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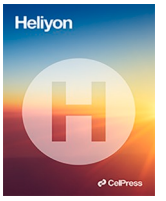

Research article

Protective effects of Pyrogallol and Caffeic acid against Fe^{2+}-Ascorbate-induced oxidative stress in the wistar rats liver: An in vitro study

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ABSTRACT

Regarding the role of oxidative damage to various tissues in various diseases, using antioxidant compounds that protect tissues from damage is proposed as an important strategy against these diseases. Liver homogenates are frequently employed as in vitro models for investigating oxidative stress owing to the liver's high metabolic activity and susceptibility to oxidative damage. In this study, we assessed the protective effects of two phenolic compounds on Fe^{2+}-ascorbate-induced oxidative stress in liver homogenates by analyzing various markers such as lipid peroxidation, protein carbonyl oxidation (PCO), reduced glutathione (GSH), and ROS levels. Catechin was used as a reference antioxidant to compare with the results. The DPPH radical scavenging activity of the compounds was also evaluated. Our findings demonstrated that co-incubation of liver homogenates with the Fe^{2+}-ascorbate system and the compounds at various concentrations (10, 25, 50, and 100 μg/mL) led to a dose-dependent reduction in lipid peroxidation, PCO levels, ROS production, and GSH depletion. Furthermore, the IC_{50} values for DPPH free radicals for pyrogallol and caffeic acid were determined to be 76.26 μg/mL and 106.31 μg/mL, respectively. Notably, pyrogallol and caffeic acid exhibited higher antioxidant activity in all assays than catechin. In most assays (DPPH, Lipid peroxidation, and PCO) and at high concentrations (50 and 100 μg/mL), the antioxidative stress activity of pyrogallol was higher than that of caffeic acid. In conclusion, this study's results suggest that these compounds can potentially ameliorate diseases associated with oxidative stress.

1. Introduction

Oxidative stress refers to an imbalance between free radicals and antioxidants in the body, leading to damage to cells and tissues [1, 2]. One of the critical mechanisms involved in oxidative stress is the production of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, and hydroxyl radicals [3]. This can lead to oxidative damage to proteins, lipids, and DNA in cells, leading to cell dysfunction and death, which has been linked to various chronic diseases such as cancer, heart disease, and neurodegenerative disorders [4].

To manage the surplus of free radicals generated by oxidative stress, humans have evolved advanced mechanisms to maintain redox homeostasis. These mechanisms involve enzymatic and non-enzymatic antioxidant systems that scavenge or detoxify ROS, inhibit their

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production, or sequester transition metals, contributing to free radical formation [5]. When the efficiency of the antioxidant system decreases during diseases, consuming exogenous antioxidants can be proposed as an appropriate strategy to strengthen the antioxidant system [6]. Antioxidants are crucial in combating oxidative stress by neutralizing ROS and preventing damage to cells and tissues [7]. Common antioxidants include vitamins C and E, beta-carotene, flavonoids, and minerals like selenium and zinc. These compounds can be obtained from various plants such as fruits, vegetables, nuts, seeds, and whole grains. Phenols, the most important antioxidants, are organic compounds containing a hydroxyl group (-OH) attached to an aromatic ring. They work through various mechanisms, including scavenging free radicals, regenerating other antioxidants, chelating metal ions, and inducing antioxidant enzymes [8].

In the present study, we used Fe^{2+}-ascorbate to induce oxidative stress in the rat's liver. The Fe-ascorbate system is significant in oxidative stress as it facilitates the generation of ROS through the redox cycling of iron and ascorbic acid (vitamin C). Studies have shown that this system can lead to oxidative modification of proteins and lipids, particularly tissues, indicating its role in promoting oxidative damage [9]. Ascorbic acid acts as an antioxidant, but in the presence of free iron, it can contribute to oxidative stress rather than alleviate it. Iron is a crucial element in many biological processes. Serum iron exists in the forms of Fe^{3+} and transferrin, which could transform into Fe^{2+} in the presence of a deoxidizer such as AA or hydroxylamine hydrochloride [10]. Moreover, the content of Fe not only depends on the amount of iron in the serum but also is associated with the transferrin-bound iron. Most iron in the body is released from foods and the aging red blood cells, and the concentration is closely related to hemolytic anemia, hemopoietic dysfunction caused by lead poisoning, necrotic hepatitis, and hemosiderosis. The excess iron can catalyze the formation of ROS through Fenton reactions, leading to oxidative stress [11].

Pyrogallol, or 1,2,3-trihydroxybenzene, is a polyphenol compound naturally found in plants such as gallnuts, tea leaves, and grapes [12]. It is a white, crystalline solid soluble in water and polar organic solvents. Pyrogallol is known for its antioxidant properties and is often used in various industries [12]. This antioxidant property makes pyrogallol a valuable compound in multiple applications where free radical scavenging is required [13]. In addition, it has anti-inflammatory, antioxidative, antibacterial, anti-amyloidogenic, and antiglycation activities [12–14]. However, pyrogallol is highly reactive and can be toxic if ingested or absorbed through the skin in large amounts. It can cause skin irritation, respiratory issues, and gastrointestinal problems. Therefore, proper precautions should be taken when handling pyrogallol.

Caffeic acid is a hydroxycinnamic acid, a polyphenol found in various plant-based foods such as coffee, fruits, vegetables, and grains. Caffeic acid is a potent antioxidant, helping to neutralize free radicals and reduce oxidative stress. This property prevents cellular damage and may affect various diseases, including cancer, and neurodegenerative and cardiovascular disorders [15,16]. In addition to its antioxidant properties, caffeic acid exhibits antimicrobial and anti-inflammatory effects [17]. It can help inhibit the growth of harmful bacteria and fungi and reduce inflammation in the body. Caffeic acid also shows potential in protecting the liver from damage caused by toxins, drugs, and diseases, possibly through its antioxidant properties and ability to enhance liver detoxification processes.

Catechin is a polyphenolic compound recognized as a significant antioxidant, exhibiting various pharmacological effects in both in vitro and in vivo settings. It also possesses structural similarities with pyrogallol and caffic acid compounds (specifically, the functional groups on the ring). In addition, pyrogallol is a part of the structure of catechin. Due to the implication of oxidative stress in the pathogenesis of various diseases, no direct study on the effects of these compounds in oxidative stress induction systems has been done so far. Therefore, we decided to study the protective and antioxidant effects of these compounds in a commen model of pathogenesis related to oxidative stress. Various parameters, including lipid peroxidation, protein carbonyl oxidation, GSH content, and ROS formation, were assessed.

2. Material and methods

2.1. Materials

Pyrogallol and caffeic acid were obtained from Merck (Darmstadt, Germany). Bovine serum albumin (BSA, 810037), 5-sulfosalicylic acid ($C_7H_6O_6S \cdot 2H_2O$, 800691), trichloroacetic acid (TCA, 100807), thiobarbituric acid (TBA, 108180), and nitro blue tetrazolium chloride (NBT, 104186) were purchased from Merck Co (Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH, 1898-66-4), catechin (7295-85-4), 5,5′-dithiobisnitro benzoic acid (DTNB, 69-78-3), 2′,7′-dichlorofluorescein diacetate (DCFH-DA, 4091-99-0), 2,4-dinitrophenylhydrazine (DNPH, 119-26-6), guanidine hydrochloride (GdnHCl), ketamin and xylazin from Sigma-Aldrich (St. Louis, MO, USA) were obtained. All other chemicals used were analytical grade.

2.2. DPPH radical scavenging assay

This method used DPPH to generate stable free radicals [18]. The compounds at different concentrations (10, 25, 50, and 100 μg/mL) were mixed with 1 mL of DPPH solution. After incubating the samples for 30 min at room temperature, the absorbance was read at a wavelength of 517 nm using the Epoch microplate reader (Biotek model/USA).

$$\text{Inhibition percentage of free radicals } (\%) = [(A_o\text{-}A_1 \ / \ A_o) \times 100]$$

where A_o represents the absorbance of DPPH and A_1 represents the absorbance of samples in the presence of DPPH. Catechin was used as a positive control. The sample's IC_{50} was determined by its ability to inhibit or scavenge 50 % of free DPPH radicals.

For the present study, we firstly selected a wide range of concentrations on the DPPH radical scavenging test (1–500 μg/mL). The

best concentrations were the selected concentrations.

2.3. Preparation of liver homogenates

Eight Male Wistar rats (Average 10 weeks old) weighing 180 ± 20 g were purchased from Lorestan University of Medical Science (Khorramabad, Iran), housed under conventional conditions, and allowed free access to food, water, and ad libitum. The conditions were carefully monitored, with a temperature of 25 °C ± 1, relative humidity of 50–60 %, and 12-h light/dark cycles. For separation of the liver, the rats were anesthetized with intraperitoneal (i.p) injection of Xylazin (10 mg/kg) and Ketamin 100 mg/kg and were killed through a surgical incision at the uterine cervix. Then, the liver was swiftly removed from the abdomen. Livers were immediately rinsed with saline, blotted on filter paper, weighed, cut into small pieces, and homogenized in phosphate buffer (50 mM, pH 7.4) with a homogenizer to give a 10 % (w/v) liver homogenate. The homogenates were centrifuged at 5000 g for 15 min at 4 °C (Beckman). The homogenates were then kept at −70 °C for further research. According the National Institute of Health's Guide for the Care and Use of Laboratory Animals, all experimental protocols were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine of Lorestan University (LU.ECRA.2024.76).

2.4. Induction of oxidative stress

The oxidant pair Fe^{2+}/ascorbate induced oxidative stress in rat liver homogenate [1]. Before conducting any test, a reaction mixture was prepared containing 0.5 mL tissue homogenate, 0.9 mL phosphate buffer (50 mM, pH 7.4), 0.25 mL $FeSO_4$ (0.01 mM), 0.25 mL ascorbic acid (0.1 mM), and 0.1 mL of different concentration of samples and the standard sample. The reaction mixture was incubated at 37 °C for 30 min. For negative control, we used from homogenates samples incubated with the Fe^{2+}-Ascorbate without compounds and homogenates samples without Fe^{2+}-Ascorbate.

2.5. Measurement of protein level

The protein level in the liver was measured using the Bradford reagent, employing bovine serum albumin (BSA) as the standard [19]. The standard solution (5 mL) and the homogenized tissue were separately poured into tubes. Subsequently, 195 μL of the Bradford reagent was added to the standard and homogenized tissue solutions. After 5 min, the absorbance of the samples was read at a wavelength of 595 nm. The protein concentration of samples was determined by plotting a standard curve.

2.6. Measurements of lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) assay is one of the most widely used methods to measure lipid peroxidation. It involves the reaction of malondialdehyde (MDA), a byproduct of lipid peroxidation, with thiobarbituric acid to form a colored complex that can be quantified spectrophotometrically [20]. Briefly, 2.5 mL of TCA solution (%10) was added to 0.5 mL of supernatant of the homogenate tissue, and it was placed in a boiling water bath at 98 °C for 15 min. After cooling to room temperature, the samples were centrifuged at 3000 g for 10 min, and 0.3 mL of each sample supernatant was transferred to a tube containing 1 mL of TBA solution (% 0.67). Each tube was then put into a boiling bain-marie for 20 min. After cooling, the resulting purple color was measured at an optical density of 535 nm in the blank solution. The concentration of MDA in the samples was calculated based on the absorbance coefficient of the TBA-MDA complex ($\varepsilon = 1.56\ 10^5\ cm^{-1}\ M^{-1}$), and results were expressed as inhibition percentage of TBARS products.

2.7. Measurements of protein carbonyl oxidation (PCO)

Dinitrophenylhydrazine (DNPH) assay is a widely used method for measuring PCO. DNPH reacts with protein carbonyls to form stable dinitrophenylhydrazone derivatives, which can be quantified spectrophotometrically at 360 nm [21]. Briefly, DNPH solution (10 mM) was freshly prepared in HCl, and 1 mL was added to the reaction mixture (2 mg protein). Samples were incubated in the dark at room temperature for 60 min, with vortex-mixing every 10–15 min. Then, 1 mL of TCA (10 %, w/v) was added to the protein samples and centrifuged at 3000 g for 10 min. The protein precipitate was washed three times with 2 mL of ethanol/ethyl acetate (1:1, v/v) and dissolved in 1 mL of guanidine hydrochloride (6 M, pH 2.3). After 10 min incubation at room temperature, the absorbance of samples was recorded at 370 nm. The PCO content was calculated based on the molar extinction coefficient of DNPH ($\varepsilon = 2.2 \times 10^4\ cm^{-1}.\ M^{-1}$), and the results were expressed as the inhibition percentage of PCO formation.

2.8. Measurements of ROS formation

2′,7′-dichlorofluorescin diacetate (DCF-DA) was used to detect ROS formation in liver homogenates. This probe is non-fluorescent until oxidized by ROS, resulting in a fluorescent signal that can be quantified using fluorescence spectroscopy [22]. Briefly, each sample consisted of 1.7 mL of phosphate buffer solution (50 mM, pH 7.4), 0.2 mL of homogenate, and 100 μL of DCFH-DA solution (10 μM), which was incubated at 37 °C temperature for 15 min. The ROS levels were detected via the formation of DCF (a highly fluorescent compound) using a spectrofluorometric (Cary Eclipse) with the excitation and emission wavelengths at 488 and 525 nm, respectively.

2.9. Measurement of GSH content

Ellman's assay (5,5′-dithiobis (2-nitrobenzoic acid) DTNB (.) is widely used for measuring GSH levels in biological samples [23]. In this method, DTNB reacts with thiol groups of GSH to form a yellow-colored product that can be quantified spectrophotometrically at 412 nm. The supernatant (0.5 mL) was mixed with sulfosalicylic acid (4 %) and incubated at 4 °C for 1 h. Then, the samples were centrifuged at 3000 g at 4 °C for 15 min, and in the following to 1 mL of supernatant, 0.1 mL of DTNB (4 mg/mL), and 0.9 mL of phosphate buffer (0.1 M, pH 7.4) were added. The absorbance was read at 412 nm using a spectrophotometer and compared with the standard curve prepared using various L-cysteine concentrations. The GSH content was expressed as the inhibition percentage of GSH oxidation.

2.10. Statistical analysis

The GraphPad Prism v10.4.0.621was applied to statistical analysis for the obtained data. The post-hoc test utilized in our analysis is Tukey's multiple comparisons test. Statistical comparisons between different groups were made using a Two-way analysis of variance (ANOVA). Otherwise stated, results were expressed as the means and standard error of the mean (mean ± SEM), and P values less than 0.05 were considered statistically significant.

3. Results and discussion

3.1. DPPH radical scavenging activity

Polyphenols are a class of compounds found in plants known for their antioxidant properties [24]. They can neutralize free radicals in the body, which helps reduce oxidative stress and prevent cell damage. The antioxidant activity of polyphenols is attributed to their chemical structure, which carries hydroxyl groups and allows the transfer of electrons or hydrogen atoms [6]. This process helps to protect cells from oxidative damage and may have various health benefits. Pyrogallol and caffeic acid are two polyphenolic compounds that possess three hydroxyl moieties in their structures. This study investigated the compounds' antioxidant activity using the DPPH test. The DPPH free radical is commonly used to evaluate the antiradical capacity of antioxidant compounds. As shown in Fig. 1, the compounds exhibited varying scavenging capacities. With increasing concentration, the percentage of free radical inhibition was increased in both compounds. At low concentrations (10 and 25 μg/mL), caffeic acid exhibited greater antioxidant activity (71.3 and 84.3 %) compared to pyrogallol (62.1 and 79.1 %). However, at higher concentrations (100 μg/mL), pyrogallol (93.3 %) demonstrated stronger antioxidant properties than caffeic acid (89.3 %). The results also indicated that the antioxidant activity of the catechin as a standard compound (31.2, 65.4, 88.7.2 % at doses 10, 25, and 100 μg/mL) was lower than that of the two compounds ($p < 0.05$). Several hydroxyl groups in compounds suggest that these moieties are responsible for the compounds' antioxidant activity. The phenolic hydroxyl group in pyrogallol has a strong hydrogen-donating property, which can capture free radicals in the reaction system to achieve an antioxidant effect.

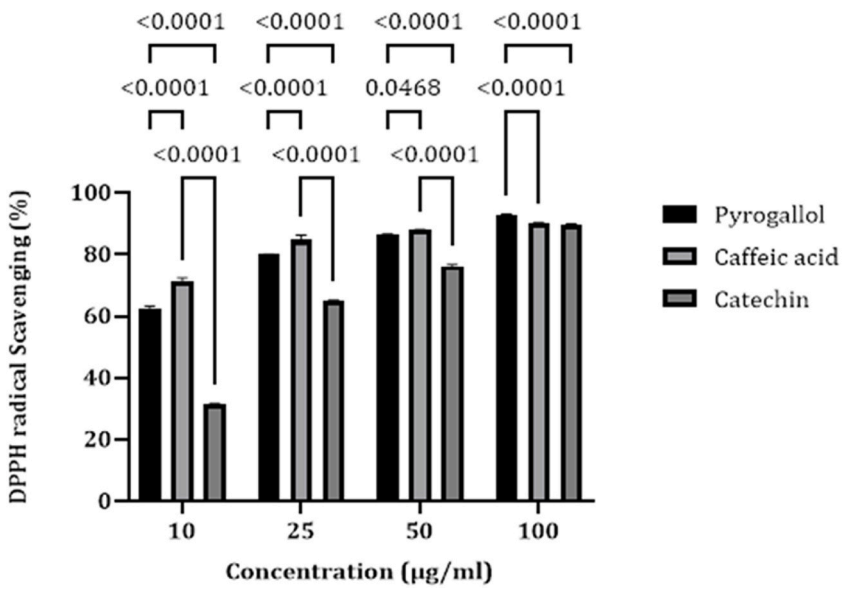

Fig. 1. Antioxidant activity of pyrogallol, caffeic acid, and catechin (as standard compound) at different concentrations using DPPH radical scavenging. The results of the three independent tests were expressed as mean ± SE. $P < 0.05$ was considered statistically significant between various concentrations and compounds.

3.2. Effect of compounds on lipid peroxidation

Lipid peroxidation is one of the important mechanisms related to the enhanced ROS production associated with the oxidative stress system [18]. MDA is the final product of unsaturated fatty acids peroxidation in cell membranes and is widely used as a reliable factor of ROS-mediated lipid peroxidation. The increased lipid peroxidation is known to cause loss of membrane fluidity, impaired iron transport, and membrane integrity, and thus can lead to cell damage and death. Non-enzymatic lipid peroxidation and formation of lipid peroxides can be initiated by adding ascorbate in the presence of oxygen and Fe or Fe ions to 3q 2q various tissue preparations such as homogenates, mitochondria, microsomes, and nuclei obtained from various tissues and species [25]. Hence, MDA levels can be considered when evaluating the oxidative damage of tissues. The amount of MDA in liver homogenates, which is assumed to be the end product of lipid peroxidation, was significantly increased in the Fe-ascorbate system. Simultaneous incubation of the Fe^{2+}-ascorbate system with compounds resulted in a decreased considerably MDA level. Our results indicate that the lipid peroxidation of liver homogenates induced by Fe^{2+}/ascorbate was suppressed to varying degrees by compounds ($p < 0.0001$). Pyrogallol (45.1, 81.2, and 84.8 %) and caffeic acid (30.4, 47.6, and 58.5 %) had the highest and lowest inhibitory effects on lipid peroxidation at concentrations 25, 50, and 100 μg/mL, respectively (Fig. 2). Based on the extent of inhibition of lipid peroxidation formation, the activity order of different compounds was found to be pyrogallol > Catechin > caffeic acid. A decrease in inhibition of lipid peroxidation can be due to hydroxyl groups in compound structure. These groups neutralize free radicals by donating electrons, breaking the chain reaction, and preventing further damage to cell membranes. These compounds may also upregulate antioxidant enzymes in the body, further enhancing the defense against lipid peroxidation. The interaction between these compounds and lipid peroxidation pathways helps protect cells from oxidative stress and maintain cellular health [2].

3.3. Effect of compounds on protein carbonyl content

Protein carbonylation is one of the irreversible oxidative protein modifications and is considered a widespread marker of severe oxidative stress, reflecting cellular damage induced by multiple forms of ROS [26]. This study confirmed that an excess of reactive oxygen species (ROS) can lead to the modification of proteins and the formation of protein carbonyl (PCO) products, which are recognized as indicators of oxidative damage to proteins [21]. The extent of PCO formation was significantly increased in our study when Fe^{2+}-ascorbate was added to the liver homogenate compared to the control sample ($p < 0.05$). However, adding pyrogallol and caffeic acid at different concentrations (25, 50, and 100 μg/mL) prevented the rise in the PCO content by various degrees (59.6, 72.7 and 79.4 %, and 65.4, 68.5 and 73.6 %, respectively). The results were compared with those of catechin as a positive control. Co-incubation of Fe^{2+}-ascorbate with Catechin resulted in a lower inhibition of PCO formation (54.6, 62.7, and 71.2 %) relative to compounds, as indicated by Fig. 3. Different studies have demonstrated that protein oxidation is a crucial function in the cell, and it can be caused by ROS or indirectly by the reactions of secondary byproducts of oxidative stress [25]. Protein carbonyl derivatives can be formed through direct oxidative attack on Lys, Arg, Pro, Thr, or secondary reaction of Cys, His, or Lys residues with reactive carbonyl compounds. Different studies have shown that polyphenols, as antioxidants, can help prevent protein carbonylation by scavenging free radicals and reducing oxidative stress. Polyphenols can protect proteins from oxidation by neutralizing reactive oxygen species and

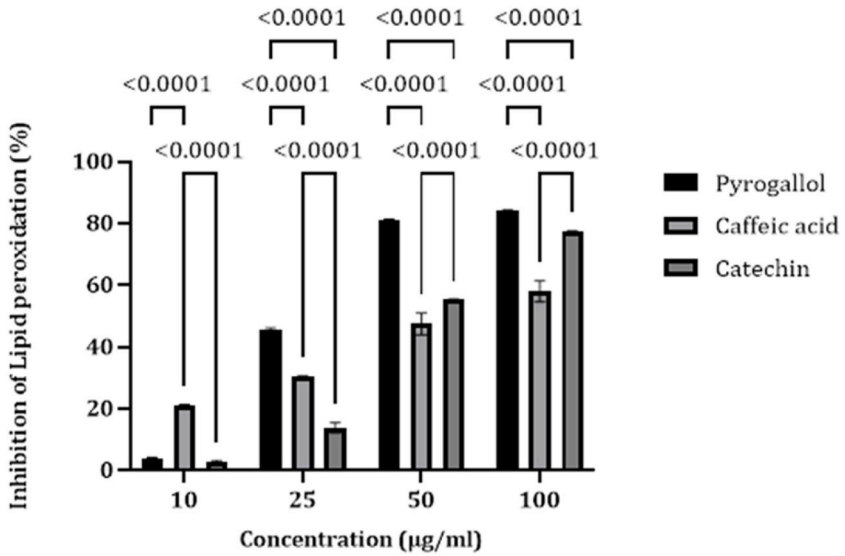

Fig. 2. Protective effects of pyrogallol, caffeic acid, and catechin, as a standard compound, against lipid peroxidation in liver homogenates induced with Fe^{2+}-ascorbate system. The data mentioned are the result of the data obtained from the effect of compounds compared to normal samples and by subtracting from the results of stressed samples. The results of the three independent tests were expressed as mean ± SE. $P < 0.05$ were considered statistically significant between various concentrations and compounds.

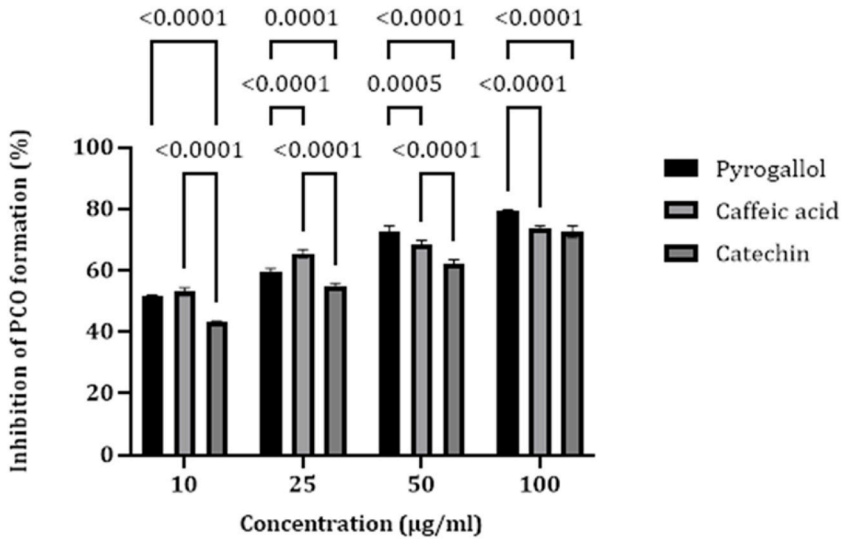

Fig. 3. Protective effects of pyrogallol, caffeic acid, and catechin, as a standard compound, against protein carbonyl oxidation in liver homogenates induced with Fe^{2+}-ascorbate system. The data mentioned are the result of the data obtained from the effect of compounds compared to normal samples and by subtracting from the results of stressed samples. The results of the three independent tests were expressed as mean ± SE. $P < 0.05$ were considered statistically significant between various concentrations and compounds.

maintaining their structural integrity and function [27]. The hydroxyl groups of the compounds probably neutralized the ROS and or protected the thiol groups of cysteine and amino groups of the His and Lys residues from oxidation in proteins [28].

3.4. *Effect of compounds on ROS formation*

According to previous evidence, Fe^{2+}-ascorbate induces oxidative damage in liver homogenates by producing remarkably free radicals [29]. The generation of ROS and the reduction of antioxidant capacity can harm vital cellular components such as proteins and membrane lipids, ultimately creating toxic compounds. In this study, the induction of liver homogenates by the Fe^{2+}-ascorbate system led to a significant increase in the ROS level. The co-incubation of Fe^{2+}-ascorbate and compounds at different concentrations significantly reduced tissue ROS levels by varying degrees ($p < 0.05$). As indicated in Fig. 4, the antioxidant effect increases with the increase in the concentration of the compounds. Catechin (47.3, 55.3, and 58.5 µg/mL) and caffeic acid (23.8, 33.5, and 45.5 %) had

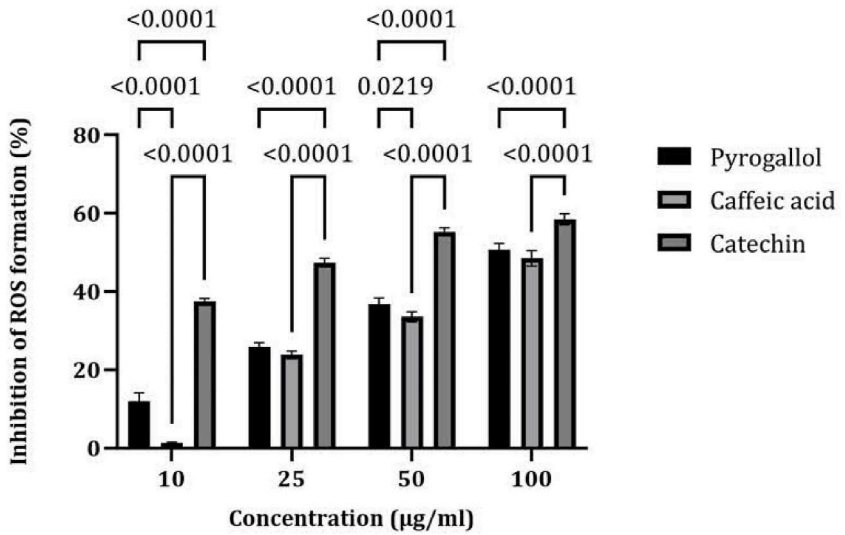

Fig. 4. Protective effects of pyrogallol, caffeic acid, and catechin, as a standard compound, against ROS formation in liver homogenates induced with Fe^{2+}-ascorbate system. The data mentioned are the result of the data obtained from the effect of compounds compared to normal samples and by subtracting from the results of stressed samples. The results of the three independent tests were expressed as mean ± SE. $P < 0.05$ were considered statistically significant between various concentrations and compounds.

the highest and lowest inhibitory on ROS formation at concentrations 25, 50, and 100 μg/mL, respectively. Pyrogallol (25.8, 36.7, and 50.7 %) had a moderate impact on inhibition of ROS formation at all concentrations. Catechin's better inhibition of ROS is likely due to the presence of five hydroxyl groups on its aromatic rings, which contribute to its proton-donating and radical-scavenging properties.

3.5. Effect of the compounds on GSH content

Tripeptide γ-glutamylcysteinylglycine or GSH, the major intracellular nonprotein thiol, is a powerful antioxidant that plays a crucial role in protecting cells from oxidative damage and maintaining cellular redox balance [29]. GSH exists either in reduced (GSH) or oxidized (GSSG) form and participates in redox reactions by the reversible oxidation of its active thiol [29–31]. Under normal cellular redox conditions, the major portion of this regulator is in its reduced form and is distributed in the nucleus, endoplasmic reticulum and mitochondria. Polyphenols can enhance GSH levels by stimulating the activity of enzymes involved in GSH synthesis, such as glutathione peroxidase and glutathione reductase [32]. Polyphenols can directly interact with GSH-related pathways, promoting the recycling of oxidized GSH (GSSG) back to its reduced form (GSH) [33]. Polyphenols increase GSH content and help bolster the body's antioxidant defense system, thereby reducing oxidative stress and supporting overall cellular health. The amount of GSH in liver homogenates induced with the Fe^{2+}-ascorbate system was significantly decreased. As indicated in Fig. 5, simultaneous incubation of the Fe^{2+}-ascorbate system with compounds at all concentrations (10–100 μg/mL) significantly ($P < 0.05$) increased GSH levels by various degrees. Caffeic acid (27.8, 32.3, and 37.2 %) and catechin (24.2, 26.2, and 28.7 %) had the highest and lowest improving effects on GSH content at concentrations 25, 50, and 100 μg/mL, respectively. Pyrogallol (26.2, 29.8 and 34.7 %) had a moderate impact on GSH content at the mentioned concentrations. Regarding the presence of hydroxyl groups in these compounds, it is suggested that these moieties protect the thiol groups from oxidation.

4. Conclusions

This study used liver homogenates as in vitro models to induce oxidative stress. In this study, the effects of pyrogallol and caffeic acid, and catechin as a standard compound against Fe^{2+}-ascorbate-induced oxidative stress in liver homogenates were evaluated by measuring several factors, such as lipid peroxidation, PCO, GSH content, and ROS formation. Catechin, pyrogallol, and caffeic acid are polyphenolic compounds that have been shown to have pharmacological effects on different diseases. Given the numerous studies demonstrating the pharmacological effects of catechin in both in vitro and in vivo conditions and the structural similarities of these compounds with catechin (specifically, the functional groups on the ring), we decided to study the protective and antioxidant effects of these compounds in a conventional model of pathogenesis related to oxidative stress. Results indicated that co-incubation of liver homogenates with the Fe^{2+}-ascorbate system and compounds decreased lipid peroxidation, PCO content, and ROS formation, as well as increased GSH content in a dose-dependent manner. All three compounds have antioxidant and protective effects but to varying degrees. The results also indicated that in most assays, the antioxidant and protective activity of pyrogallol and caffeic acid was higher than that of catechin. In some tests, such as lipid peroxidation and protein oxidation, pyrogallol performed better; in others, such as glutathione content, caffeic acid performed better. Catechin was more effective than those in reducing ROS. These results indicate that there is no strong correlation between the effects of the compounds. Aromatic rings and hydroxyl groups on the ring seem essential for activity. However, the presence of linkers between the rings will reduce activity (Catechin). Regarding the presence of multiple hydroxyl groups and their scavenging activity, it is suggested that these moieties are responsible for the compounds' antioxidant properties. Due to their antioxidative stress properties and previous proven pharmacological effects, these compounds can be suggested for treating most diseases related to oxidative stress.

CRediT authorship contribution statement

Seifollah Bahramikia: Writing – original draft, Validation, Supervision, Project administration. **Nasrin Shirzadi:** Investigation, Formal analysis, Data curation. **Vali Akbari:** Writing – review & editing, Formal analysis, Conceptualization.

Ethical approval

The Animal Ethics Committee of the Faculty of Veterinary Medicine of Lorestan University approved this study (LU. ECRA.2024.76).

Data availability

This study's data are included in the article, and the corresponding author can provide the primary data.

Funding

No funding was received for this work.

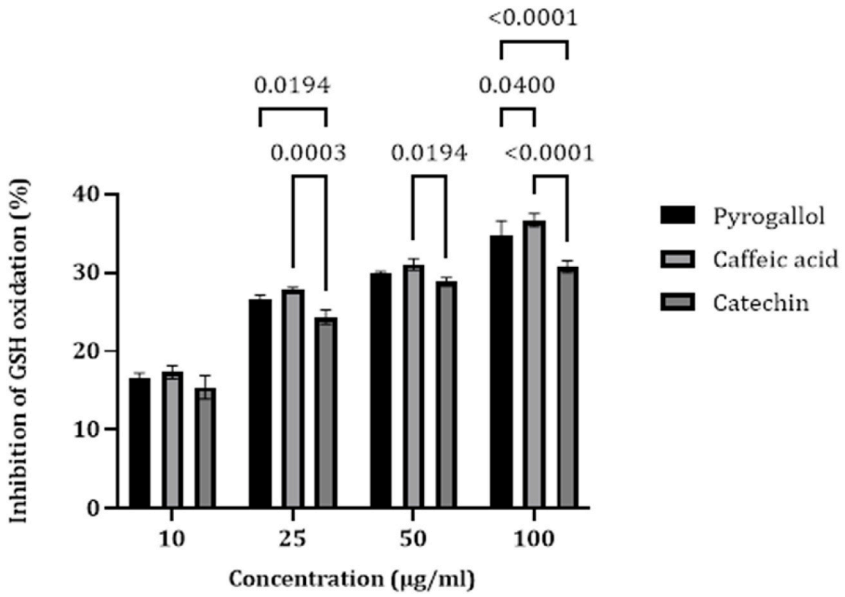

Fig. 5. Protective effects of pyrogallol, caffeic acid, and catechin, as a standard compound, against GSH oxidation in liver homogenates induced with Fe^{2+}-ascorbate system. The data mentioned are the result of the data obtained from the effect of compounds compared to normal samples and by subtracting from the results of stressed samples. The results of the three independent tests were expressed as mean ± SE. $P < 0.05$ were considered statistically significant between various concentrations and compounds.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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