Indian Journal of Experimental Biology
Vol. 63, September 2025, pp. 738-745
DOI: 10.56042/ijeb.v63i09.18020

Investigation of the protective effects of geraniol on cyclophosphamide-induced hepatotoxicity in rats

Halime Tuba Canbaz[1]*, Serpil Kalkan[2], Rahim Kocabas[3], Mehmet Enes Sozen[4] & Gokhan Cuce[2]

[1]Department of Histology and Embryology, Hamidiye Faculty of Medicine, University of Health Sciences, Istanbul, Turkey
[2]Department of Histology and Embryology, Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey
[3]Department of Medical Biochemistry, Faculty of Medicine, Karamanoglu Mehmetbey University, Karaman, Turkey
[4]Department of Histology and Embryology, Faculty of Medicine, Alanya Alaaddin Keykubat University, Antalya, Turkey

Received 18 March 2025; revised 03 June 2025

This study aimed to investigate the efficacy of geraniol (Ge) on improving cyclophosphamide (CP)-induced hepatotoxicity in rats. A total of 42 rats in 6 groups, group I (control); group II (CP at 20 mg/kg); group III (CP at 20 mg/kg + Ge at 100 mg/kg); group IV (CP at 20 mg/kg + Ge at 200 mg/kg); group V (Ge at 100 mg/kg); and group VI (Ge at 200 mg/kg), were used. Ge was administered orally via gavage daily for 14 days. Intraperitoneal CP was administered from day 8 to 14. Blood serums were analysed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), total antioxidant status (TAS), and total oxidant status (TOS) over a 14-day period. Liver sections were stained using hematoxylin and eosin (H&E). NFκB expression was assessed using immunohistochemistry. Bax-Bcl immunofluorescence was employed to identify apoptosis, whereas TUNEL was utilised to detect DNA fragmentation. Group III and group IV exhibited significantly decreased histopathological scores, NFκB expression, and DNA breaks compared to group II. The OSI and AST levels were significantly reduced in group IV compared to group II. The current investigation indicates that Ge possesses anti-inflammatory and antioxidant effects on CP-induced liver damage.

Keywords: Chemoprevention, Antioxidants, Histopathology, Liver, Apoptosis

Cyclophosphamide (CP) is a cytotoxic and strong alkylating agent commonly employed to immunosuppress and treat different malignancies[1]. Due to its immunosuppressive qualities, it is utilized in organ transplantation for the treatment of autoimmune disorders including as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus[2].

The therapeutic use of CP is associated with numerous undesirable consequences, including organ destruction[3]. CP toxicity results from deleterious byproducts of CP metabolism, such as phosphoramide and acrolein. These metabolic products exhibit chemical reactivity and induce cytotoxicity by alkylating DNA crosslinks and the DNA molecule itself. Reduced glutathione (GSH) can interact with acrolein, leading to the accumulation of reactive oxygen species (ROS), oxidative stress, and lipid peroxidation[4].

The next phase entails the activation of the nuclear factor kappa B (NF-κB) signaling pathway and an elevation in the production of inflammatory cytokines. NF-κB is a transcription factor chiefly accountable for modulating inflammation and apoptosis[4]. Studies have shown that compounds possessing antioxidant and free radical scavenging properties provide protection against CP-induced hepatotoxicity and oxidative stress[6,7].

Geraniol (Ge) is an acyclic monoterpene alcohol included in the essential oils of rose, lavender, lemon, and several other plants[8]. In addition to its widespread use in consumer products such as fragrances and cosmetics, Ge offers numerous health benefits, including antibacterial, anti-inflammatory, antioxidant, antiulcerative, anticarcinogenic, neuroprotective, and cardioprotective properties. Its pharmacological profile encompasses a broad spectrum of activities[8,9]. Ge has been shown to regulate signaling molecules and pathways associated with several biological processes, including the cell cycle, apoptosis, autophagy, and metabolism[10]. Ge is a potent antioxidant, and its hepatoprotective properties have been reported[11].

This study aimed to investigate the antiapoptotic and anti-inflammatory effects of Ge in rats with CP-induced liver damage.

*Correspondence:
Phone: +90 216 7778777
E-mail: halimetuba.canbaz@sbu.edu.tr

Materials and Methods

Animals and experimental design

Forty-two male Wistar Albino rats, aged 4 months and weighing between 250-300 g, were sourced from the Necmettin Erbakan University KONÜDAM Experimental Medicine Application and Research Center. The rats were administered a regular diet and supplied with tap water. The research was performed under regulated environmental parameters of room temperature (22 ± 2°C), 60% humidity, and a 12-hour light/dark cycle.

Six groups were formed from rats: Group I (n = 7) served as the control group; Group II (n = 7) was administered CP 20 mg/kg; Group III (n = 7) received CP 20 mg/kg combined with Ge 100 mg/kg; Group IV (n = 7) was given CP 20 mg/kg along with Ge 200 mg/kg; Group V (n = 7) received Ge 100 mg/kg; and Group VI (n = 7) was administered Ge 200 mg/kg.

The duration of the trial was 14 days. Ge (Sigma Aldrich, 163333) was administered orally by gavage once daily for 14 consecutive days at two different doses: 100 mg/kg and 200 mg/kg[12]. From days 8 to 14, CP (Endoxan, 500 mg IV solution prepared for infusion) was administered intraperitoneally once daily at a dose of 20 mg/kg[13].

At the end of the experiment, intraperitoneal injections of 10 mg/kg xylazine and 90 mg/kg ketamine were administered to produce anesthesia for the collection of blood samples from the animals' hearts. Subsequently, the rats were euthanised via cervical dislocation while still anaesthetized. Subsequent to the sacrificial procedure, the animals' abdomens were incised, and their livers were extracted and preserved in a neutral formaldehyde solution. The tissues were excised for processing following a 48-hour fixation period.

Biochemical analyses including oxidative stress parameters

To extract serum, the blood samples were placed in sterile tubes with anticoagulant, labelled, and centrifuged for 10 minutes at 3500 rpm. Before further analysis, the separated sera were aliquoted into Eppendorf tubes and stored at -40°C.

The ALT and AST values of the obtained sera were analysed. The total oxidant status (TOS) and total antioxidant status (TAS) of serum samples were assessed using ELISA kits in accordance with the manufacturer's instructions. Subsequently, the oxidative stress index (OSI) was calculated using the formula: OSI = (TOS/TAS) × 100.

Histopathological evaluation of liver

Liver tissue was fixed for 48 hours in 10% neutral formaldehyde, sliced to the proper size, coded, and arranged on cassettes. Subsequently, paraffin tissue blocks were prepared for each animal using standard tissue processing methods. Each paraffin block was sliced to a thickness of 4 µm using a Microm HM325 rotary microtome. After mounting and labeling, tissue sections three sections per slide were created. Samples from each animal were stained with H&E to assess changes in the liver tissue.

H & E-stained slides were examined microscopically at various magnifications to identify histological alterations, including sinusoidal dilation, lymphocyte infiltration, and congestion. The slides were subsequently evaluated and scored using the following criteria: 0 expresses = none; 1 expresses = mild; 2 expresses = moderate; and 3 expresses = intense[14].

Immunohistochemical staining

Sections of 4 µm in thickness were extracted from the prepared paraffin blocks and mounted on slides coated with poly-L-lysine. Following an overnight deparaffinization procedure, antigen retrieval was performed utilizing citrate buffer, inhibiting endogenous peroxidase activity with 3% hydrogen peroxide, and super blocking was executed. Thermo Scientific, RB-9034-P, primer antibody-Anti NFκB was incubated overnight at +4°C. The secondary antibody was administered to the sections for 20 minutes. Streptavidin-peroxidase was employed for 20 minutes to identify the antigen-antibody complex. DAB chromogen was administered for 15 minutes. The staining of the sections was evaluated according to the following criteria: 0 indicates none, 1 indicates mild, 2 indicates moderate, and 3 indicates strong[15].

TUNEL assay and the assessment of APOI

To evaluate DNA fragmentation and apoptotic cell death, cells were stained with the *in situ* apoptosis detection kit (ApopTag Plus, *In Situ* Apoptosis Detection Peroxidase kit, S7101-KIT, Chemicon).

TUNEL labelling was performed to evaluate apoptosis in liver tissue slices from all experimental groups. The apoptotic index (APOI) was calculated by dividing the count of positively stained nuclei by the total number of nuclei in the field[13].

Immunofluorescence staining

Sections of 4 µm in thickness were extracted from the prepared paraffin blocks and mounted on slides coated with poly-L-lysine. Following an overnight

Table 1 — TAS, TOS, and OSI values

Groups	TAS (mean±SEM)	TOS (mean±SEM)	OSI (mean±SEM)
Control	1.51±0.02	1.14±0.27	0.07±0.01
CP	1.17±0.14 [a]	2.45±0.78 [a]	0.22±0.06 [a]
CP+Ge100	1.21±0.05 [b]	1.8±0.28 [b]	0.14±0.02 [b]
CP+Ge200	1.34±0.01 [b]	1.67±0.22 [b]	0.12±0.01 [c]
Ge100	1.52±0.02 [d]	1.42±0.16 [d]	0.09±0.01 [d]
Ge200	1.42±0.02 [d]	1.41±0.28 [d]	0.09±0.01 [d]

Oxidative stress index, OSI; Total antioxidant situation, TAS; Total oxidant situation, TOS. One Way Anova, Duncan, HSD.[[a]P<0.05 with respect to control group; [b]P>0.05 with respect to CP group; [c]P<0.05 with respect to CP group; [d]P>0.05 with respect to control group]

deparaffinization procedure, antigen retrieval was performed utilizing citrate buffer, inhibiting endogenous peroxidase activity with 3% hydrogen peroxide, and super blocking was executed. Bax (ABclonal A19684) Bcl-2 (ABclonal A21592) applied and waited overnight at +4°C. Alexa Fluor® 488 (ab150077) used as secondary antibody for 1 hour at room temperature. DAPI used for nuclear staining. The intensity observed using an inverted Axio Scope A1 Microscope (Zeiss, Germany) equipped with a digital camera.

Statistical analyses

The Statistical Package for Social Sciences (version 25.0) was utilized for the statistical study. A P-value of less than 0.05 was deemed indicative of statistical significance. Continuous variables were presented as the mean ± standard error of the mean (SEM). One-Way ANOVA and Tukey HSD tests were conducted on TAS, TOS, OSI, ALT, and AST results. The One-Way ANOVA test was utilized for histopathology data, NFκB expression, Bax and Bcl-2 expression intensities and TUNEL application data, while matched groups were reassessed using the Independent t-Test.

Results

Biochemical findings and oxidative stress parameters

At the end of the experiment, blood serums were sent to Necmettin Erbakan University Meram Medical Faculty Biochemistry laboratory for oxidative stress marker (TAS and TOS) analysis. The results obtained are given in the Table 1.

The serum TOS level in group II was higher compared to group I (P<0.05). However, there was no statistically significant difference observed between the group II and either group III or group IV (P>0.05 for both).

The serum TAS level was significantly lower in group II compared to group I (P<0.05). There was no statistically significant difference observed between group II and either group III or group IV (P>0.05 for both).

Table 2 — AST, ALT values

Groups	AST (mean±SEM)	ALT (mean±SEM)
Control	72.14±6.48	36.57±4.65
CP	99.00±6.16 [a]	47.00±3.23 [a]
CP+Ge100	91.14±3.48 [b]	43.14±1.37 [b]
CP+Ge200	81.85±4.28 [c]	43.42±1.41 [b]
Ge100	69.57±6.99 [d]	39.57±3.13 [d]
Ge200	75.14±4.10 [d]	38.42±3.60 [d]

One Way Anova, Duncan, HSD.[[a]P<0.05 with respect to control group; [b]P>0.05 with respect to CP group; [c]P<0.05 with respect to CP group; [d]P>0.05 with respect to control group]

OSI values were statistically significantly higher in group II compared to group I (P<0.05). However, while there was no statistically significant difference observed between group II and group III (P>0.05), OSI values were significantly lower in group IV compared to group II (P<0.05).

The serum results obtained from biochemistry laboratory are presented in the Table 2. Serum AST levels were significantly elevated in group II compared to group I (P<0.05). No statistically significant difference was detected between group III and group II (P>0.05); however, a statistically significant difference was noted between group IV and group II (P<0.05).

Serum ALT levels were significantly lower in the control group compared to group II, group III, and group IV (P<0.05 for all comparisons). Nonetheless, no statistically significant difference detected between the group II and either the group III or group IV (P>0.05 for both comparisons).

Histopathological scores

The histopathological score was considerably elevated in group II compared to control group (P<0.05). The histopathological score was considerably reduced in group III and group IV

Table 3 — Histopathological scores, NFκB expression, and the intensities of Bax and Bcl-2 expression in groups

Groups	Histopathological score	NFκB expression	Bax intensity	Bcl-2 intensity
Control	0±0	0±0	34.54±0.82	32.73 ± 1.97
CP	2.42±0.20 [a]	2.42±0.20 [a]	66.97 ± 1.10 [a]	13.34 ± 0.47 [a]
CP+GE100 mg/kg	1.28±0.18 [b]	1.28±0.18 [b]	44.37 ± 0.68 [b]	28.56 ± 0.66 [b]
CP+GE200 mg/kg	1.00±0.21 [b]	1.00±0.21 [b]	41.37 ± 0.53 [b]	36.65 ± 1.52 [b]
GE100 mg/kg	0±0	0±0	29.45 ± 2.27	29.86 ± 0.48
GE200 mg/kg	0±0	0±0	31.30 ± 1.27	31.36 ± 1.21

One Way Anova, TUKEY HSDa. [[a]P<0.05 with respect to control group; [b]P>0.05 with respect to CP group]

Table 4 — Numbers of TUNEL positive cells in groups

Groups	TUNEL positive cells (mean±SEM)
Control	3.00±0.53
CP	16.42±1.26 [a]
CP+GE100 mg/kg	9.14±0.59 [b]
CP+GE200 mg/kg	8.42±0.61 [b,c]
GE100 mg/kg	3.57±0.48
GE200 mg/kg	3.42±0.59

One Way Anova, TUKEY HSDa. [[a]P<0.05 with respect to control group; [b]P<0.05 with respect to CP group; [c]P>0.05 with respect to CP+GE 100 mg/kg]

compared to group II (P<0.05 for both). However, there is no significant difference between group III and group IV (P>0.05). The histopathological scores for all groups are displayed in Table 3 and shown in Fig. 1.

Immunohistochemical staining

The NFκB expression scores align with the histopathology scores presented in Table 3 and shown in Fig. 2, indicating a notable rise in group II and a significant decrease in the CP+Ge groups, regardless of Ge dosage.

TUNEL

Cells exhibiting stained nuclei were recognised as apoptotic cells subsequent to TUNEL labeling. TUNEL staining results were evaluated using One-Way ANOVA (Table 4).

The number of TUNEL-positive cells was significantly elevated in group II compared to group I (P<0.05). In contrast, the number of TUNEL-positive cells was significantly lower in both the group III and group IV compared to group II (P<0.05 for both). However, there was no statistically significant difference between group III and group IV (P>0.05).

Immunofluorescence staining

The intensity of Bax and Bcl-2 expressions calculated using Image J. Bax signaling is

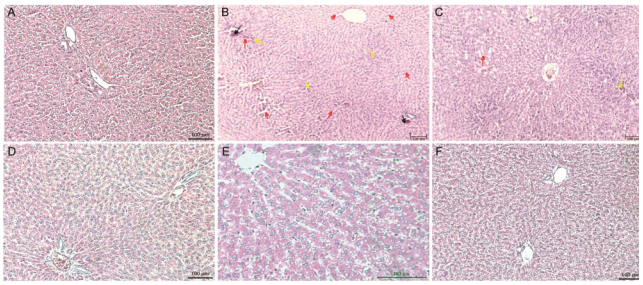

Fig. 1 — H&E-stained liver sections. (A) Group 1, Control; (B) Group 2, CP; (C) Group 3, CP+Ge 100mg/kg; (D) Group 4, CP+Ge 200mg/kg; (E) Group 5, Ge 100mg/kg; (F) Group 6, Ge 200mg/kg. Yellow arrows show congestion, black arrows show lymphocyte infiltration and red arrows show sinusoid dilatations (Scale bars = 100 μm).

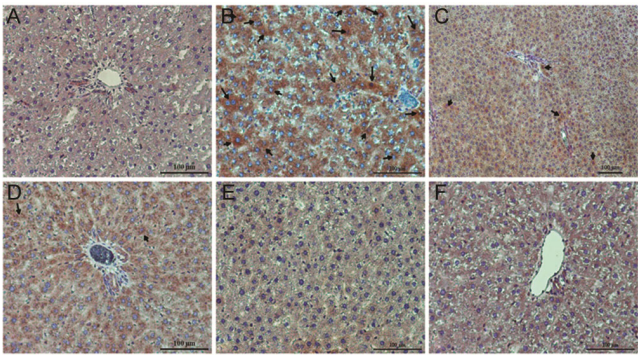

Fig. 2 — NFκB expression. (A) Group 1, Control; (B) Group 2, CP; (C) Group 3, CP+Ge 100mg/kg; (D) Group 4, CP+Ge 200mg/kg; (E) Group 5, Ge 100mg/kg; (F) Group 6, Ge 200mg/kg. Black arrows show expressions (Scale bars = 100 μm).

significantly increased in group II compared to group I, II and III. And conversely the intensity of Bcl-2 signaling is significantly decreased in group II compared to group I, II and III (Table 3, Fig. 3).

Discussion

CP is a highly toxic pharmaceutical agent commonly used in chemotherapy and immunosuppression[16,17]. A substantial body of research has focused on agents with free radical scavenging and antioxidant properties, with the goal of reducing and preventing the toxicity associated with CP use[18,19].

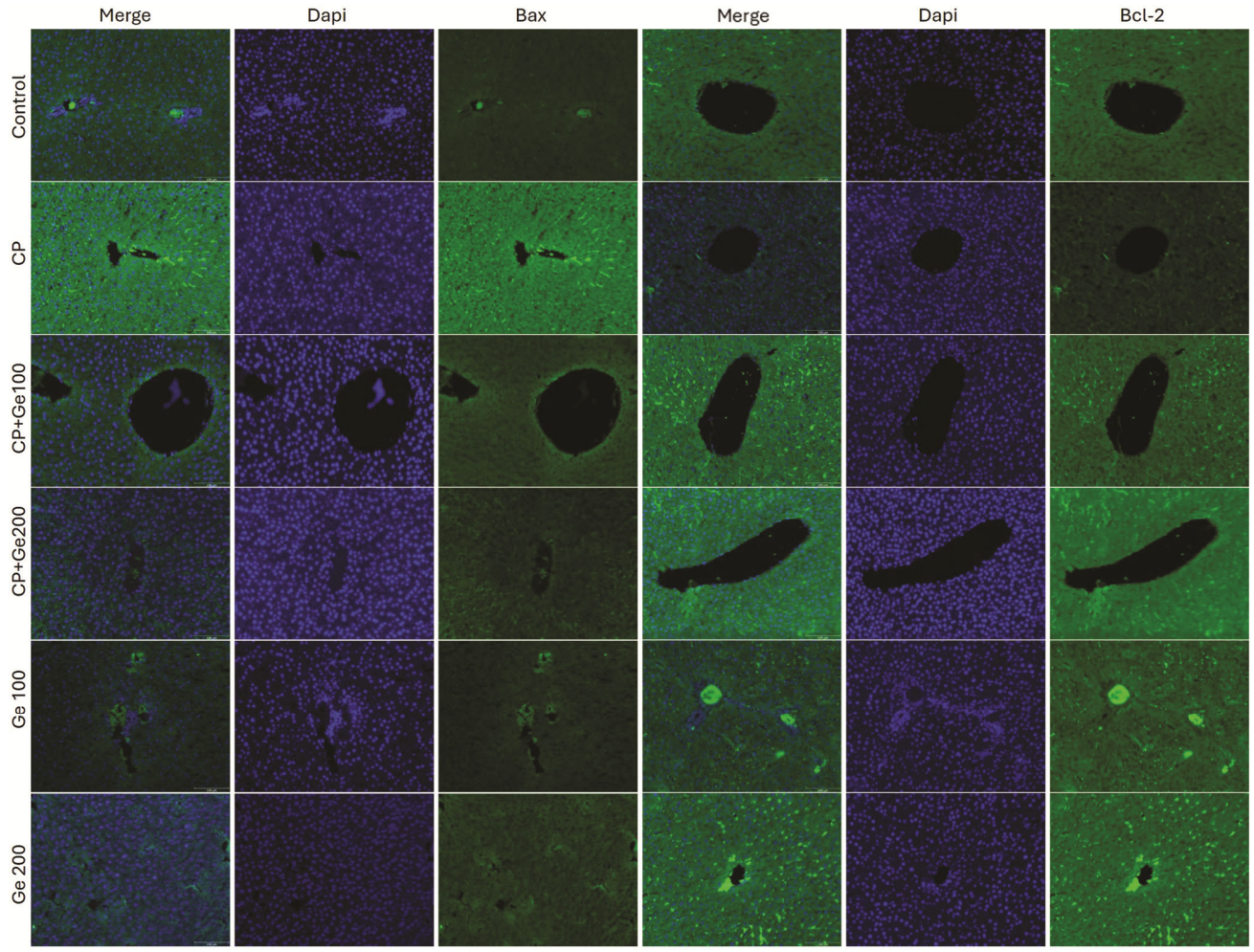

Fig. 3 — Immunofluorescence staining reveals the expression intensity of Bax and Bcl-2 (Scale bars = 100 μm).

The liver is one of the organs most affected by CP toxicity. For this reason, this study focused on liver examination and utilized a CP dosage derived from prior research, administering a quantity enough to elicit toxicity[13]. In this study; histopathological examination revealed that CP caused disruption of liver structure, degeneration of hepatocytes, lymphocyte infiltration, congestion, and sinusoid dilatation. These pathologic changes are consistent with previous studies[20,21]. The histopathological damage scores in the present study were significantly higher in the CP-treated group compared to the other groups. Ge significantly reduced the damage in both dosages used. The damaging consequences of CP are mainly facilitated by the oxidative stress it induces, resulting in numerous disturbances in cellular functions[22]. These effects of oxidative stress can be alleviated with the use of antioxidants[23,24]. Previous studies have demonstrated that Ge functions as an antioxidant[25,26], and this study revealed its antioxidant effect through the enhancement of hepatocyte structure, constriction of dilated sinusoids, alleviation of congestion, and a reduction in inflammatory cells in the Ge-treated groups subjected to CP treatment.

CP metabolises its products and generates ROS, which induce oxidative stress[27,28]. Hepatotoxicity induced by oxidative stress is characterised by increased TOS levels and decreased TAS levels, as reported in the literature and confirmed in this study[29]. The equilibrium between oxidant and antioxidant levels is disrupted with CP, and Ge repairs this imbalance by bolstering antioxidant levels, particularly Ge at a dose of 200 mg/kg significantly ameliorates OSI levels in CP treatment.

Excessive production of ROS initiates the inflammatory process. The NFκB signaling pathway is one of the most important ways to observe the progression to inflammation. Studies revealed that CP

treatment causes elevation in NFκB signaling, NFκB related to inflammation[4,30]. Immunohistochemical staining results of this study also showed the same results, NFκB expression in CP is too intense in connection with the inflammation process, regardless of the dose taken Ge decreased the intensity of NFκB expression in CP treated group because of the anti-inflammatory effect of Ge. The results of NFκB expression level scoring and histopathologic scoring in this study groups were found to be mutually supportive.

It has been observed that Ge administered prior to inflammation increases the anti inflammatory effect[31]. In this study; the anti-inflammatory effect observed with both doses of Ge can be explained by the fact that CP was started while the body's antioxidant metabolism dominated the toxicity, because Ge was started before CP and the antioxidant support continued during the time CP was administered.

Free radicals generated by oxidative stress stimulate apoptosis and cause mitochondrial damage. Bax is a pro-apoptotic protein that disrupts the mitochondrial membrane, leading to caspase activation and ultimately cell death. In contrast, Bcl-2, a member of the same protein family, exerts an antiapoptotic effect by binding to Bax and inhibiting apoptosis. It has been reported that Bax levels increase, while Bcl-2 levels decrease, in liver damage induced by oxidative stress[32]. CP treatment promotes apoptosis and increases Bax expression. In this study, immunofluorescence staining revealed elevated Bax expression in the CP-treated group[33]. TUNEL staining indicates apoptosis by labelling of DNA fragments, also shows significantly increased APOI in CP treated group. Ge one of the antiapoptotics used in previous studies[34,35] also showed its antiapoptotic property and increase the expression of Bcl-2 in this study, the CP groups which administered Ge has significantly lower APOI in TUNEL staining and this results supports the antiapoptotic effect of Ge in both two doses.

Previous studies have demonstrated that CP impairs liver function tests and antioxidants have been reported to decrease the levels of enzymes[36,37]. Ge also decrease the elevated levels of AST and ALT[38]. In this study CP significantly elevates the AST and ALT levels. However, despite not reaching the anticipated peak, Ge has been observed to mitigate this effect in this study, with Ge at a dose of 200 mg/kg exhibiting a significant improvement in AST levels. In instances of ischemia and toxicity, the AST/ALT ratio tends to increase[14]. However, the administration of Ge has been shown to reverse this ratio, indicating a potential for therapeutic benefit. Considering previous studies and this study, it was concluded that endogenous metabolism of Ge may also slightly increase these levels.

Conclusion

This study demonstrated that the CP induces oxidative stress in the liver, whereas Ge administration effectively ameliorates hepatotoxicity by scavenging free radicals and enhancing antioxidant metabolism. The hepatoprotective effect of Ge is likely mediated through its antiapoptotic and anti-inflammatory properties, including suppression of NFκB and Bax, beside the enhacement of Bcl-2 activity, with efficacy observed at both 100 mg/kg and 200 mg/kg doses. While both dosages alleviated hepatotoxic effects, the 200 mg/kg dose exhibited superior biochemical efficacy, evidenced by greater antioxidant activity and a reduced apoptotic index. These findings underscore the significant role of Ge administration in mitigating CP-induced liver damage and suggest a dose-dependent protective effect.

Ethical statement

Experimental procedures were performed with approval obtained from Necmettin Erbakan University KONUDAM Experimental Medicine and Research Center Local Ethics Committee for Animal Experiments, Konya, Turkey (Approval number: 2017-022).

Funding statement

This study was supported by Necmettin Erbakan University Scientific Research Projects Coordinatorship with decision number 171418005.

Conflict of interest

The authors declare no conflicts of interest.

References

1 Jali AM, Alam MF, Hanbashi A, Mawkili W, Abdlasaed BM, Alshahrani S, Qahl AM, Alrashah ASS & Shahi HA. Sesamin's therapeutic actions on cyclophosphamide-induced hepatotoxicity, molecular mechanisms, and histopathological characteristics. *Biomedicines*, 11 (2023) 3238.

2 Highley MS, Landuyt B, Prenen H, Harper PG & De Bruijn EA. The Nitrogen Mustards. *Pharmacol Rev*, 74 (2022) 552

3 Alruhaimi RS. Protective effect of arbutin against cyclophosphamide-induced oxidative stress, inflammation,

and hepatotoxicity via Nrf2/HO-1 pathway in rats. *Environ Sci Pollut Res Int*, 30 (2023) 68101.

4 Mostafa RE, Morsi AH & Asaad GF. Piracetam attenuates cyclophosphamide-induced hepatotoxicity in rats: amelioration of necroptosis, pyroptosis and caspase-dependent apoptosis. *Life Sci*, 303 (2022) 120671.

5 Golmohammadi MG, Banaei S, Timar M & Abedi A. Saponin protects against cyclophosphamide-induced kidney and liver damage via antioxidant and anti-inflammatory actions. *Physiol Int*, 110 (2023) 108.

6 Althunibat OY, Abukhalil MH, Jghef MM, Alfwuaires MA, Algefare AI, Alsuwayt B, Alazragi R, Abourehab MAS, Almuqati AF, Karimulla S & Aladaileh SH. Hepatoprotective effect of taxifolin on cyclophosphamide-induced oxidative stress, inflammation, and apoptosis in mice involvement of Nrf2/HO-1 signaling. *Biomol Biomed*, 23 (2023) 649.

7 Özatik FY, Özatik O, Tekşen Y, Koçak H, Arı NS & Çengelli Ünel Ç. Dose-dependent effect of hydrogen sulfide in cyclophosphamide-induced hepatotoxicity in rats. *Turkish J Gastroenterol*, 34 (2023) 626.

8 Maczka W, Winska K & Grabarczyk M. One hundred faces of geraniol. *Molecules*, 25 (2020) 3303.

9 El Azab EF, Saleh AM, Yousif SO, Mazhari BBZ, Abu Alrub H, Elfaki EM, Hamza A & Abdulmalek S. New insights into geraniol's antihemolytic, anti-inflammatory, antioxidant, and anticoagulant potentials using a combined biological and *in silico* screening strategy. *Inflammopharmacology*, 30 (2022) 1811.

10 Ben Ammar R. Potential effects of geraniol on cancer and inflammation-related diseases: a review of the recent research findings. *Molecules*, 28 (2023) 3669.

11 Tunç E, Durgun V, Akıncı O, Ergün S, Şimşek O, Bolayırlı IM & Kepil N. The role of geraniol on hepatic ischemia-reperfusion injury in rats. *Ulus Travma Acil Cerrahi Derg*, 30 (2024) 390.

12 Alasmari AF, Ali N, Alharbi M, Alqahtani F, Alasmari F, Almoqbel D, AlSwayyed M, Alshammari A, Alanazi MM, Alhoshani A & Al-Harbi NO. Geraniol ameliorates doxorubicin-mediated kidney injury through alteration of antioxidant status, inflammation, and apoptosis: potential roles of NF-κB and Nrf2/HO-1. *Nutrients*, 14 (2022) 1620.

13 Cuce G, Çetinkaya S, Koc T, Esen HIH, Limandal C, Balcı T, Kalkan S & Akoz M. Chemoprotective effect of vitamin E in cyclophosphamide-induced hepatotoxicity in rats. *Chem Biol Interact*, 232 (2015) 7.

14 Karabekir SC, Gultekin B, Ayan IC, Savas HB, Cuce G & Kalkan S. Protective effect of astaxanthin on histopathologic changes induced by bisphenol A in the liver of rats. *Pak Vet J*, 44 (2024) 244.

15 Cuce G, Esen HH, Koc T, Canbaz HT, Limandal C, Kalkan S & Gurbilek M. Vitamin E partially ameliorates cyclophosphamide-induced nephrotoxicity in rats. *Prog Nutr*, 18 (2016) 140.

16 Maleki R, Noorbakhsh MF, Kazemipour N, Masoudian M, Namazi F & Nazifi S. The hepatoprotective effects of sitagliptin against cyclophosphamide-induced hepatotoxicity in rat. *Mol Biol Res Commun*, 13 (2024) 193.

17 Cengiz M, Kutlu HM, Peker Cengiz B & Ayhancı A. Escin attenuates oxidative damage, apoptosis and lipid peroxidation in a model of cyclophosphamide-induced liver damage. *Drug Chem Toxicol*, 45 (2022) 1180.

18 Cuce G, Canbaz H, Sozen M, Yerlikaya F & Kalkan S, Vitamin E and selenium treatment of monocrotaline induced hepatotoxicity in rats. *Biotech Histochem*, 92 (2017) 59.

19 Patwa J, Khan S & Jena G. Nicotinamide attenuates cyclophosphamide-induced hepatotoxicity in SD rats by reducing oxidative stress and apoptosis. *J Biochem Mol Toxicol*, 34 (2020) e22558.

20 Akamo AJ, Rotimi SO, Akinloye DI, Ugbaja RN, Adeleye OO, Dosumu OA, Eteng OE, Amah G, Obijeku A & Cole OE. Naringin prevents cyclophosphamide-induced hepatotoxicity in rats by attenuating oxidative stress, fibrosis, and inflammation. *Food Chem Toxicol*, 153 (2021) 112266.

21 Selamoglu Z & Yilmaz I. The investigation of the antioxidative properties of the synthetic organoselenium compounds in liver tissue of rat with histological and biochemical analyses. *J Pharm Care*, 2 (2014) 162.

22 Huang Y, Ma C, Zhu L, Kong L, Huang C, Yang W, He J, Yang M, Huang L, Yuan L & Yi J. The ameliorative effect of betulinic acid on oxidative stress in mice of cyclophosphamide-induced liver damage. *Environ Toxicol*, 40 (2025) 608.

23 Turedi S. Protective/preventive effects of quercetin against cyclophosphamide-induced hepatic inflammation, apoptosis and fibrosis in rats. *Hepatol Forum*, 4 (2023) 135.

24 Alam MF, Ajeibi AO, Safhi MH, Alabdly AJA, Alshahrani S, Rashid H, Qadri M, Jali AM, Alqahtani S, Nomier Y, Moni SS, Khalid M & Anwer T. Therapeutic potential of capsaicin against cyclophosphamide-induced liver damage. *J Clin Med*, 12 (2023) 911.

25 Alzahrani SA, Bekhet GM, Ammar R Ben, Abdallah BM, Ali EM, Al Ramadan SY, Althumairy D & Rajendran P. The inhibitory effect of geraniol on CCL4-induced hepatorenal toxicity in pregnant mice through the PI3K/AKT signaling pathway. *Saudi J Med Med Sci*, 12 (2024) 17.

26 Algefare AI, Alfwuaires M, Famurewa AC, Elsawy H & Sedky A. Geraniol prevents CCL4-induced hepatotoxicity via suppression of hepatic oxidative stress, pro-inflammation and apoptosis in rats. *Toxicol Reports*, 12 (2024) 128.

27 Al-Amarat W, Abukhalil MH, Alruhaimi RS, Alqhtani HA, Aldawood N, Alfwuaires MA, Althunibat OY, Aladaileh SH, Algefare AI, Alanezi AA, AbouEl-Ezz AM, Ahmeda AF & Mahmoud AM. Upregulation of Nrf2/HO-1 signaling and attenuation of oxidative stress, inflammation, and cell death mediate the protective effect of apigenin against cyclophosphamide hepatotoxicity. *Metabolites*, 12 (2022) 648.

28 Ijaz MU, Mustafa S, Batool R, Naz H, Ahmed H & Anwar H. Ameliorative effect of herbacetin against cyclophosphamide-induced nephrotoxicity in rats via attenuation of oxidative stress, inflammation, apoptosis and mitochondrial dysfunction. *Hum Exp Toxicol*, 41 (2022) .

29 Sozen ME, Savas HB & Cuce G. Protective effects of selenium against acrylamide-induced hepatotoxicity in rats. *Pak Vet J*, 44 (2024) 274

30 Bokhary T, Refaat B, Bakr ES, Baz S, Rajab B, Gadalla H & El-Boshy M. Salvadora persica extract attenuates cyclophosphamide-induced hepatorenal damage by modulating oxidative stress, inflammation and apoptosis in rats. *J Integr Med*, 20 (2022) 348.

31 Marcuzzi A, Pontillo A, Leo L De, Tommasini A, Decorti G, Not T & Ventura A, Natural isoprenoids are able to reduce inflammation in a mouse model of mevalonate kinase deficiency. *Pediatr Res*, 64 (2008) 177.

32 Badr G, Sayed EA, Waly H, Hassan KAH, Mahmoud MH & Selamoglu Z. The therapeutic mechanisms of propolis against CCL4-mediated liver injury by mediating apoptosis of activated hepatic stellate cells and improving the hepatic architecture through PI3K/AKT/mTOR, TGF-β/Smad2, Bcl2/BAX/P53 and iNOS signaling pathways. *Cell Physiol Biochem*, 53 (2019) 301.

33 Alfwuaires MA. Rosmarinic acid protects against cyclophosphamide-induced hepatotoxicity via inhibition of oxidative stress, inflammation, and apoptosis and upregulation of Nrf2 in mice. *J Mol Histol*, 56 (2024) 49.

34 El-Emam SZ, Soubh AA, Al-Mokaddem AK & Abo El-Ella DM. Geraniol activates Nrf-2/HO-1 signaling pathway mediating protection against oxidative stress-induced apoptosis in hepatic ischemia-reperfusion injury. *Naunyn Schmiedebergs Arch Pharmacol*, 393 (2020) 1849.

35 Mohamed ME, Elmorsy MA & Younis NS. Renal ischemia/reperfusion mitigation via geraniol: the role of Nrf-2/HO-1/NQO-1 and TLR2,4/MYD88/NFκB pathway. *Antioxidants*, 11 (2022) 1568.

36 El Beheiry KM, El Sayed El-Sayad M, El Masry TA & Elsisi AE. Combination of metformin and hesperidin mitigates cyclophosphamide-induced hepatotoxicity. emerging role of PPAR-γ/Nrf-2/NF-κB signaling pathway. *Int Immunopharmacol*, 117 (2023) 109891.

37 Elshater AEA, Haridy MAM, Salman MMA, Fayyad AS & Hammad S. Fullerene C_{60} nanoparticles ameliorated cyclophosphamide-induced acute hepatotoxicity in rats. *Biomed Pharmacother*, 97 (2018) 53.

38 Khoshnazar SM, Mohagheghi M, Rahimi S, Dabiri S, Shahrokhi N & Shafieipour S. Geraniol modulates inflammatory and antioxidant pathways to mitigate intestinal ischemia–reperfusion injury in male rats. *Naunyn Schmiedebergs Arch Pharmacol*, 398 (2025) 8713.