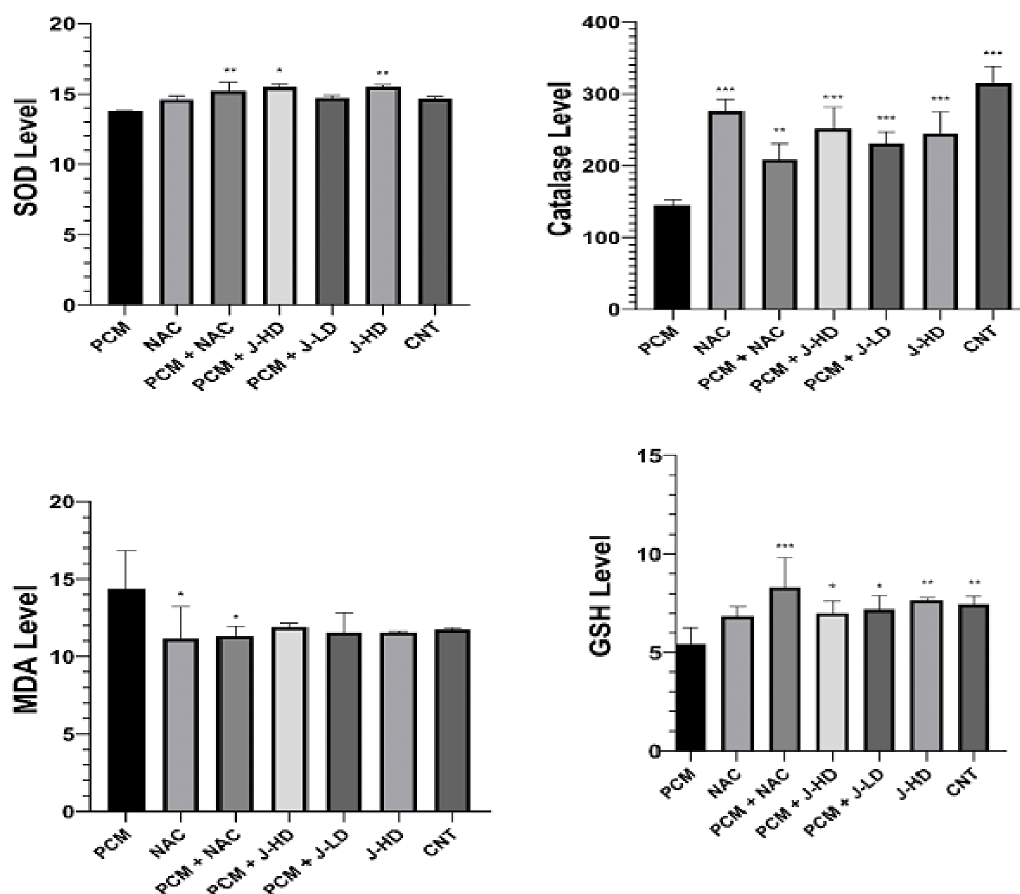


Figure 2. Effects on SOD, CAT, GSH and MDA levels in liver tissue.

DISCUSSION

Acetaminophen (paracetamol), a commonly prescribed analgesic and antipyretic, relieves mild to moderate pain and fever and is also used in combination with opioids in the treatment of chronic pain[20,21]. Drug-induced tissue damage frequently occurs in the liver[22]. Paracetamol overdose causes hepatotoxicity. Jervine, one of the steroidal alkaloids isolated from *Veratrum album*, has been used as a herbal folk medicine to treat hypertension, epilepsy, and lymphangitis[23]. In addition, jervine has various pharmacological activities including anti-inflammatory, anti-platelet, anti-tumor, and anti-adipogenic effects[24,25]. The primary objective of our study was to investigate the potential protective effect of Jervine on high-dose paracetamol-induced hepatotoxicity using histopathologic and biochemical methods.

In our study, while the liver tissues of the control group were normal, degeneration, inflammation, and hemorrhagic regions were detected in the liver tissue of the 2 mg/kg paracetamol-administered group. Administration of NAC, low and high dose Jervine 30 minutes before high dose paracetamol administration decreased the severity of paracetamol toxicity and prevented liver damage. It was found that SOD, CAT, and GSH levels, which decreased in the high-dose paracetamol group, increased significantly by administration of NAC and jervine 30 minutes before paracetamol administration and almost reached the control group levels. Increased MDA levels in the paracetamol group were significantly decreased with the administration of N-acetylcysteine

30 minutes before paracetamol. However, although Jervine decreased MDA levels, this difference was not significant.

The processing of exogenous substances in the liver occurs primarily by adding polar groups to the processed molecule by oxidation, reduction, or hydrolysis. These reactions are catalyzed by the cytochrome P450 (CYP) system present in centrilobular hepatocytes. These reactions allow drugs to be converted into active compounds but also lead to the formation of toxic by-products, such as ROS[26]. Various enzymes conjugate these molecules with glucuronic acid, sulfates, or glutathione (GSH). Most of the active conjugates for paracetamol are excreted in bile and urine. In contrast, approximately 5% to 9% of the inactive conjugates undergo oxidative conversion via CYPs to the toxic metabolite N-acetyl-p-benzoquinonimine (NAPQI), which is highly reactive[27,28]. NAPQI, which increases as a result of an overdose of paracetamol, consumes GSH in hepatocytes and after depletion of GSH stores, it binds to cellular proteins, thus disrupting the liver's detoxification capacity and increases the risk of hepatotoxicity[29]. Excessive CYP activation can lead to increased ROS generation, resulting in liver tissue damage[30,31]. When GSH reserves are reduced by approximately 80%, the liver's detoxification ability is exceeded. Consequently, NAPQI accumulates and interacts with the liver and other cells, causing tissue damage[29]. In our study, it was observed that Jervine administration in paracetamol toxicity increased the antioxidant systems activity such as SOD and CAT, increased GSH levels, and significantly contributed to the protection of liver tissue from the harmful effects of free radicals.

Dumlu et al. reported that in the inflammation model induced by carrageenan injection into rat paws, Jervine given orally at a dose of 400 mg/kg showed a more effective anti-inflammatory effect, reducing proapoptotic agents such as TNF-α and IL-1β compared to NSAIDs like diclofenac and indomethacin. In the same study, SOD and CAT levels increased, and GSH levels decreased after carrageenan injection. Jervine administration reduced SOD and CAT levels and increased GSH levels[9]. SOD catalyzes the dismutation of superoxide anion (O_2^-) to H_2O_2, and H_2O_2 is converted to H_2O and O_2 by CAT enzyme. Thus, the harmful effects of superoxide ions are eliminated. Although these findings do not seem to be compatible with our results except for the increase in GSH levels, both studies showed that Jervine administration results in SOD, CAT, and GSH levels approached the control group values. In a study consistent with our results, ischemia-reperfusion (IR) injury was induced in liver tissue, and it was observed that SOD activity decreased. In this study, SOD activity increased when *Veratrum nigrum L. var. ussuriense Nakai*, which also contains Jervine alkaloid, was given before IR injury. Similarly, edema, hemorrhage, and inflammatory cell infiltration caused by IR injury decreased due to the administration of *Veratrum nigrum L. var. ussuriense Nakai* alkaloid[32].

Paracetamol overdose is a well-known harmful factor for hepatotoxicity. NAC is a drug widely used in the clinic for paracetamol detoxification. However, its effectiveness is limited to the early stages of hepatotoxicity and often causes side effects such as vomiting, nausea, and even shock[33]. Therefore, it is important to elucidate the mechanism of paracetamol-induced hepatotoxicity and develop additional drugs for treatment. Our current study, through histopathological analyses, revealed that jervine significantly prevented paracetamol-induced liver damage including degeneration, inflammation, and hemorrhage. The upregulation of anti-apoptotic markers further confirmed the effectiveness of jervine in protecting against liver damage. A limitation of this study is that it was an animal experiment and the effects of jervine on humans may vary. Another limitation is that the effects of Jervine on other tissues have not been evaluated. Future studies should examine the long-term benefits, optimal dosage, and side effects of Jervine in terms of clinical safety and effectiveness. Jervine may lead to improved clinical outcomes for the prevention of liver damage. These results provide a framework for the development of innovative strategies in the clinic to treat hepatotoxicity, especially those induced by paracetamol overdose.

Author Contributions: Concept/Design : SY, MY; Data acquisition: SY; Data analysis and interpretation: TA, LD, AAK; Drafting manuscript: SY, SB, İY; Critical revision of manuscript: SAB; Final approval and accountability: SY, TA, SB, SAB, MY, LD, İY, AAK; Technical or material support: SY; Supervision: SY, SAB; Securing funding (if available): n/a.

Ethical Approval: Ethical approval was obtained from the Chairman of the Local Ethics Committee of Animal Experiments of Kafkas University with the decision dated 27.07.2021 and numbered 2021/129.

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Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support

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