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Black radish root extract alleviates sodium valproate–induced liver damage *via* inhibiting mitochondrial membrane potential collapse and oxidative stress in miceMohammad Hadi Zarei^{1✉}, Sami Akbulut², Maryam Zafari³, Elham Saghaei⁴, Zahra Lorigooini¹, Hossein Amini Khoei¹, Somaye Khosravi⁵, Elham Bijad¹¹Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran²Department of Liver Transplant Institute and Biostatistics, Inonu University Faculty of Medicine, 44280, Malatya, Turkey³Student Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran⁴Physiology and Pharmacology Department, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran⁵Department of Anatomical Sciences, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran

ABSTRACT

Objective: To explore the effect of black radish (*Raphanus sativus* L. var niger) root extract on liver enzymes, oxidative stress, and histopathological alterations in mice with sodium valproate-induced hepatotoxicity.

Methods: Thirty-two mice were divided into four groups: the control group received drinking water by gavage, the second group was administered with 100 mg/kg of sodium valproate, the third group received 300 mg/kg of black radish root extract, and the fourth group was given both sodium valproate (100 mg/kg) and black radish root extract (300 mg/kg). After 28 days, the mice were euthanized, and serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), along with liver malondialdehyde (MDA), reactive oxygen species (ROS), mitochondrial parameters, tumor necrosis factor- α (TNF- α) gene expression, and histopathological changes were assessed.

Results: Sodium valproate caused hepatic damage in mice, characterized by elevated serum levels of liver enzymes, increased MDA and ROS levels and TNF- α gene expression, as well as histopathological alterations. The black radish root extract significantly alleviated sodium valproate-caused hepatic injury by decreasing the serum levels of ALT and AST, MDA, ROS, TNF- α gene expression, as well as mitochondrial impairment, but did not have a significant effect on sodium valproate-induced histopathological changes.

Conclusions: The black radish root extract demonstrates protective effects against sodium valproate-induced liver injury, possibly

through mitigating oxidative stress, mitochondrial impairment, and inflammatory mediator expression.

KEYWORDS: Black radish; *Raphanus sativus*; Sodium valproate; Hepatotoxicity; Oxidative stress; Anti-inflammation

1. Introduction

Sodium valproate (SVP) is a commonly prescribed antiepileptic medication utilized for the management of epilepsy, seizures,

Significance

The beneficial effects of *Raphanus* species on liver damage have been reported in previous investigations. In the present study, the results show that black radish root extract could mitigate sodium valproate-induced liver damage by reducing oxidative stress, mitochondrial impairment, and inflammatory mediator expression.

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and migraine[1]. Despite its clinical benefits, the administration of SVP has been associated with severe adverse effects, notably hepatotoxicity[2,3]. The precise mechanisms underlying SVP-induced liver injury remain incompletely understood. However, it is well-established that SVP-induced hepatotoxicity involves a complex interplay of pathways, including oxidative stress, inflammation, and fibrosis[4,5]. The pathological processes linked to SVP-induced hepatotoxicity often result from the overproduction of reactive oxygen species (ROS) and disruption in the equilibrium of antioxidant defenses, leading to oxidative stress. Oxidative stress triggers the activation of various transcription factors that regulate the expression of genes involved in inflammatory cascades. Inflammatory responses triggered by oxidative stress are implicated in the pathogenesis of numerous chronic disorders. The inflammatory and immune reactions incited by SVP treatment prompt the secretion of inflammatory cytokines, with TNF- α playing a prominent regulatory role. Previous investigations have highlighted a notable upregulation in TNF- α expression after SVP exposure[6,7].

The liver, a key metabolic organ responsible for various biochemical processes, is commonly targeted by a range of toxic substances[8]. One of the liver's crucial functions is the conversion of lipophilic toxins into hydrophilic metabolites, facilitating their elimination from the body through urine[9]. Liver damage represents a significant global health concern, underscoring the importance of identifying effective preventive agents to mitigate liver injury. Despite the absence of definitive pharmaceutical interventions for liver protection, herbal remedies have emerged as potential candidates for managing various liver disorders. Herbal supplements are often recognized for their hepatoprotective effects, fostering the natural healing mechanisms associated with liver ailments[10].

Black radish (*Raphanus sativus* L. var *niger*), a member of the Brassicaceae family, is a widely utilized vegetable in culinary practices and traditional medicine globally, known for its therapeutic potential in addressing metabolic disorders encompassing lipid imbalances, respiratory ailments, digestive issues, and urinary system dysfunctions[11,12]. This cruciferous vegetable is notably rich in glucosinolates, sulfates, and cysteine-rich proteins, acting as precursors for glutathione synthesis[13]. The presence of isothiocyanate in black radishes has been linked to ameliorating the severity of non-alcoholic fatty liver disease induced by a high-fat diet in a rat model[14]. Administration of black radish has been shown to enhance the activity of detoxification enzymes in experimental and clinical studies[15–17]. Furthermore, the plant constituents, including anthocyanins, polyphenols, glucosinolates, and the metabolite isothiocyanate, are closely associated with antioxidant properties[18]. Hence, this investigation was undertaken to assess the potential hepatoprotective effects of black radish extract against SVP-induced hepatotoxicity.

2. Materials and methods

2.1. Chemicals

Hank's Balanced Salt Solution, bovine serum albumin, rotenone, D-mannitol, N-(2-hydroxyethyl) piperazine-N'-(2-ethane sulfonic acid) (HEPES), sucrose, rhodamine 123, 2',7'-dichlorofluorescein diacetate, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoic acid)], 3-(N-morpholino) propane sulfonic acid, dimethyl sulfoxide (DMSO), 2-amino-2-hydroxymethyl-propane-1,3-diol, potassium chloride, sodium succinate, magnesium chloride and monopotassium phosphate were purchased from Sigma (St. Louis, MO USA).

2.2. Black radish root extract

The black radish root was procured from a local store and its identity was verified by a trained botanist. Then, a voucher specimen was deposited at the Herbarium of the Medical Plants Research Center at Shahrekord University of Medical Sciences, Shahrekord, Iran (SKUMS-1050). The root extract was prepared utilizing the maceration technique. The dried plant material was powdered using an electric mill, with the resultant powder combined with a mixture of water (30%) and 97% methanol alcohol (70%). Subsequently, after a 72-hour maceration period, the liquid extract was filtered employing a Buchner funnel and underwent extraction using a rotary apparatus. Following extraction, the extract was dried in an incubator, collected, and then prepared by dissolution in an appropriate solvent (distilled water).

2.3. Ethical statement

This investigation was carried out at Shahrekord University of Medical Sciences and obtained approval from the Animals Ethics Committee of the university, with the reference number IR.SKUMS.AEC.1401.039 (approval date: 2023-01-08).

2.4. Laboratory animals and grouping

A total of 32 male mice weighing approximately 20 to 30 g were purchased from the Pasteur Institute of Iran (Tehran, Iran). The mice were housed under standardized conditions with a consistent light cycle of 12 hours, a temperature maintained at $(23 \pm 2)^\circ\text{C}$ and free access to food and water. The mice were categorized into four groups ($n=8$). Mice in the control group received distilled water orally once every 24 hours for 28 d. The SVP group was given SVP at a dosage of 100 mg/kg orally once every 24 hours for 28 d[19]. The extract group received black radish extract at a dose of 300 mg/kg orally once every 24 hours for 28 d[20]. In the treatment group (SVP+

black radish extract), mice were treated with 100 mg/kg SVP and 300 mg/kg black radish extract at a 3-hour interval, once every 24 hours for 28 d. At the end of the experimental period, mice were anesthetized *via* ketamine (40 mg/kg) and xylazine (15 mg/kg) injection. Blood samples were collected from the heart to assess the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Furthermore, liver tissue samples were extracted for the evaluation of malondialdehyde (MDA) concentration, ROS levels, mitochondrial parameters, and histopathological indexes.

2.5. Measurement of ALT, AST, and ALP activities

The enzymatic activities of ALT, AST, and ALP were assessed in the serum samples utilizing commercially available kits (ParsAzmon Company) and an autoanalyzer (BT3500, Italy).

2.6. Measurement of MDA concentration

A quantity (10 mg) of tissue sample was placed in a 20 mL glass tube and incubated at a controlled temperature of $(37 \pm 1)^\circ\text{C}$ using a metabolic shaker (Kavosh Teb) for 60 min. Following the incubation period, 1 mL of 5% tetrachloroacetic acid and 1 mL of 67% thiobarbituric acid were successively added to the tube, ensuring thorough mixing after each addition. The contents of each vial were then transferred to a centrifuge tube and centrifuged at $2000 \times g$ for 15 min. Subsequently, the resulting supernatant was carefully transferred to another tube and subjected to heat treatment (100°C) in a boiling water bath. After 10 minutes of heating, the test tubes were cooled, and the absorbance of each solution was measured at a wavelength of 535 nm[21].

2.7. ROS measurement

To quantify the levels of ROS, the dichlorofluorescein diacetate probe was employed[22]. This probe transforms within the cellular environment: initially, dichlorofluorescein diacetate salt is converted to non-fluorescent dichlorofluorescein by cellular esterases after penetration into the cell. Subsequently, upon reacting with active oxygen radicals, this compound is converted to dichlorofluorescein, a highly fluorescent molecule whose concentration can be readily assessed using a spectrofluorimetric technique. For the experimental procedure, 3 mL of homogenized tissue was centrifuged at $1000 \times g$ for 1 min, following which 3 mL of the dichlorofluorescein diacetate solution was added to the sediment of each sample. The resultant mixture was gently vortexed and incubated in a water bath (37°C) for 10 min. Then the quantity of generated ROS was determined through a fluorimetric analysis based on the fluorescence intensity unit. Excitation and emission wavelengths used for the measurement were set at 470

nm and 540 nm, respectively.

2.8. Real-time PCR analysis

In this study, the *TNF- α* gene expression was evaluated through real-time PCR analysis. Initially, RNA was isolated from the samples utilizing the RNX product manufactured by Cinagen Company. To assess the purity and concentration of the RNA samples quantitatively, optical density readings were taken at A 260/280 and A 260/230 wavelengths using a nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, cDNA synthesis was performed from the isolated RNA samples following the instructions provided by the cDNA production kit (Thermofisher cDNA synthesis kit). Finally, to determine the expression level of the target gene, real-time PCR reactions were conducted. Target cDNAs were amplified using a light cycler device (Roche Diagnostics, Mannheim, Germany) for 40 cycles following an initial denaturation step of 10 min at 95°C . The conditions used for amplification were 25°C for 5 min, 42°C for 60 min and 70°C for 5 min. The results were analyzed using the $2^{-\Delta\Delta\text{Ct}}$ method to calculate the relative gene expression level. Specific forward (5'-TCTTCTCATTCTGCTTGTC-3') and reverse (5'-TGATCTGAGTGTGAGGGTCTGG-3') primers were designed and procured using Primer 3 software version 0.4.0 for the desired genes, while beta-2 microglobulin (*$\beta 2M$*) gene was selected as the housekeeping gene for normalization purposes.

2.9. Histopathological studies

Following the experimental procedures, the mice were euthanized, and liver tissue specimens were harvested for histopathological analysis. The liver specimens from each experimental group were rinsed with phosphate-buffered saline (PBS) and subsequently fixed in a formalin solution of 10%. Subsequently, tissue slides were prepared from the liver samples and subjected to hematoxylin and eosin (H&E) staining. These stained tissue sections were then examined by a trained pathologist under an Nikon optical microscope for the presence of pathological alterations. The extent of tissue damage was assessed and quantified utilizing the Suzuki scoring system, a standard method for evaluating liver injury in research studies[23].

2.10. Isolation of mitochondria from the liver

Mitochondria were isolated from mouse liver using a process of differential centrifugation. The liver tissue was extracted and finely minced within a cold mannitol solution containing 0.225 M *D*-mannitol, 75 mM sucrose, and 0.2 mM EDTA. Following this, the minced tissue was homogenized and subsequently centrifuged to eliminate nuclei, unbroken cells, and other non-subcellular debris.

The resulting supernatant underwent further centrifugation, and the upper layer which contained the isolated mitochondria, was meticulously separated from the pellet[24].

2.11. Succinate dehydrogenase (SDH) activity

The molecular functionality of mitochondria was assessed using an MTT assay to quantify SDH activity. Isolated liver mitochondria were resuspended in a mitochondrial assay buffer containing 0.5 mmol/L KH_2PO_4 , 0.5 mmol/L EGTA, 20 mmol/L HEPES, 140 mmol/L KCl, 10 mmol/L NaCl, and 2 mmol/L MgCl_2 , supplemented with 10 mmol/L succinate and 1 mg/mL rotenone. For MTT assay, 25 μL of MTT solution (0.5 mg/mL) were mixed with 100 μL of isolated mitochondria (100 μg per well) in 96-well plates and incubated at 37°C for 30 min. Finally, 100 μL of DMSO was added to each well and the absorbance was measured at 570 nm using an ELISA reader (Tecan, Rainbow Thermo)[24].

2.12. Mitochondrial swelling

The evaluation of isolated mitochondrial swelling was conducted by measuring light scatter at a wavelength of 540 nm. Reduction in absorbance at this particular wavelength, indicative of mitochondrial swelling[24], was assessed using isolated liver mitochondria. Isolated liver mitochondria were incubated in a freshly prepared mitochondrial swelling buffer containing 20 mmol/L HEPES, 0.5 mmol/L KH_2PO_4 , 0.5 mmol/L EGTA, 140 mmol/L KCl, 10 mmol/L NaCl, and 2 mmol/L MgCl_2 , supplemented with 10 mmol/L succinate and 1 mg/mL rotenone. The absorbance of the isolated liver mitochondria (100 μg /well) was subsequently measured at 540 nm.

2.13. Mitochondrial membrane potential (MMP) collapse

MMP was evaluated utilizing rhodamine 123, a fluorescent dye[24]. Mitochondria, at a protein concentration of 1 mg/mL, were

suspended and incubated in an MMP buffer containing the following constituents: 5 mM KH_2PO_4 , 68 mM *D*-mannitol, 220 mM sucrose, 10 mM HEPES, 2 μM rotenone, 50 μM EGTA, 2 mM MgCl_2 , 5 mM sodium succinate, 10 mM KCl and 5 μM rhodamine 123, at 37°C for 15 min. After this initial incubation, the MMP buffer was removed and rhodamine 123-free MMP buffer was added. Samples were incubated for another 15 min. Finally, the fluorescence intensity of rhodamine 123 was quantified using a fluorescence spectrophotometer (Shimadzu RF5000U) at an excitation wavelength of 470 nm and an emission wavelength of 540 nm.

2.14. Statistical analysis

The statistical analysis was carried out using GraphPad Prism5 software (Graphpad software, La Jolla, CA). The data were analyzed using one-way ANOVA followed by Tukey's *post hoc* test. The results are presented as mean $\pm$ SEM, with statistical significance established at $P < 0.05$.

3. Results

3.1. Effect of black radish extract on liver enzymes

Treatment of the animals with SVP resulted in a significant ($P < 0.01$) elevation in ALT and AST serum levels compared to the control group. Co-administration of the root extract of black radish with SVP led to a significant ($P < 0.01$) reduction in ALT and AST levels in comparison to the group administered with SVP alone (Figure 1A and 1B). Furthermore, the findings indicated that SVP significantly ($P < 0.05$) increased the serum level of ALP in comparison with the control group (Figure 1C). However, the co-administration of the root extract of black radish with SVP caused a non-significant ($P > 0.05$) decrease in ALP serum level compared to the SVP group.

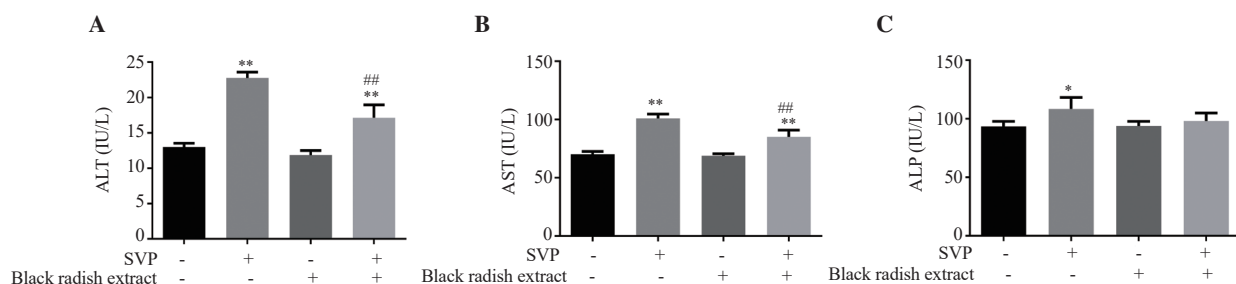


Figure 1. Effect of black radish extract on (A) ALT, (B) AST, and (C) ALP levels in mice with sodium valproate-induced liver damage. The results are presented as mean $\pm$ SEM ($n=5$) and analyzed using one-way ANOVA followed by Tukey's *post hoc* test. * $P < 0.05$, ** $P < 0.01$ compared with the control group. ## $P < 0.01$ compared with the SVP group. SVP: sodium valproate; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase.

3.2. Effect of black radish extract on lipid peroxidation

SVP administration resulted in a significant ($P < 0.01$) elevation in hepatic MDA levels in comparison with the control group. Conversely, treatment with the root extract of black radish markedly ($P < 0.01$) reduced MDA level compared to the SVP group (Figure 2A).

3.3. Effect of black radish extract on ROS level

As shown in Figure 2B, it was observed that SVP administration significantly ($P < 0.01$) elevated ROS levels compared to the control group. Conversely, the black radish extract significantly ($P < 0.01$) decreased ROS production in liver tissue in comparison to the SVP group. Furthermore, the administration of root extract of black radish alone significantly ($P < 0.01$) decreased ROS level compared to the control group.

3.4. Effect of black radish extract on $TNF-\alpha$ gene expression

$TNF-\alpha$ gene expression was notably upregulated ($P < 0.01$) following SVP administration in mice. In contrast, treatment with the root extract of black radish along with SVP significantly ($P < 0.01$) downregulated $TNF-\alpha$ gene expression as compared with the SVP group (Figure 2C).

3.5. Effect of black radish extract on liver histopathology

The histopathological examination revealed a substantial increase in necrosis, congestion, and vacuolation in the group administered with SVP compared to the control group. Administration of the root extract of black radish showed a non-significant reduction in histopathological damage in comparison to the SVP group (Figure 3A). Additionally, the severity of the histopathological changes was graded using the Suzuki scoring method. As depicted in Figure 3B, SVP administration resulted in a significant elevation in Suzuki

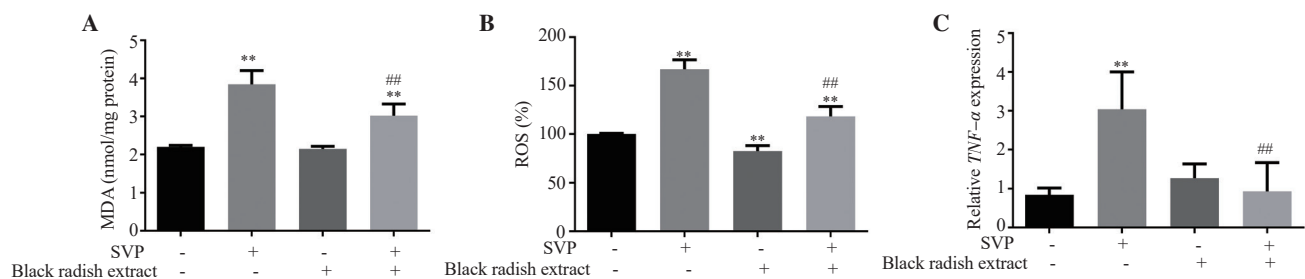


Figure 2. Effect of black radish extract on lipid peroxidation, ROS generation and $TNF-\alpha$ gene expression in mice with sodium valproate-induced liver damage. $TNF-\alpha$ level is normalized against beta-2 microglobulin ($\beta 2M$) as the housekeeping gene. The results are presented as mean $\pm$ SEM ($n=5$) and analyzed using one-way ANOVA followed by Tukey's *post hoc* test. ** $P < 0.01$ compared with the control group. ## $P < 0.01$ compared with the SVP group.

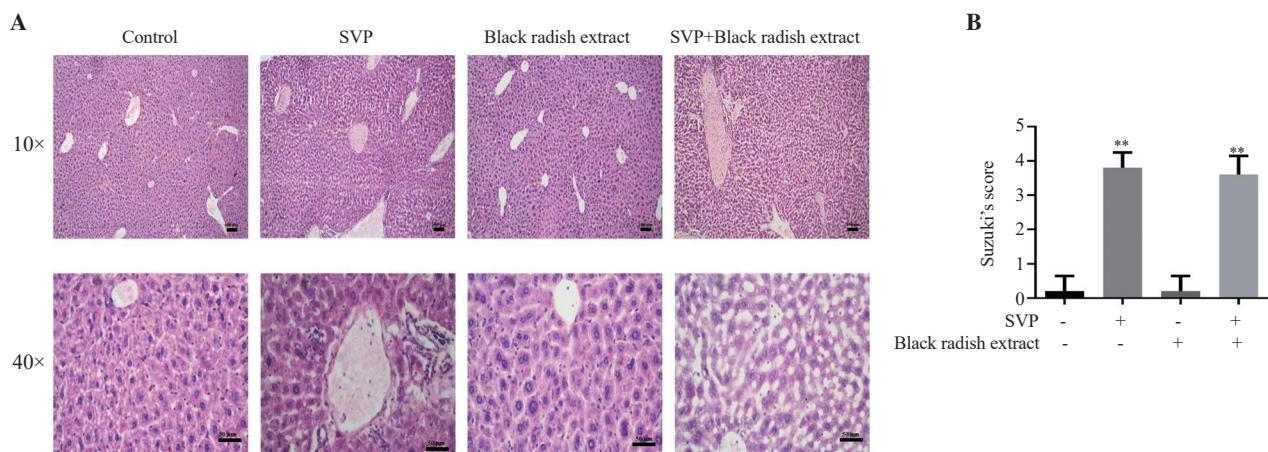


Figure 3. Effect of black radish extract on (A) histopathological changes (H&E staining) and (B) Suzuki's score in the liver of mice. The black radish extract alone group shows no significant changes in liver structure compared with the control group. The SVP group and the SVP+black radish extract group show a significant increase in necrosis, congestion, and vacuolation compared with the control group. The results are presented as mean $\pm$ SEM ($n=5$) and analyzed using one-way ANOVA followed by Tukey's *post hoc* test. ** $P < 0.01$ compared with the control group.

score, whereas the co-administration of the root extract of black radish did not significantly alleviate the SVP-induced changes in Suzuki score.

3.6. Effect of black radish extract on mitochondrial function

As presented in Figure 4A, a significant ($P < 0.01$) decrease in mitochondrial SDH activity was found in mice receiving SVP alone, which was significantly ($P < 0.01$) mitigated by treatment with 300 mg/kg of black radish extract.

3.7. Effect of black radish extract on mitochondrial swelling

As depicted in Figure 4B, a significant ($P < 0.01$) increase in mitochondrial swelling was observed in the SVP group when compared to the control group. However, a considerable reduction ($P < 0.01$) was observed in mitochondrial swelling in mice that received 300 mg/kg of black radish extract along with SVP, in comparison to the SVP group.

3.8. Effect of black radish extract on mitochondrial membrane potential (MMP)

Figure 4C demonstrates that SVP administration caused a significant ($P < 0.01$) collapse of the MMP in the liver of mice. Interestingly, treatment with the root extract of black radish significantly ($P < 0.01$) decreased MMP collapse compared with the SVP group.

4. Discussion

The current study revealed a significant elevation in ALP, AST, and ALT levels in the serum of mice administered with SVP compared to the control group, indicative of hepatotoxicity in these animals. Conversely, concurrent administration of black radish root extract

significantly decreased serum levels of ALT and AST in mice with SVP-induced liver damage, with no significant differences observed in ALP levels. These findings are consistent with previous studies which demonstrated the efficacy of black radish in reducing serum levels of ALT, AST, and ALP under different conditions such as carbon tetrachloride (CCl_4)-induced hepatic injury and ethanol intoxication[20,25,26]. Our findings also indicate that SVP significantly elevated ROS levels and promoted lipid peroxidation, thereby inducing oxidative stress and hepatic toxicity. The root extract of black radish reduced SVP-induced increase in ROS and MDA levels, thus mitigating oxidative stress. Various studies have linked SVP with oxidative stress, a phenomenon implicated in diverse health complications, including hepatotoxicity, and neurodegenerative conditions[27,28]. Oxidative stress triggered by SVP encompasses multifaceted mechanisms; one such pathway involves the induction of SOD2 acetylation, which can disrupt cell signaling and contribute to the onset of developmental anomalies like neural tube defects[29]. Another mechanism includes excessive ROS generation or compromised antioxidant defense mechanisms, leading to mitochondrial dysfunction and hepatotoxicity[28]. Ahn *et al.* investigated the levels of catalase, superoxide dismutase (SOD), and MDA in the liver without and with pretreatment using black radish extract. Their outcomes demonstrated that pre-administration of this extract mitigated lipid peroxidation and enhanced SOD and catalase levels in livers afflicted by CCl_4 [20], thus shielding the liver from CCl_4 -induced oxidative stress. These findings are consistent with the current study results. Other previous research has also outlined the hepatoprotective attributes of black radish extract. Enkhtuya *et al.* ascertained that black radish exhibited antioxidant efficacy under laboratory conditions[30]. A previous investigation explored the protective properties of radish leaf extract against oxidative damage in human fetal lung fibroblast cells (MRC-5)[31]. In this study, F2, a component of radish leaf extract, exhibited superior free radical scavenging capabilities and possessed the highest total polyphenol content. Pretreatment with F2 resulted in a substantial increase in cell viability, enhanced internal antioxidant enzyme activity, reduced

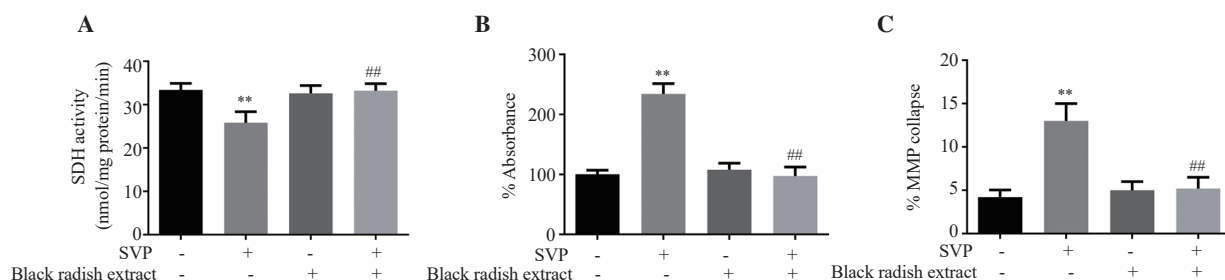


Figure 4. Effect of black radish extract on (A) SDH activity, (B) mitochondrial swelling and (C) collapse of mitochondrial membrane potential in mice with sodium valproate-induced liver damage. The results are presented as mean $\pm$ SEM ($n=5$) and analyzed using one-way ANOVA followed by Tukey's *post hoc* test. ** $P < 0.01$ compared with the control group. ## $P < 0.01$ compared with the SVP group. SDH: succinate dehydrogenase.

MDA content, decreased intracellular ROS levels, and restored the loss of MMP induced by H_2O_2 [31]. Furthermore, Hwang *et al.* demonstrated that radish extract mitigated acetaminophen-induced liver damage in rats by suppressing oxidative stress and apoptosis[32]. This antioxidative efficacy was attributed to compounds such as polyphenols, flavonoids, and anthocyanins. Numerous studies have confirmed mitochondrial dysfunction in SVP-induced hepatotoxicity toward liver cells[33–35]. Our study corroborates these observations by revealing the protective effects of black radish on mitochondrial functionality. Moreover, black radish root extract mitigated SVP-induced mitochondrial swelling, MMP collapse, and increased SDH activity. Mitochondrial dysfunction can trigger the release of ROS, further exacerbating the hepatotoxicity associated with SVP. Preventing such mitochondrial damage holds the potential to inhibit liver injury.

In this study, SVP administration led to a significant rise in *TNF- α* levels compared to the control group whereas co-administration of black radish root extract with SVP effectively mitigated the rise in *TNF- α* levels. A previous investigation delved into the anti-inflammatory properties of black radish extract concerning inflammatory responses mediated by interleukin (IL)-6 and lipopolysaccharide (LPS) in RAW 264.7 cell lines. This research revealed that black radish extract effectively suppressed the production of inflammatory cytokines such as *TNF- α* , IL-10, IL-6, and IL-1 β , along with inflammatory mediators like prostaglandin E_2 (PGE $_2$) and nitric oxide[36]. Similarly, the effects of radish sprout ethanol extract on inflammatory responses in macrophages and a mouse model of colitis have been investigated. The administration of radish sprout ethanol extract effectively inhibited the phosphorylation of I κ B and, in turn, the production of pro-inflammatory enzymes and cytokines in LPS-stimulated macrophages[37]. In dextran sulfate sodium-induced colitis mice, the extract prevented a reduction in body weight and colon length, while decreasing inflammation and mucosal necrosis[37].

The histopathological analysis of the present study revealed that SVP administration led to severe liver damage, as indicated by high Suzuki scores. Surprisingly, the administration of black radish root extract did not significantly mitigate the liver damage induced by SVP. These findings are in contrast to findings of previous research which highlighted the hepatoprotective effects of radish extract in various models of liver damage. For instance, Hwang *et al.* demonstrated that radish extracts ameliorated liver histopathology in acetaminophen-induced damage by reducing necrosis and inflammation[32]. Another study displayed that fermented black radish alleviated methionine-caused non-alcoholic fatty liver disease and choline deficiency, ameliorating features such as fat accumulation, inflammation, and liver fibrosis associated with the condition[38]. Additionally, it has been elucidated that black radish

extract inhibits fat accumulation, prevents acute hepatotoxicity, and mitigates lipid peroxidation and serum ALT and AST elevation, along with associated histopathological alterations and increased oxidative stress[38]. These favorable impacts on liver damage may be attributed to the presence of antioxidant compounds and detoxifying enzymes. The disparity between the histopathological outcomes of our study and the findings of other researchers might be attributed to the severe effects of the high dose of SVP utilized in our investigation. Moreover, the protective effects of black radish extract on liver damage might be dose-dependent, a factor that was not explored in the present study.

There are some limitations in the present study. The total extract of black radish root was used which limits us from investigating the compounds (or fraction) of total extract responsible for the observed effects. Furthermore, only one dose of black radish root extract was used for assessment of its hepatoprotective effects which cannot represent the possible dose-response relationship between black radish root extract and SVP-induced liver damage.

In conclusion, the findings show that the hepatoprotective effects of black radish extract on SVP-induced liver damage are probably due to its ability to alleviate oxidative stress, mitigate mitochondrial impairment, suppress inflammatory mediator expression, and reduce the elevation of ALT, AST, and ALP levels. Nevertheless, treatment with black radish extract at 300 mg/kg following SVP administration had little ameliorative effect on histopathological changes in the liver. Further study using different doses is warranted to gain a comprehensive understanding of the mechanisms underlying the action of the extracts and to determine the optimal dosage for therapeutic applications.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

MHZ monitored and supervised the project, developed the project conceptualization, and obtained ethical approval. MHZ and SA reviewed the draft manuscript. MZ conducted the experimental work. MHZ, ES and SK were responsible for experimental design, and protocol preparation. ZL revised manuscript. HAK analyzed the data, and provided research facilities and resources. EB prepared the draft manuscript.

References

- [1] Liampas I, Siokas V, Brotis A, Zintzaras E, Stefanidis I, Dardiotis E. Intravenous sodium valproate in status epilepticus: Review and meta-analysis. *Int J Neurosci* 2021; **131**(1): 70-84.
- [2] Walker CP, Deb S. Rhabdomyolysis and hepatotoxicity from valproic acid. *J Pharm Pract* 2021; **34**(4): 648-552.
- [3] Meseguer ES, Elizalde MU, Borobia AM, Ramírez E. Valproic acid-induced liver injury: A case-control study from a prospective pharmacovigilance program in a tertiary hospital. *J Clin Med* 2021; **10**(6). doi: 10.3390/jcm10061153.
- [4] Utrecht J. A review of the mechanisms of valproate-induced liver injury with an emphasis on the role of reactive metabolites discovered by Tom Baillie. *Med Chem Res* 2023; **32**(9): 1995-2000.
- [5] Ezhilarasan D, Mani U. Valproic acid induced liver injury: An insight into molecular toxicological mechanism. *Environ Toxicol Phar* 2022; **95**(1). doi: 10.1016/j.etap.2022.103967.
- [6] Oztupuz O, Turkon H, Buyuk B, Coskun O, Sehitoğlu MH, Ovali MA, et al. Melatonin ameliorates sodium valproate-induced hepatotoxicity in rats. *Mol Biol Rep* 2020; **47**(1): 317-325.
- [7] Abdelkader NF, Elyamany M, Gad AM, Assaf N, Fawzy HM, Elesawy WH. Ellagic acid attenuates liver toxicity induced by valproic acid in rats. *J Pharmacol Sci* 2020; **143**(1): 23-29.
- [8] Garcia-Cortes M, Robles-Diaz M, Stephens C, Ortega-Alonso A, Lucena MI, Andrade RJ. Drug induced liver injury: An update. *Arch Toxicol* 2020; **94**: 3381-3407.
- [9] Coleman MD. *Human drug metabolism*. Hoboken, NJ, USA: John Wiley & Sons; 2020.
- [10] Datta S, Aggarwal D, Sehrawat N, Yadav M, Sharma V, Sharma A, et al. Hepatoprotective effects of natural drugs: Current trends, scope, relevance and future perspectives. *Phytomedicine* 2023; **121**. doi: 10.1016/j.phymed.2023.155100.
- [11] Yüçetepe A, Altın G, Özçelik B. A novel antioxidant source: Evaluation of *in vitro* bioaccessibility, antioxidant activity and polyphenol profile of phenolic extract from black radish peel wastes (*Raphanus sativus* L. var. niger) during simulated gastrointestinal digestion. *Int J Food Sci Tech* 2021; **56**(3): 1376-1384.
- [12] Zrouri H, Elbouzidi A, Bouhrim M, Bencheikh N, Kharchoufa L, Ouahhoud S, et al. Phytochemical analysis, antioxidant activity, and nephroprotective effect of the *Raphanus sativus* aqueous extract. *Mediterr J Chem* 2021; **11**(1): 84-94.
- [13] Gamba M, Asllanaj E, Raguindin PF, Glisic M, Franco OH, Minder B, et al. Nutritional and phytochemical characterization of radish (*Raphanus sativus*): A systematic review. *Trends Food Sci Tech* 2021; **113**: 205-218.
- [14] You H, Hao R, Li R, Zhang L, Zhu Y, Luo Y. The effect of radish sourced 4-(Methylthio)-3-butenyl isothiocyanate on ameliorating the severity of high fat diet induced nonalcoholic fatty liver disease in rats. *Int J Clin Exp Med* 2015; **8**(9): 15910-15919.
- [15] Shin T, Ahn M, Kim GO, Park SU. Biological activity of various radish species. *Oriental Pharm Exp Med* 2015; **15**: 105-111.
- [16] Pocasap P, Weerapreeyakul N, Wongpoomchai R. Thai rat-tailed radish prevents hepatocarcinogenesis in rats by blocking mutagenicity, inducing hepatic phase II enzyme, and decreasing hepatic pro-inflammatory cytokine gene expression. *Cancers* 2023; **15**(6). doi: 10.3390/cancers15061906.
- [17] Chen CH. Dietary inducers of detoxification enzymes. In: Chen CH (eds.) *Xenobiotic metabolic enzymes: Bioactivation and antioxidant defense*. Cham, Switzerland: Springer; 2020, p. 221-234.
- [18] Kim HY, Shin TK, Ahn MJ, Kim GO, Chun JY. Characterization of black radish (*Raphanus sativus* L. var. niger) extracts according to different extraction and drying methods. *Resour Sci Res* 2021; **3**(1): 46-54.
- [19] Gai Z, Krajnc E, Samodelov SL, Visentin M, Kullak-Ublick GA. Obeticholic acid ameliorates valproic acid-induced hepatic steatosis and oxidative stress. *Mol pharmacol* 2020; **97**(34): 314-323.
- [20] Ahn M, Kim J, Hong S, Kim J, Ko H, Lee NH, et al. Black radish (*Raphanus sativus* L. var. niger) extract mediates its hepatoprotective effect on carbon tetrachloride-induced hepatic injury by attenuating oxidative stress. *J Med Food* 2018; **21**(9): 866-875.
- [21] Abolhassanzadeh Z, Ansari S, Lorigooini Z, Anjomshoa M, Bijad E, Ramezannezhad P, et al. The nephrotoxicity of *Aristolochia rotunda* L. in rats: Mitochondrion as a target for renal toxicity of aristolochic acids-containing plants. *Heliyon* 2023; **9**(11). doi: 10.1016/j.heliyon.2023.e21848.
- [22] Seydi E, Mahzari F, Zarei MH, Ramazani M, Pourahmad J. Hexavalent chromium induced oxidative stress and toxicity on isolated human lymphocytes. *Int Pharm Acta* 2020; **3**(1): e1. doi: 10.22037/ipa.v3i1.28616.
- [23] Suzuki S, Nakamura S, Koizumi T, Sakaguchi S, Baba S, Muro H, et al. The beneficial effect of a prostaglandin 12 analog on ischemic rat liver. *Transplantation* 1991; **52**(6): 979-983.
- [24] Salimi A, Khezri S, Helmi K, Maleki P, Sajjadi Alehashem SH, Razmjou M. Evaluation of toxicity of thymoquinone, as a bioactive compound of *Nigella sativa* using isolated mitochondria from different organs. *Toxin Rev* 2024; **43**: 1-11.
- [25] Kang KK, Seo MS, Sung SE, Choi JH, Lee S, Kim K, et al. Effects of

- Raphanus sativus* var. *niger* (black radish) fermented with *Lactobacillus plantarum* on alcohol metabolism in rats. *Food Suppl Biomater Health* 2021; **1**(2): 1-8.
- [26] Kim J, Ahn M, Choi Y, Ekanayake P, Kim GO, Shin T. Antifibrotic effects of fermented black radish (*Raphanus sativus* L. var. *niger*) on chronic liver injury in rats. *Adv Tradit Med* 2020; **20**(1): 53-59.
- [27] Gao Y, Jiang D, Wang C, An G, Zhu L, Cui C. Comprehensive analysis of metabolic changes in male mice exposed to sodium valproate based on GC-MS analysis. *Drug Des Devel Ther* 2022; **16**(1): 1915-1930.
- [28] Salimi A, Alyan N, Akbari N, Jamali Z, Pourahmad J. Selenium and L-carnitine protects from valproic acid-induced oxidative stress and mitochondrial damages in rat cortical neurons. *Drug Chem Toxicol* 2022; **45**(3): 1150-1157.
- [29] Hansen JM, Lucas SM, Ramos CD, Green EJ, Nuttall DJ, Clark DS, et al. Valproic acid promotes SOD2 acetylation: A potential mechanism of valproic acid-induced oxidative stress in developing systems. *Free Radical Res* 2021; **55**(11-12): 1130-1144.
- [30] Enkhtuya E, Tsend M. The effect of peeling on antioxidant capacity of black radish root. *Ital J Food Sci* 2020; **32**(3): 701-711.
- [31] Luo X, Zhang H, Duan Y, Chen G. Protective effects of radish (*Raphanus sativus* L.) leaves extract against hydrogen peroxide-induced oxidative damage in human fetal lung fibroblast (MRC-5) cells. *Biomed Pharmacother* 2018; **103**: 406-414.
- [32] Hwang KA, Hwang Y, Hwang HJ, Park N. Hepatoprotective effects of radish (*Raphanus sativus* L.) on acetaminophen-induced liver damage via inhibiting oxidative stress and apoptosis. *Nutrients* 2022; **14**(23). doi: 10.3390/nu14235082.
- [33] Celik E, Tunali S, Gezginci-Oktayoglu S, Bolkent S, Can A, Yanardag R. Vitamin U prevents valproic acid-induced liver injury through supporting enzymatic antioxidant system and increasing hepatocyte proliferation triggered by inflammation and apoptosis. *Toxicol Mech Method* 2021; **31**(8): 600-608.
- [34] Caiment F, Wolters J, Smit E, Schroorders Y, Kleinjans J, van den Beucken T. Valproic acid promotes mitochondrial dysfunction in primary human hepatocytes *in vitro*; impact of C/EBP α -controlled gene expression. *Arch Toxicol* 2020; **94**: 3463-3473.
- [35] Sanaei M, Kavooosi F. Effect of valproic acid on the class I histone deacetylase 1, 2 and 3, tumor suppressor genes p21WAF1/CIP1 and p53, and intrinsic mitochondrial apoptotic pathway, pro-(Bax, Bak, and Bim) and anti-(Bcl-2, Bcl-xL, and Mcl-1) apoptotic genes expression, cell viability, and apoptosis induction in hepatocellular carcinoma HepG2 cell line. *Asian Pac J Cancer Prev* 2021; **22**(S1): 89-95.
- [36] Jeon H, Yang D, Lee NH, Ahn M, Kim G. Inhibitory effect of black radish (*Raphanus sativus* L. var. *niger*) extracts on lipopolysaccharide-induced inflammatory response in the mouse monocyte/macrophage-like cell line RAW 264.7. *Prev Nutr Food Sci* 2020; **25**(4): 408-421.
- [37] Kim G, Jang M, Hwang I, Cho J, Kim S. Radish sprout alleviates DSS-induced colitis via regulation of NF- κ B signaling pathway and modifying gut microbiota. *Biomed Pharmacother* 2021; **144**. doi: 10.1016/j.biopha.2021.112365.
- [38] Ahn M, Kim J, Choi Y, Ekanayake P, Chun JY, Yang D, et al. Fermented black radish (*Raphanus sativus* L. var. *niger*) attenuates methionine and choline deficient diet-induced nonalcoholic fatty liver disease in mice. *Food Sci Nutr* 2019; **7**(10): 3327-3337.

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