

Effects of *Helichrysum maracandicum* polyphenols on mitochondrial membrane permeability integrity in a rat model of toxic hepatitis

Saidaxon Ahmedova^{1*}, Muzaffar Asrarov², and Sobitjon Mirzakulov¹

¹National University of Uzbekistan, 100174, Tashkent, Uzbekistan

²Institute of Biophysics and Biochemistry, National University of Uzbekistan, 100174, Tashkent, Uzbekistan

Abstract. We examined whether polyphenol fractions from *Helichrysum maracandicum* could influence passive ion permeability of liver mitochondrial membranes in rats exposed to carbon tetrachloride. Mitochondrial permeability was evaluated indirectly by monitoring time-dependent changes in optical density of the mitochondrial suspension, which reflect changes in ion flux across the inner membrane. Treatment with the extracts influenced the passive transport of monovalent cations, including potassium (K⁺), sodium (Na⁺), and hydrogen (H⁺). The most evident changes were detected in K⁺ permeability at a dose of 20 mg, whereas alterations in Na⁺ and H⁺ transport were comparatively moderate. Overall, our data show that the extracts reduced the severity of membrane damage, although mitochondrial parameters did not completely return to control values.

1 Introduction

The maintenance of cellular energy balance, particularly in liver tissue, is closely linked to mitochondrial ATP synthesis through oxidative phosphorylation processes [1,2]. Proper regulation of K⁺, Na⁺, Ca²⁺, and H⁺ transport across the inner mitochondrial membrane is essential for preserving membrane potential and metabolic stability [3]. During pathological states such as toxic injury, hypoxia, or metabolic disorders, mitochondrial membranes frequently exhibit increased passive ion permeability, which compromises energy metabolism [4,5]. This phenomenon has been observed in experimental models of carbon tetrachloride (CCl₄)-induced hepatitis, where oxidative stress is associated with lipid peroxidation and structural changes in membrane proteins, resulting in impaired ion selectivity [6]. For this reason, compounds that can limit oxidative damage and support mitochondrial membrane stability remain an important focus of experimental studies [7]. Polyphenols have been widely reported to protect mitochondrial structures by limiting oxidative injury and preserving membrane function [8,9]. Experimental data from *Helichrysum* species further indicate that these plants possess antioxidant properties that may support mitochondrial energy metabolism [10–12]. Ahmedova et al.'s study indicates that polyphenol extracts from *H. maracandicum* enhance the activity of antioxidant enzymes, including glutathione peroxidase (GPx), catalase, and superoxide dismutase (SOD). They also lower lipid peroxidation, and bring oxidative phosphorylation back to normal in liver mitochondria.

Furthermore, polyphenols can influence passive ion transport across the mitochondrial membrane. They regulate the ability of the mitochondrial membrane potential ($\Delta\psi_m$), by adjusting the concentrations of K⁺, Na⁺, and H⁺ ions [13–15]. Polyphenols maintain membrane stability at low concentrations; nevertheless, at elevated levels, they may activate the mitochondrial permeability transition pore (MPTP). Additionally, increased permeability to divalent ions, such as Ca²⁺ and Mg²⁺ is a crucial factor in mitochondrial swelling and the initiation of apoptotic pathways [16]. To date, the influence of *H. maracandicum* polyphenols on mitochondrial ion permeability during toxic hepatitis has not been specifically examined. This study aims to investigate how polyphenol extracts from *Helichrysum maracandicum* affect the passive permeability of liver mitochondrial inner membranes to mono- and divalent cations (K⁺, Na⁺, H⁺, Ca²⁺, and Mg²⁺).

While plant polyphenols' hepatoprotective and antioxidant benefits are widely documented, little study has been conducted on how they affect the passive ion permeability of liver mitochondrial membranes during severe hepatitis. Previous research has predominantly focused on oxidative stress measurements, while the current study investigates carbon tetrachloride-induced alterations in mitochondrial membrane permeability and assesses the ameliorative effects of polyphenol extracts derived from *Helichrysum maracandicum*.

2 Materials and methods

* Corresponding author: saidaxon.axmedova@gmail.com

2.1 Isolation of Mitochondria

Thirty male outbred rats weighing between 180 and 220 grams were used in the experimental study. The animals were kept in the vivarium at the Institute of Bioorganic Chemistry's Department of Pharmacology in Tashkent, Uzbekistan. The National University of Uzbekistan's Institute of Biophysics and Biochemistry said that all animal-related procedures followed the guidelines for laboratory animal research set by the institution and the country (Protocol No. 3, 01 November 2021). Differential centrifugation was used to get liver mitochondria from white non-breeding rats [17]. The animals were beheaded, and the liver was promptly taken out and put in an ice-cold isolation medium (IM) with 250 mM sucrose, 1 mM EDTA, and 10 mM Tris-HCl buffer at pH 7.4. After weighing, the liver tissue was gently squeezed through a mechanical press and mixed with six times the amount of the isolation medium in a Teflon homogenizer. We initially used an angular rotor centrifuge (IJP-1) to spin the homogenate at $450 \times g$ for 7 minutes at 0 ± 2 °C. This got rid of nuclei and cell debris. Then, the supernatant was spun at $4000 \times g$ for 15 minutes at 0 ± 2 °C. The mitochondrial pellet was resuspended in isolation medium without EDTA at a ratio of 10:1. During the experimental procedures, the suspension was maintained on ice to preserve functional stability.

The doses of *helmar-1* and *helmar-2* were selected according to preliminary experiments and previously published data on their biological activity. These concentrations did not produce detectable mitochondrial toxicity and allowed us to compare moderate versus higher levels of polyphenol exposure while maintaining membrane integrity.

Five experimental groups (n = 5) were randomly assigned to the animals: the control group, the toxic hepatitis group (CCl₄), the CCl₄ + *helmar-1*, the CCl₄ + *helmar-2*, and the CCl₄ + silymarin. For the duration of the experiment, treatments were given once a day.

2.2 Determination of protein concentration

Protein content in mitochondrial samples was determined using a modified Lowry method following Peterson's procedure [18].

2.3 Assessment of Passive Membrane Permeability

The passive permeability of the inner mitochondrial membrane to various ions was assessed photo metrically by recording changes in the optical density of the mitochondrial suspension over time at 540 nm. The kinetics of ion permeability (for H⁺, K⁺, Na⁺, and Ca²⁺) were measured in iso-osmotic nitrate solutions of the corresponding cations using de-energized mitochondria [19].

By tracking mitochondrial swelling as variations in optical density at 540 nm, changes in passive membrane permeability were evaluated. Despite being indirect, this technique is frequently employed as a sensitive measure of the integrity of the mitochondrial membrane. Future

research will incorporate functional mitochondrial assays, which were not used in this study.

2.4 Statistical Evaluation

Experimental data are presented as mean \pm standard error (SEM). Statistical comparisons between groups were performed using one-way ANOVA, followed by appropriate post hoc tests to determine specific intergroup differences. Differences were considered statistically significant at p values below 0.05. Data processing and visualization were performed using the OriginPro 8.6 analytical platform (OriginLab, USA). The kinetics of mitochondrial swelling were normalized to the maximal response within each experiment and averaged across 4 to 7 independent trials. In selected comparisons between control, treated, and treated plus compound groups, significance was further examined using Student's t-test. Statistical notation was applied as follows: *p < 0.05, **p < 0.01, and ***p < 0.001.

3 Results and Interpretation

According to the data, the permeability of liver mitochondria from rats with CCl₄- induced toxic hepatitis (Group II) increased significantly compared with the control group (Group I) when measured in iso-osmotic media containing KNO₃, NaNO₃, and NH₄NO₃ salts. Under CCl₄ - induced toxic hepatitis, passive ion permeability of mitochondria increased by 67.6% for K⁺, 72.7% for Na⁺, and 32.1% for H⁺ ions compared with the control group (Fig. 1). Our data suggest under experimental toxic hepatitis, liver mitochondria exhibit enhanced passive permeability for K⁺, Na⁺, and H⁺ cations. Animals in Groups III and IV showed partial restoration of passive permeability after receiving 10 days of treatment with *Helichrysum maracandicum* polyphenol extract (*helmar-1*, 20 mg/kg) and silymarin (20 mg/kg), respectively. Specifically, compared to the CCl₄-treated group (Group II), mitochondrial passive permeability in the *helmar-1* treated group decreased by 15.8% in KNO₃ medium, 18.1% in NaNO₃ medium, and 10.8% in NH₄NO₃ medium. In the silymarin-treated group, these parameters decreased by 26.4% (KNO₃), 36.9% (NaNO₃), and 21.6% (NH₄NO₃), respectively (Fig. 1).

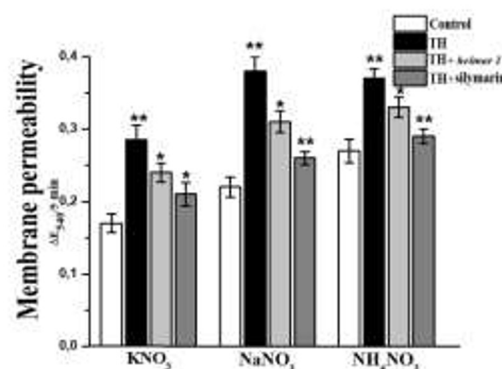


Fig. 1. Effects of *helmar-1* extract compared with silymarin on passive permeability of hepatic

mitochondria for monovalent cations under conditions of experimental toxic hepatitis (*-P<0.05; **-P<0.01; n=5).

The obtained results show that polyphenol extracts from *Helichrysum maracandicum* exhibit a membrane-stabilizing activity, partially normalizing mitochondrial ion permeability that is impaired under toxic hepatitis conditions.

In subsequent experiments, the passive ion permeability of liver mitochondria was also looked at in rats from Groups III and IV, who got treatment with the *helmar-2* extract and silymarin, respectively (Fig. 2). In these treatment groups, mitochondrial ion permeability was partially restored compared with the CCl₄-induced toxic hepatitis group (Group II). Specifically, in *helmar-2* treated rats, mitochondrial permeability was 19.3% in KNO₃ medium, 23.7% in NaNO₃ medium, and 16.2% in NH₄NO₃ medium, compared with the CCl₄ group. In the silymarin-treated group, these parameters decreased by 33.3% (KNO₃), 36.9% (NaNO₃), and 24.3% (NH₄NO₃), respectively (Fig. 2).

Our observations support the idea that *helichrysum maracandicum* polyphenol extract (*helmar-2*), like silymarin, exerts a membrane-protective effect by reducing excessive passive ion permeability of mitochondria under toxic hepatitis conditions.

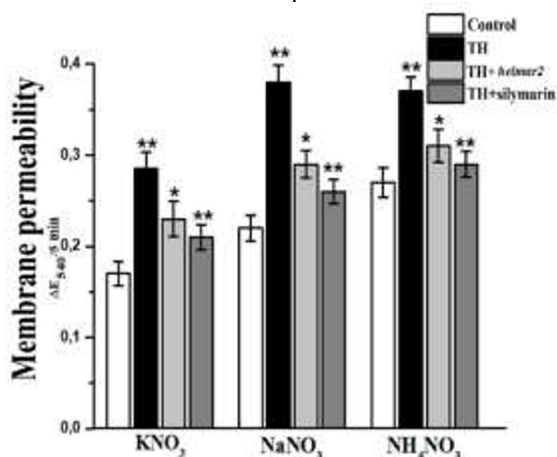


Fig. 2. Effects of *helmar-2* extract compared with silymarin on passive permeability of hepatic mitochondria for monovalent cations under conditions of experimental toxic hepatitis (*-P<0.05; **-P<0.01; n=5).

Under experimental toxic hepatitis (TH) conditions, increased lipid peroxidation (LPO), mitochondrial membrane structural alterations, and decreased mitochondrial membrane potential ($\Delta\psi_m$) collectively contribute to enhanced passive ion permeability. Correction of hepatitis-induced damage in laboratory animals with polyphenol extracts restored K⁺ and H⁺ cation transport in mitochondria, which in turn may help stabilize $\Delta\psi_m$.

The impact of the polyphenol extract on impaired mitochondrial permeability to Ca²⁺ and Mg²⁺ was further assessed in the experimental hepatitis model. Compared with the control animals (Group I), rats receiving CCl₄ (Group II) demonstrated higher mitochondrial

membrane permeability to these cations. The silymarin group was included as a standard for comparison (Fig. 3).

Specifically, under CCl₄-induced TH, the passive permeability increased by 52.1% for Ca²⁺ ions and 40.2% for Mg²⁺ ions compared to control values. Pharmacotherapy of rats with toxic hepatitis (Groups III and IV) using *Helichrysum maracandicum* polyphenol extract (*helmar-1*) and silymarin (20 mg/kg, 10 days) exerted a corrective effect on mitochondrial passive permeability, leading to partial restoration of Ca²⁺ and Mg²⁺ ion transport across the mitochondrial membrane (Fig. 3).

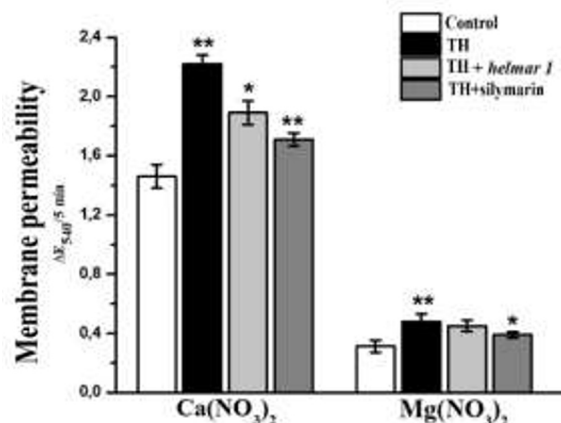


Fig. 3. Modulation of liver mitochondrial permeability to divalent cations (Ca²⁺, Mg²⁺) by *helmar-1* extract and silymarin in rats with induced toxic hepatitis (*P<0.05; **-P<0.01 n=5).

In laboratory animals of groups III and IV, corrected with *Helichrysum maracandicum* polyphenol extracts, the passive permeability of liver mitochondria was inhibited by 14.9% and 22.9% for Ca²⁺ ions and by 15.2% and 23.9% for Mg²⁺ ions compared to group II rats. The data presented here point to under toxic hepatitis conditions, increased transport of Ca²⁺ and Mg²⁺ ions disrupts cellular ion homeostasis and Ca²⁺-dependent processes (Fig. 3).

The passive permeability of liver mitochondria corrected with the *helmar-2* extract and silymarin was restored by 20.7% and 23.3% for Ca²⁺ ions, and by 17.4% and 26.1% for Mg²⁺ ions, respectively, compared to the CCl₄-induced toxic hepatitis group (Group II) (Fig. 4).

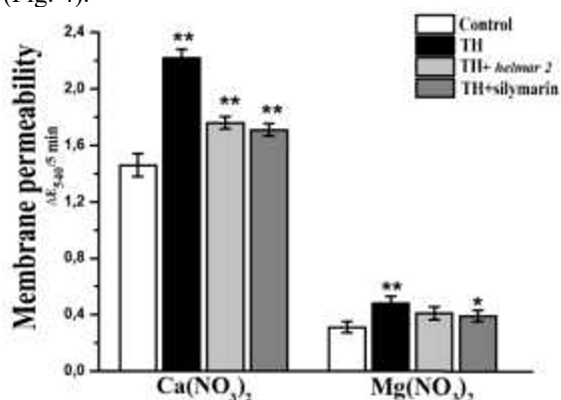


Fig. 4. Modulation of liver mitochondrial permeability to divalent cations (Ca²⁺, Mg²⁺) by *helmar-2* extract and silymarin in rats with induced toxic hepatitis (*P<0.05; **-P<0.01 n=5).

silymarin in rats with induced toxic hepatitis (*- $P < 0.05$; **- $P < 0.01$ $n=5$).

Our results demonstrate that increased passive ion permeability to K^+ , Na^+ , H^+ , Ca^{2+} , and Mg^{2+} , dramatically increased mitochondrial vulnerability in CCl_4 -induced toxic hepatitis. Loss of ion selectivity is usually associated with structural and functional damage of the inner mitochondrial membrane. Such changes are mainly related to oxidative stress and lipid peroxidation processes, which negatively affect mitochondrial ion channels and transport systems [3,6,11]. In the present work, special attention was paid to the membrane-stabilizing action of *H. maracandicum* polyphenols. In rats with toxic hepatitis, administration of *helmar-1* and *helmar-2* extracts led to a decrease in excessive mitochondrial ion permeability. This finding indicates that mitochondria become more resistant to oxidative damage after polyphenol treatment. The reduced permeability to monovalent (K^+ , Na^+ , H^+) and divalent (Ca^{2+} , Mg^{2+}) ions may be explained by the antioxidant properties of polyphenols. These compounds are able to limit free radical damage, protect membrane phospholipids, and partially preserve mitochondrial protein structures [8,10,12]. Consistent with our observations, Ahmedova et al. [6] previously reported that treatment with *H. maracandicum* extracts supported mitochondrial bioenergetic function in the liver. This was reflected by stimulation of oxidative phosphorylation, suppression of lipid peroxidation processes, and enhancement of endogenous antioxidant enzyme activity. These findings support the protective role of polyphenols under conditions of oxidative stress. In our experiments, polyphenols also affected passive ion transport in liver mitochondria. Changes were observed in mechanisms related to the control of mitochondrial volume and membrane potential ($\Delta\psi_m$). A marked decline in Ca^{2+} accumulation was observed following polyphenol treatment. Given that Ca^{2+} overload plays a central role in apoptosis induction and in the activation of the mitochondrial permeability transition pore, this finding supports the mitochondria-stabilizing properties of the extract [14,16]. Polyphenols can affect mitochondrial permeability by influencing Ca^{2+} and Mg^{2+} balance. When these ions remain more stable, mitochondrial swelling is reduced, and membrane potential ($\Delta\psi_m$) is better preserved. Similar effects of natural polyphenols on mitochondrial permeability transition have been described in earlier experimental studies [7,9,15]. In most measured parameters, the effects of the polyphenol extracts were weaker than those of silymarin. However, the general direction of the changes was similar. This suggests that *H. maracandicum* may contain compounds with hepatoprotective properties.

Helmar-2 demonstrated a somewhat stronger stabilizing effect compared to *helmar-1* in several ion transport parameters. The difference is likely related to differences in the composition of the extracts. In rats with toxic hepatitis, both preparations influenced mitochondrial function, mainly at the level of ion movement and membrane properties. Changes were detected in Ca^{2+} accumulation and mitochondrial

membrane potential ($\Delta\psi_m$), suggesting involvement of permeability transition processes.

Extracts obtained from *Helichrysum maracandicum* appear to modify mitochondrial membrane permeability under toxic stress. At the same time, our observations are confined to isolated mitochondrial measurements. Because in vivo biochemical and histological analyses were not performed, the conclusions of this study remain limited to isolated mitochondrial observations.

4 Conclusion

In the present study, we evaluated the effect of polyphenol fractions from *H. maracandicum* on mitochondrial membrane permeability in rats treated with carbon tetrachloride.

In the CCl_4 -treated group, permeability of the mitochondrial membrane was higher than in controls. Increased movement of mono- and divalent cations was detected, together with alterations in ionic balance.

Administration of *helmar-1* and *helmar-2* partially reduced these changes, leading to a measurable reduction in excessive permeability to K^+ , Na^+ , H^+ , Ca^{2+} , and Mg^{2+} ions. Although the normalization was not complete, several mitochondrial indicators approached control levels after administration of the extracts. The treated animals showed protective changes that appear to be related to the antioxidant properties of the polyphenol fractions. Lipid peroxidation decreased, and membrane potential ($\Delta\psi_m$) was better preserved.

Although silymarin produced a stronger response, the general trend was similar.

The findings point to an effect of *H. maracandicum* polyphenols on mitochondrial membrane stability during toxic injury. At the same time, the study was limited to isolated mitochondria, and direct assessment of permeability transition pore activity was not carried out. Liver histology was not examined. Further in vivo studies are needed to better understand the overall hepatic response.

References

1. Nicholls DG, Ferguson SJ. Bioenergetics. 5th ed. London: Academic Press; 2020.
2. Brand MD, Nicholls DG. Assessing mitochondrial dysfunction in cells. *Biochem J.* 2011; 435:297–312.
3. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial permeability transition and cell death. *Biochim Biophys Acta.* 2014;1837:1343–53.
4. Brownlee M. The pathobiology of diabetic complications. *Diabetes.* 2005;54:1615–25.
5. Cadenas S. ROS and redox signaling in myocardial ischemia–reperfusion injury. *Free Radic Biol Med.* 2018; 117:76–89.
6. Ahmedova S, Asrarov M. Evaluation of the hepatoprotective and antioxidant properties of an aqueous extract of plant polyphenols from *Helichrysum maracandicum*. *IOP Conf Ser Mater Sci Eng.* 2021; 939:012045.

7. Liu J, Zhang X, Wang Y, Chen L, Zhao H, Li Q, et al. Natural polyphenols modulate mitochondrial pathways in metabolic disorders. *Nutrients*. 2023;15:1234.
8. Singh CK, Ahmad N. Mitochondrial targets of natural polyphenols: therapeutic implications. *Pharmacol Res*. 2019; 146:104317.
9. Wang Y, Liu J, Chen X, Zhang L, Li H, Zhao Q, et al. Polyphenols and mitochondrial function in health and disease. *Front Nutr*. 2022;9:876543.
10. Halliwell B. Free radicals and antioxidants: updating a personal view. *Nutr Rev*. 2012; 70:257–65.
11. Chen Q, Vazquez EJ, Moghaddas S, Hoppel CL, Lesnfsky EJ. Production of reactive oxygen species by mitochondria. *J Biol Chem*. 2003; 278:36027–31.
12. Korshunov SS, Skulachev VP, Starkov AA. The antioxidant function of mitochondria. *FEBS Lett*. 1997;416:15–18.
13. Szewczyk A, Wojtczak L. Mitochondria as a pharmacological target. *Pharmacol Rev*. 2002; 54:101–27.
14. Bernardi P, Rasola A, Forte M, Lippe G. The mitochondrial permeability transition pore: molecular nature and role as a target in pharmacology. *Curr Med Chem*. 2015; 22:2073–89.
15. Petronilli V, Penzo D, Scorrano L, Bernardi P, Di Lisa F. Regulation of the permeability transition pore. *J Bioenerg Biomembr*. 2001;33:381–89.
16. Halestrap AP. What is the mitochondrial permeability transition pore? *J Mol Cell Cardiol*. 2009;46:821–31.
17. Schneider WC, Hageboom GH, Palade GE. Isolation of intact mitochondria from rat liver. *J Biol Chem*. 1948; 172:619–35.
18. Peterson GL. A simplification of the protein assay method of Lowry et al. *Anal Biochem*. 1977;83:346–56.
19. Brierley GP. Passive permeability and energy-linked ion movements in isolated heart mitochondria. *Ann N Y Acad Sci*. 1974; 227:398–410.