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Antioxidant and cardioprotective properties of polyphenolic plant extract of *Rhus glabra L.*

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Abstract

This research looks at the heart-healthy and antioxidant effects of a polyphenol mixture made from *Rhus glabra* by using a lot of different types of experiments. First, the DPPH assay was used to test the antiradical activity. It showed strong free radical scavenging abilities, with an IC50 value of 18.4 ± 1.4 µL. Mitochondria isolated from rat liver were utilized to examine antioxidative effects on mitochondrial K+ATP ion channels and the mitochondrial permeability transition pore (mPTP). Lipid peroxidation (LPO) was measured via malondialdehyde (MDA) production, indicating that the preparation inhibited LPO and oxidative stress in mitochondrial membranes. Further, the study employed an adrenaline-induced ischemia model to evaluate cardioprotective effects. Treatment with the polyphenol preparation significantly reduced enzyme markers such as ALT, AST, CK, and LDH compared to the ischemic group, highlighting its potential in mitigating ischemic damage. Creatine kinase activity assays indicated improved cellular energy metabolism and biochemical profiling revealed enhancements in atherogenic, cardioprotective, and coronary risk indices. Statistical analyses confirmed the significance of these findings, demonstrating that *Rhus glabra* exhibits potent antioxidant activity, mitigates oxidative stress, and provides cardioprotective benefits. The study's results suggest that *Rhus glabra* holds substantial therapeutic potential for enhancing cardiovascular health and combating oxidative stress through its antioxidant properties and ability to improve mitochondrial function and protect against ischemic injury.

Keywords

Antioxidant; cardioprotective; DPPH assay; ischemia; lipid peroxidation; mitochondrial dysfunction; *Rhus glabra*

Introduction

Cardiovascular diseases (CVD) are a leading cause of global morbidity and mortality. Natural products, particularly those derived from plants like *Rhus glabra*, offer promising therapeutic potential due to their diverse biologically active compounds, such as vitamins, flavonoids, and xanthones. In particular, *Rhus glabra* compounds like quercetin and rutin are powerful antioxidants that help fight oxidative stress, which is linked to CVD. Biochemical markers such as creatine kinase (CK) and the enzymes AST, ALT, and LDH play vital roles in diagnosing and monitoring myocardial

damage. Mitochondrial ATP-sensitive potassium channels (mitoKATP) are also crucial, as they regulate cellular energy metabolism and provide cardioprotective effects during ischemic conditions (1-13). This study focuses on exploring the antioxidant and cardioprotective effects of *Rhus glabra*'s polyphenolic fraction, aiming to contribute to the development of novel therapeutic approaches for managing cardiovascular diseases.

Materials and Methods

Chemicals

KCl (125 mM), Hepes (10 mM), succinate (5 mM), MgCl2 (1 mM), K2HPO4 (2.5 mM), KH2PO4 (2.5 mM), rotenone (0.005 mM), and oligomycin (0.001 mM) (14). Polyphenols were added at concentrations ranging from 1-50 μM, with 10 mM ATP serving as control, TBA, malondialdehyde, EDTA-free medium, 1,1-diphenyl-2-picrylhydrazyl

Determination of antiradical activity

The impact of a composite polyphenol preparation on the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was assessed following the methodology outlined by Yokozawa T. In this approach, the ethanol solution of the tested preparations was introduced into the control cuvette containing 100 μM DPPH. After swift agitation, the kinetics of absorbance change were promptly recorded at 517 nm over a half-hour period (14).

Tissue preparation

Animal handling and experimental procedures were conducted by the European Directive 2010/63/EU on the protection of animals used for scientific purposes (14). The specific protocol received approval from the Animal Ethical Committee of the Institute of Bioorganic Chemistry, AS RUz (Protocol Number: 133/1a/h, dated August 4, 2014). All surgical procedures were performed under sodium pentobarbital anesthesia, and all possible measures were taken to reduce animal suffering. The experiments involved white male rats weighing between 200 and 250 grams.

Isolation of mitochondria

Mitochondria were isolated from rat liver using differential centrifugation, following the protocol established by Schneider et al. (15). Initial centrifugation at 600 g for 7 minutes efficiently removed nuclei and cellular fragments. Subsequent centrifugation of the supernatant at 10,000 g for 15 minutes at -4 -0 °C temperature resulted in the pelleting of mitochondria. The mitochondrial pellet underwent two washes in an isolation EDTA-free medium. The mitochondrial protein content was quantified using the Lowry method, as modified by Peterson (16).

Determination of MDA in liver tissue

Thiobarbituric Acid Reactive Substances (TBARS) Measurement:

TBARS levels, indicative of malondialdehyde (MDA) production and lipid peroxidation, were assessed in tissues following the method of Heath and Packer (17). Tissue supernatant (1 ml) was combined with 4 ml of 20% trichloroacetic acid (TCA) containing 0.5% Thiobarbituric acid (TBA). After heating at 95°C for 30 min and centrifugation at 10,000 g for 10 min, the MDA_TBA complex was measured at 532 nm using a spectrophotometer.

Lipid Peroxidation Measurement

Lipid peroxidation (LPO) experiments were conducted using thiobarbituric acid (TBA). Mitochondrial suspensions were centrifuged after adding 0.220 ml of 70% trichloroacetic acid. To the supernatant, 1 ml of 75% TBA solution was added and incubated at 37°C for 30 min. After cooling, absorbance was measured at 540 nm. MDA quantity was calculated, and the LPO rate was expressed in nM of MDA/mg of protein per hour (18).

mitoKATP Current Recording

MitoKATP channel current recording at 540 nm was performed in an external solution containing KCl (125 mM), Hepes (10 mM), succinate (5 mM), $MgCl_2$ (1 mM), K_2HPO_4 (2.5 mM), rotenone (0.005 mM), and oligomycin (0.001 mM) (19). Polyphenols were added at concentrations ranging from 1-50 μM, with 10 mM ATP serving as control.

Adrenaline Ischemia Model

Sixty minutes after drug and water administration, acute ischemia was induced by a single intraperitoneal injection of adrenaline at 100 mg/kg.

Creatine Kinase Activity Determination

Creatine kinase activity in heart tissue was determined using the Cypress Diagnostics Creatine Kinase NAC kit. The reagents were mixed, and absorbance measurements at 340 nm were conducted using a spectrophotometer. A mixture of 40 μL of the sample and 1 mL of working reagent was incubated, and absorbance was recorded every minute for 3 minutes.

Calculation

At 25-30 ºC ΔA/min × 4127 = U/L CK

Determination of Profile Biomarkers

Transaminases (AST/ALT) were analyzed using the colorimetric method (20), and creatine kinase activity was assayed by the Nelson method. The p-nitrophenol protocol was used to evaluate ALP activity using the Randox test kit (21). Atherogenic, cardioprotective, and coronary risk indices were calculated as previously (22). In brief, the atherogenic index (AI), coronary risk index (CRI), and cardioprotective index (CPI) were calculated as

AI = Log [TG/HDL-C],

CRI = TC/HDL-C,

CPI= [HDL-C/LDL-C],

Extraction and origin of PC-5

Rhus glabra, or smooth sumac, is originally from North America. It has been introduced to Uzbekistan, where it thrives due to its adaptability and resilience.

Medicinal Properties: *Rhus glabra* is valued for its numerous medicinal benefits:

1. **Antioxidant:** Rich in vitamin C and antioxidants, helping to combat oxidative stress.
2. **Astringent:** Useful for treating minor skin irritations and wounds.
3. **Anti-inflammatory:** Reduces inflammation, aiding conditions like arthritis.
4. **Antimicrobial:** Prevents and treats infections with its antimicrobial properties.

Digestive Aid: Soothes digestive issues such as diarrhea and stomach cramps.

In Uzbekistan, smooth sumac is used in traditional remedies, taking advantage of its health benefits while also serving ornamental and ecological purposes.

We extracted 1000 g of *Rhus glabra* plant raw materials in chloroform at 450 °C for 2 hours in a water bath with reflux cooling to remove lipophilic compounds three times. We filtered the extracts and dried the crude material at room temperature until the chloroform odour vanished. Next, we extracted the raw material thrice in 70% aqueous acetone, maintaining a temperature of 450 ° C for two hours. We filtered the extracts and separated the aqueous fraction by driving off acetone under vacuum at 35–40 °C. We extracted the ethyl acetate from the aqueous fraction. We dried this fraction over anhydrous Na2SO4, filtered it, and extracted it with a rotary evaporator to yield an ethyl acetate concentrate. We isolated a total of 15.8% of polyphenols from the dry mass of the plant by precipitating the concentrate with chloroform in a ratio of 1:4. Column chromatography separated the sum of the isolated polyphenols into individual compounds.

Data analysis

Statistical analyses were performed using the statistical package Origin 8.5 (OriginLab Corporation, USA). The data were evaluated using parametric Student's t-test, which we expressed as M ± m. Deemed authentic results are expressed at * - P<0.05; ** - P<0.01; ***- P<0.001.

Results and Discussion

When the test compound is introduced into an alcohol solution of DPPH, the free radical molecules transform into a non-radical form, causing the initially intense violet DPPH solution to lose its color. Figure 1 illustrates the kinetics of optical density changes in a DPPH solution upon the addition of PC-5 at different concentrations. Upon analysis of the results, it is evident that the addition of PC-5 to the alcohol solution of DPPH leads to a rapid reduction in the optical density, indicative of a high Antiradical Activity (ARA) for this compound (Fig. 1).

To quantify the antiradical activity, we employed the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), along with parameters such as t50, representing the time required for the studied drugs to reduce the initial radical concentration by 50%, and IC_{50}, denoting the concentration of the substance required for a 50% reduction in the initial radical concentration. Detailed results are presented in Table 1. Thus, the obtained

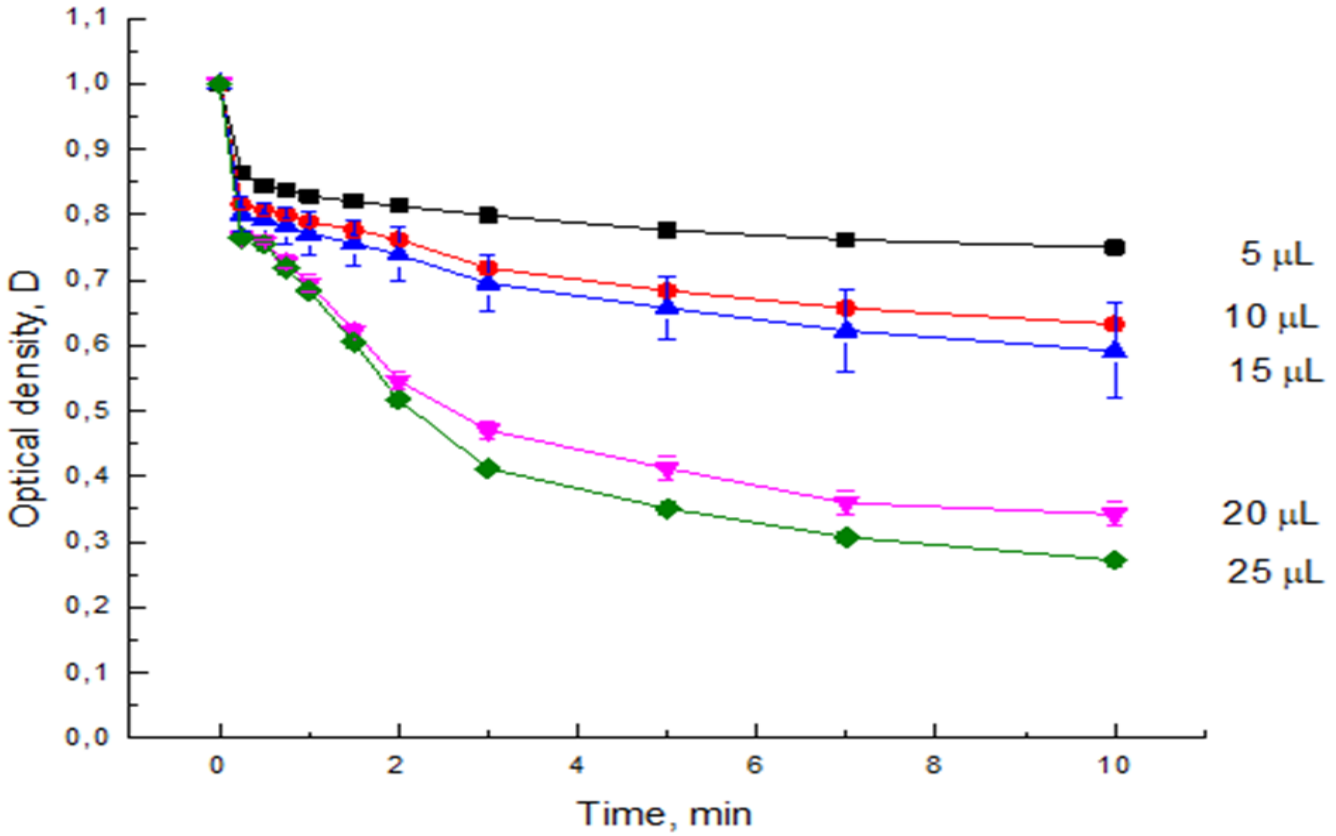

Fig. 1 The Effect of *Rhus glabra* L. (PC-5). The graph illustrates the change in optical density of a 0.1 mM DPPH alcohol solution relative to the control over time upon the addition of the test compound. Measurements were conducted at 20°C immediately after introducing the test drug, with an initial test compound concentration of 1 mg/ml.

Table 1. 50% inhibitory concentration (IC50) and time required to reduce the concentration of DPPH by 50% (t50) when reacting with the test substance

IC_{50}, µL	t_{50}, seconds				
	5 µL	10	15 µL	20 µL	25 µL
PC-5	PC-5	PC-5	PC-5	PC-5	PC-5
18,4 ± 1,4	-	-	-	165 ±	122 ± 5,6

experimental results indicate that PC-5 has a high ARA about the free radical DPPH.

LPO in liver homogenate. Further research consisted of studying the effect of PC-5 on the process of lipid peroxidation (LPO) of membranes. For this purpose, we used a technique based on the induction of lipid peroxidation in liver homogenate by the Fe^{2+}/ascorbate system. The addition of the Fe^{2+}/ascorbate system to the liver homogenate induces LPO, resulting in the formation of malondialdehyde (MDA). Under conditions of LPO induction, adding PC-5 to the incubation medium at a concentration of 1 µL from a previously prepared mg/ml solution, a slight inhibition of LPO was observed. A gradual increase in the concentration of PC-5 in the incubation medium led to further inhibition of the lipid peroxidation process, which indicated its antioxidant properties (Fig. 2).

The induction of Lipid Peroxidation (LPO) in mitochondria results in alterations in membrane permeability, a decline in membrane potential, and the uncoupling of Oxidative Phosphorylation (OP) and ATP hydrolysis (5). The impact of LPO on mitochondrial functions manifests at both the level of direct influence by LPO products on the lipid matrix of membranes and through various indirect effects (23). The ATP-dependent potassium channel (mitoKATP) is a pivotal mitochondrial membrane channel governing matrix volume and playing a crucial role in membrane potential formation. Subsequently, this study explores the influence of PC-5 on mitoKATP mitochondrial activity. Analysis of experimental data reveals that, at a concentration of 5 µl, PC-5 demonstrates a mild activating effect on this channel. As the PC-5 concentration increases to 10 and 20 µl, mitoKATP activity experiences a substantial surge, reaching 2-2.5 times that of the control (Fig. 4).

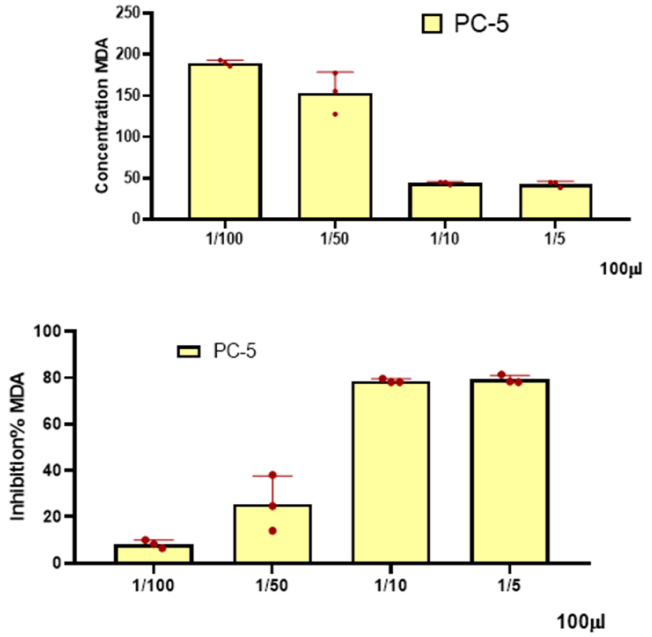

Fig.2. The Effect of *Rhus glabra* L. (PC-5) on Fe^{2+}/ascorbate-induced lipid peroxidation in rat liver homogenate.

LPO in mitochondria. The introduction of the Fe^{2+}/ascorbate system to the incubation medium triggers Lipid Peroxidation (LPO), leading to a disruption in the barrier function of mitochondrial membranes and a significant swelling of the organelles compared to the control (Fe^{2+}/ascorbate) (Fig. 3). When PC-5 is added to the incubation medium under conditions of LPO induction, starting from a concentration of 1 µl from a pre-prepared mg/ml solution, it effectively inhibits mitochondrial swelling. The impact of PC-5 on LPO in mitochondrial membranes is concentration-dependent, with an increase in its concentration resulting in a higher percentage of inhibition. Complete inhibition of mitochondrial swelling, indicative of the LPO process, is observed at a PC-5 concentration of 20 µl. The concentration causing half-maximal inhibition of LPO (IC_{50}) for PC-5 is determined to be 6.08±0.06 µl. These experiments demonstrate the antioxidant properties of PC-5.

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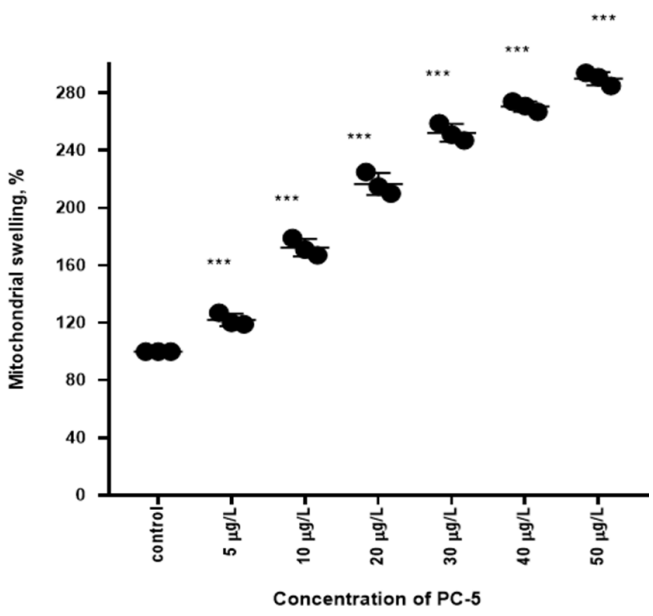

Fig. 4. The Effect of *Rhus glabra* L. (PC-5) on the activation of the mitoKATP channel. Incubation medium is (mM): KCl- 125, Hepes-10, succinate-5, $MgCl_2$-1, K_2HPO_4 -2.5, KH_2PO_4-2.5, rotenone-0.005.

demonstrates a mild activating effect on this channel. As the PC-5 concentration increases to 10 and 20 µl, mitoKATP activity experiences a substantial surge, reaching 2-2.5 times that of the control (Fig. 4).

Subsequently, the effectiveness of PC-5 on enzyme markers associated with cardiopathologies was assessed. The results in Table 2, presented as mean ± SEM (standard error of the mean) from five animals in each group, demonstrated a significant ($P < 0.05$) increase in the activities of ALT, AST, CK, and LDH under ischemic conditions (Group II) compared to the control group on a standard diet (Group I). However, coadministration of PC-5 with adrenaline in Groups III to IX resulted in a significant ($P < 0.05$) decrease in the activities of ALT, AST, and CK compared to the ischemic group (Group II).

Values are presented as mean ± SEM (standard error of the mean). SD denotes the standard diet, AD represents adrenaline administration, and PC-5 signifies the polyphenolic fraction extract of *Rhus Glabra*. The group treated with PC-5 only exhibited no significant ($P < 0.05$) changes in enzyme markers when compared to the normal control (Group I). A significance level of $P < 0.05$ indicates higher values compared to the standard diet control and lower values compared to the adrenaline control.

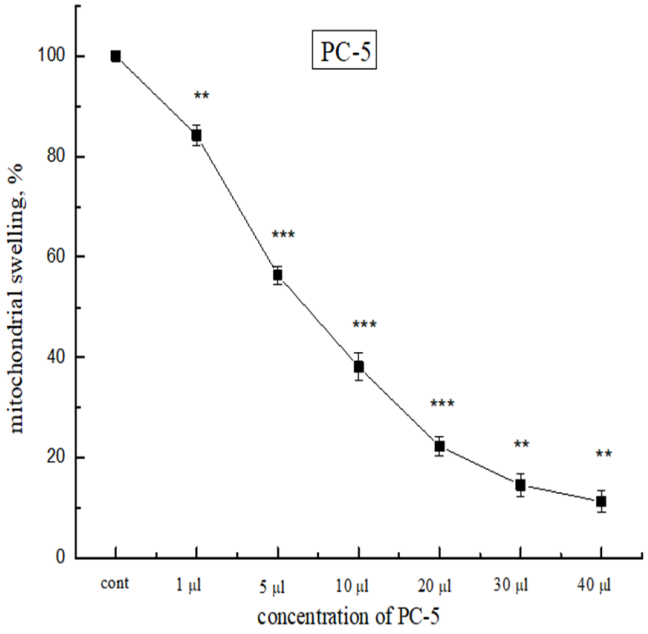

Fig. 3. The Effect of *Rhus glabra* L. (PC-5) on Fe^{2+}/ascorbate -induced swelling of rat liver mitochondria. LPO solution: KCl - 125, Tris-HCl - 10, pH 7.4; $FeSO_4$ – 0.01, ascorbate – 0.6; mitochondrial protein 0.3-0.4 mg/ml; (** - P<0.01, *** - P<0.001; n=4).

The current experiments underscored the potential cardioprotective and antioxidant effects of the polyphenolic fraction extract derived from *Rhus Glabra* in both in vitro and in vivo models. *Rhus Glabra*, a widely recognized medicinal plant in Central Asia, boasts diverse biological activities and pharmaceutical functions, particularly its robust antioxidant properties.

Antioxidants play a crucial role by interacting with various reactive oxidants, including reactive oxygen species and free radicals, leading to their partial or complete inactivation (6). The classification of antioxidants is continually expanding, with two main groups recognized:

Group I: Encompassing high molecular weight compounds, including antioxidant enzymes such as superoxide dismutase (SOD), ceruloplasmin, catalase, and glutathione-dependent enzymes. It also includes proteins capable of binding Fe and Cu ions, catalysts for free radical processes.

Group II: Comprising low-molecular-weight fat- and water-soluble antioxidants like α-tocopherol, vitamins A, K, P, urea, uric acid, glutathione, ascorbic acid, sulfur-containing amino acids, bilirubin, etc. (24).

It's noteworthy that the 2nd group, consisting of low-molecular-weight antioxidants, is widely used in clinical and experimental medicine (25). The compound under study, PC-5, extracted from the *Rhus Glabra* plant and belonging to hydrolyzable tannins, falls into this category. As such, it can be reasonably postulated that PC-5 exhibits antioxidant activity, resulting in the inhibition of lipid peroxidation.

Understanding the role of lipid peroxidation (LPO) in regulating crucial cellular functions holds significant interest for various reasons. Electron transfer along the mitochondrial respiratory chain (RC) is known to generate reactive oxygen species (ROS), initiating peroxidation reactions of lipids, proteins, and nucleic acids (26-27). Concurrently, there is evidence suggesting the involvement of mitochondria in shielding cells from oxidative stress (OS) (28). Considering these factors, the capacity of PC-5 to mitigate free radicals was assessed. Employing diverse methods to determine antiradical activity, it was established that PC-5 possesses the

Table 2. The Effect of *Rhus glabra* L. (PC-5) on the Tissue Enzyme Markers of ischemia

Group	ALT (IU/L)	AST (IU/L)	CK (IU/L)	LDH (IU/L)
I. Standard diet (SD)	21.53 ± 0.11	105.90 ± 1.02	174.60 ± 6.46	210.30 ± 6.36
II. adrenaline administration (AD)	102.30 ± 4.36	182.20 ± 6.33	479.20 ± 7.30	577.40 ± 9.51
III AD + 10 mg/kg PC-5	95,2 ± 0,23	166,2 ± 2,33	375,0 ± 6,37	489,4 ± 7.84
IV AD + 20 mg/kg PC-5	82,4 ± 0,87	157,5 ± 3,24	348,4 ± 4,23	443,2 ± 6.23
V AD + 30 mg/kg PC-5	69,1 ± 1,46	149,3 ± 2,65	325,1 ± 3,34	412,7 ± 3.34
VI AD + 40 mg/kg PC-5	55,1 ± 0,97	141,7 ± 1,21	207,9 ± 5,20	385,6 ± 4.25
VII AD + 50 mg/kg PC-5	46,7 ± 1,64	137,8 ± 2,18	182,6 ± 4,89	326,0 ± 8.14
VIII AD + 75 mg/kg PC-5	38,3 ± 2,02	128,2 ± 3,61	170,4 ± 7,14	294,4 ± 6.30
IX AD + 100 mg/kg PC-5	32,6 ± 1,86	127,3 ± 2,78	256,8 ± 3,89	278,8 ± 5.78

capability to quench free radicals. Given that antioxidants may operate through varied mechanisms, a comprehensive study of their activity using diverse methods is recommended.

Subsequently, the impact of various concentrations of PC-5 on the process of lipid peroxidation in mitochondrial membranes induced by the Fe^{2+}/ascorbate system was investigated in in vitro experiments. The results highlight the pronounced antioxidant activity of PC -5, effectively suppressing the effects of LPO inducers.

The observation that PC-5 not only inhibits the accumulation of lipid peroxidation products in liver homogenate but also exhibits high antiradical activity signifies its true antioxidant nature. The mechanism of action involves the donation of mobile hydrogen to free radicals, disrupting the LPO reaction chain. This assertion is further supported by a correlation coefficient (r) of 0.94 between the manifestation of antioxidant and antiradical properties.

Contemporary research increasingly suggests that the activation of mitoKATP channels plays a pivotal role in the body's adaptation to various pathological conditions, including oxygen starvation and ischemia/reperfusion (12). Furthermore, therapeutic drugs often target mitoKATP channels (29), with literature supporting the notion that mitoKATP activators hold potential as cardioprotectors. The pharmacological activation of mitoKATP is anticipated to replicate the endogenous cardioprotective mechanism. Numerous studies, including those by Akopova and Oldenburg O, confirm the cardioprotective effect associated with the opening of the mitochondrial K^+ATP channel. Notably, ischemic adaptation (preconditioning) is linked to mitoKATP channel activation, as evidenced by the reproducibility of the protective effect with activators like diazoxide and pinacidil and its elimination by blockers such as glibenclamide and 5-hydroxydecanoic acid (30). The activation of the K^+ATP channel influences various aspects of mitochondrial function, encompassing mitochondrial volume, oxygen consumption, Ca^{2+} transport, oxidative phosphorylation, and the generation of reactive oxygen species (ROS) (31-34). Studies indicate that K^+ATP channel activation reduces Ca^{2+} accumulation, suppresses mitochondrial permeability transition pore (mPTP) formation, and affects ROS production through mechanisms involving protein kinase C and hydroperoxide (35-37). While some research suggests a reduction in ROS formation and a subsequent cardioprotective effect (32), our results indicate the activation of mitoKATP under the influence of PC-5, suggesting potential cardioprotective activity. Despite these observed effects likely contributing to a complex cardioprotective mechanism, the exact protective action of K^+ATP channel activators remains insufficiently understood. Therefore, our ongoing work delves into the impact of PC-5 on changes in the activity of cardiac marker enzymes.

It is established that various cardiovascular diseases can be early diagnosed by assessing biochemical parameters in blood plasma (8). For instance, creatine phosphokinase (CPK) acts as a crucial enzyme maintaining the ATP and ADP ratio, catalyzing ATP conversion, and releasing energy for diverse biochemical processes in living systems. Additionally, enzymes like aspartate aminotransferase and alanine aminotransferase serve as biochemical markers for myocardial damage in the coronary sinus.

In our study, intraperitoneal administration of adrenaline to rats resulted in an elevation of enzyme marker activities associated with diagnosing myocardial ischemia, including creatine kinase, lactate dehydrogenase, ALT, and AST in both the blood and heart homogenate of rats, compared to the standard diet control (Table 2). Notably, treatment with PC-5 decreased the activities of these enzyme markers, implying a potential cytoprotective mechanism of PC-5. Moreover, PC -5 exhibited no significant changes in enzyme activity compared to the standard diet control

Conclusion

This study highlights the potent antioxidant and cardioprotective properties of the polyphenolic fraction extract (PC-5) from *Rhus Glabra*. The investigation demonstrated that PC-5 effectively inhibited lipid peroxidation (LPO) in liver and mitochondrial membranes, underscoring its robust antioxidant activity. PC-5's ability to reduce malondialdehyde (MDA) formation and its significant impact on the ATP-dependent potassium channel (mitoKATP) activity further emphasize its role in protecting against oxidative stress and ischemia/ reperfusion injury. PC-5 exhibited a dose-dependent inhibitory effect on LPO, with a marked reduction in mitochondrial swelling and enhancement of mitoKATP channel activity. These findings indicate that PC-5 mitigates oxidative damage by donating hydrogen to free radicals, thereby breaking the LPO chain reaction. The correlation between the antioxidant and antiradical properties of PC-5 reinforces its therapeutic potential. In vivo studies using an adrenaline-induced ischemia model in rats revealed that PC-5 significantly decreased the activities of key enzyme markers of myocardial injury, such as creatine kinase (CK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). This reduction in enzyme activity suggests a cytoprotective mechanism, highlighting PC-5's potential in cardio protection. Overall, PC-5 from *Rhus Glabra* shows promise as a nutraceutical with significant antioxidant and cardioprotective effects, warranting further exploration in clinical settings for managing cardiovascular diseases and oxidative stress-related conditions.

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cardiovascular system based on *Herba leonuri, Gnaphalii uliginosi herba, Chamomillae recutie flores, Crataegi flores* medicinal plants")

Authors' contributions

The author was involved in every aspect of the work, including experimental design, data analysis, interpretation of the results, and writing the entire manuscript.

Compliance with ethical standards

Conflict of interest: The author declares that they have no competing interests.

Ethical issues: None.

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