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Therapeutic potential of protocatechuic acid in *in silico* evaluation, antioxidant activity and anti-inflammatory effects for cardiovascular health

Bhavadharseny Uma Shanmugasundaram[1], Subashini Ragunathan[*2]

[1]*Department of Biochemistry and Biotechnology, Annamalai University, Annamalai Nagar, Tamil Nadu, India*
[2]*Department of Biochemistry and Biotechnology (Deputed), Annamalai University, Annamalai Nagar, Tamil Nadu, India*

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ABSTRACT

Protocatechuic acid, a naturally occurring phenolic compound found in a variety of fruits, vegetables, and medicinal plants, has garnered attention for its potential therapeutic properties, particularly in the prevention and management of cardiovascular diseases. This study aimed to evaluate the pharmacokinetic, toxicological, antioxidant, and anti-inflammatory properties of protocatechuic acid. *In silico* tools, including SwissADME and STopTox, were used to assess the compound's ADME (absorption, distribution, metabolism, and excretion) and toxicity profiles. Experimental assays were conducted to measure protocatechuic acid's antioxidant activity, including ferric reducing power, hydrogen peroxide scavenging, metal chelation, and free radical scavenging. Additionally, its anti-inflammatory potential was assessed using a protein denaturation assay. The results revealed that Protocatechuic acid exhibited favourable ADME properties, moderate toxicity, and significant antioxidant and anti-inflammatory activities, with IC_{50} values comparable to standard compounds such as ascorbic acid and aspirin. These findings suggest that protocatechuic acid holds promise as a natural agent for mitigating oxidative stress and inflammation in cardiovascular disorders, with potential for oral therapeutic applications.

***Corresponding author:** Subashini Ragunathan ✉ drsubashini05@gmail.com
* https://orcid.org/0009-0004-5873-6632

First author:
B. U. Shanmugasundaram: https://orcid.org/0009-0007-6762-4941

INTRODUCTION

Phenolic compounds, a diverse group of secondary metabolites found throughout the plant kingdom, have attracted significant attention for their therapeutic potential in the management of chronic diseases (Spagnuolo, 2020). Among these, protocatechuic acid, chemically known as 3,4-dihydroxybenzoic acid, is a simple phenolic acid widely distributed in edible fruits, vegetables, and traditional medicinal herbs (Kakkar and Bais, 2014; Li *et al.*, 2009). As a primary metabolite of complex polyphenols such as anthocyanins and procyanidins, protocatechuic acid is frequently detected in human plasma at concentrations significantly higher than its parent compounds, making it a central figure in the health benefits associated with a polyphenol-rich diet (Cañas *et al.*, 2022; Hidalgo *et al.*, 2012).

The pharmacological importance of protocatechuic acid is particularly pronounced in cardiovascular diseases, where oxidative stress and chronic inflammation are fundamental drivers of vascular dysfunction and atherosclerosis (Kakkar and Bais, 2014; Li *et al.*, 2025). Oxidative stress, characterized by an excess of reactive oxygen species, reduces nitric oxide (NO) bioavailability, a hallmark of endothelial dysfunction (Chook *et al.*, 2023; Festa *et al.*, 2024). Protocatechuic acid has been shown to protect vascular endothelial function by improving NO bioavailability through the Akt/eNOS signalling pathway and by directly scavenging superoxide radicals (Chook *et al.*, 2023; Festa *et al.*, 2024; Graton *et al.*, 2022).

Furthermore, protocatechuic acid exerts potent anti-atherosclerotic effects by inhibiting the NF-κB pathway, which, in turn, downregulates the expression of pro-inflammatory cytokines and adhesion molecules such as VCAM-1 and ICAM-1 (Bhattacharjee *et al.*, 2017; Hidalgo *et al.*, 2012; Speciale *et al.*, 2014). These molecular actions contribute to improved vasodilation and the stabilisation of atherosclerotic plaques, highlighting protocatechuic acid as a promising natural intervention for hypertension and coronary heart disease (Chook *et al.*, 2023; Graton *et al.*, 2022; Speciale *et al.*, 2014).

Despite its robust biological activity, the clinical development of protocatechuic acid requires a detailed understanding of its pharmacokinetics and safety profile. In modern drug discovery, *in silico* tools such as SwissADME and STopTox are essential for predicting a compound's absorption, distribution, metabolism, excretion, and potential toxicity at an early stage (Borba *et al.*, 2022; Oliveira *et al.*, 2022). These models provide critical data on membrane permeability, gastrointestinal absorption, and systemic safety, which are vital for determining the viability of protocatechuic acid as an oral therapeutic or nutraceutical agent (Borba *et al.*, 2022; Cañas *et al.*, 2022).

The present study aims to evaluate the therapeutic potential of protocatechuic acid through computational and experimental approaches. Specifically, we assess its ADME and toxicity profiles using SwissADME and STopTox models to evaluate its oral safety. Additionally, we investigate its antioxidant and anti-inflammatory capacity through various *in vitro* assays.

MATERIALS AND METHODS

In silico ADME and toxicity prediction

The physicochemical properties, pharmacokinetic profile, and drug-likeness of Protocatechuic acid (Protocatechuic acid) were assessed using the SwissADME web server (http://www.swissadme.ch). The canonical SMILES string for protocatechuic acid was submitted to the server to generate key parameters, including lipophilicity (iLOGP), water solubility, molecular weight, and topological polar surface area. The BOILED-Egg model was used to predict gastrointestinal absorption and blood-brain barrier penetration.

Furthermore, the acute toxicity profile, covering oral, dermal, and inhalation toxicity, as well as skin and eye irritation/corrosion, was evaluated using the STopTox web portal (https://stoptox.mml.unc.edu/).

Antioxidant activity assays

All *in vitro* antioxidant assays for protocatechuic acid and the reference standard, ascorbic acid, were performed at concentrations ranging from 25 to 400 µM in triplicate.

Ferric reducing power assay

The reducing power of protocatechuic acid was determined using the potassium ferricyanide method. Protocatechuic acid (2.5 mL) at varying concentrations was mixed with phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide. After incubation at 50°C for 20 minutes, 10% trichloroacetic acid was added, and the mixture was centrifuged. The supernatant was then mixed with distilled water and 0.1% ferric chloride, and the absorbance was measured at 700 nm.

Hydrogen peroxide (H_2O_2) scavenging assay

A 40 mM solution of H_2O_2 was prepared in phosphate buffer (50 mM, pH 7.4). Protocatechuic acid at various concentrations was added to the H_2O_2 solution, and the absorbance was measured at 230 nm after 10 minutes of incubation, with a blank solution as a reference.

Ferrous ion chelating activity

The metal-chelating activity of protocatechuic acid was assessed using the ferrozine method. Protocatechuic acid was incubated with a 2 mM ferrous chloride solution, and the reaction was initiated by adding 5 mM ferrozine. The mixture was then incubated in the dark at room temperature for 5 to 10 minutes. The absorbance of the Fe^{2+}-ferrozine complex was measured at 562 nm.

Nitric oxide radical scavenging assay

Nitric oxide radicals were generated from sodium nitroprusside in phosphate-buffered saline at physiological pH. Protocatechuic acid was incubated with 50 mM SNP for 60 minutes at 37°C. The nitrite produced was measured by adding Griess reagent (1% sulfanilamide and 0.1% NED in 5% H_3PO_4) and measuring the absorbance at 540 nm.

Superoxide anion radical scavenging assay

Superoxide radicals were generated in a Tris-HCl buffer (16 mM, pH 8.0) using the PMS-NADH-NBT system. The reaction mixture contained NBT (0.3 mM), NADH (0.936 mM), and Protocatechuic acid at various concentrations. The reaction was initiated by adding phenazine methosulfate (PMS, 0.12 mM), and the absorbance was measured at 560 nm after incubation.

Anti-inflammatory activity: Protein denaturation assay

The anti-inflammatory potential of protocatechuic acid and the reference drug, aspirin (25–400 µM), was evaluated by assessing their ability to inhibit the denaturation of bovine serum albumin (BSA). The reaction mixture, containing 1% aqueous BSA and protocatechuic acid, was adjusted to pH 6.3. The samples were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes to induce denaturation. After cooling, the turbidity was measured spectrophotometrically at 660 nm.

Statistical analysis

All experiments were conducted in triplicate, and the results are expressed as mean ± standard deviation (SD). The percentage of inhibition or scavenging activity was calculated for each assay. The inhibitory concentration required to achieve 50% activity (IC_{50}) was determined by linear regression.

RESULTS

ADME profile

Fig. 1 presents the physicochemical properties and pharmacokinetic profile of protocatechuic acid assessed by SwissADME. Fig. 1A shows a radar plot of key molecular descriptors, including flexibility (FLEX), size (SIZE), lipophilicity (LIPO), polarity (POLAR), solubility (INSOLU), and insaturation (INSATU). The redder region in the plot highlights protocatechuic acid's moderate flexibility and lipophilicity, indicating its potential interaction with biological membranes. These properties are consistent with a moderate ability to pass through cellular barriers. Fig. 1B illustrates the BOILED-Egg model, which predicts the compound's gastrointestinal absorption (HIA) and blood-brain barrier (BBB) penetration. The yellow region

indicates the compound's predicted ability to be absorbed in the gastrointestinal tract, while the white region suggests that protocatechuic acid is unlikely to cross the BBB, as indicated by its position outside the BBB permeant zone. Additionally, protocatechuic acid is located within the non-P-glycoprotein (PGP-) substrate area, confirming that it does not interact with P-glycoprotein efflux mechanisms. In terms of its physicochemical properties, protocatechuic acid has a molecular weight of 154.12 g/mol and a topological polar surface area (TPSA) of 77.76 Å², suggesting moderate water solubility.

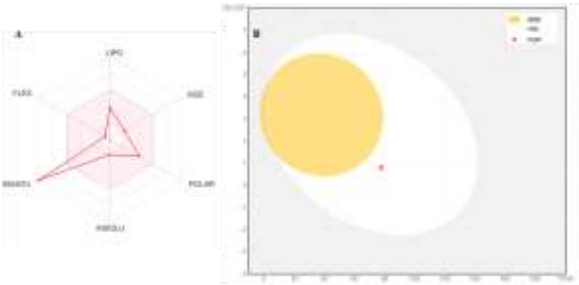

Fig. 1. SwissADME analysis of protocatechuic acid. A) Radar plot and B) BOILED-Egg model

The compound exhibits lipophilicity (iLOGP = 0.66), indicating low to moderate membrane permeability. Its bioavailability score is 0.56, suggesting moderate bioavailability potential in rats. Protocatechuic acid adheres to the Lipinski and Veber filters, suggesting its viability as an oral drug candidate, though it violates the Ghose filter due to its molecular weight.

Furthermore, it does not act as a P-glycoprotein substrate and is predicted to have limited central nervous system access due to its inability to permeate the BBB. These characteristics highlight protocatechuic acid as a promising compound for oral administration, with moderate pharmacokinetic properties.

STopTox model

According to the STopTox model (Fig. 2), protocatechuic acid is predicted to be non-toxic for acute inhalation exposure, with a 78% confidence in the non-toxic prediction. It is also classified as non-toxic for acute oral and dermal exposures, both with a 52% confidence.

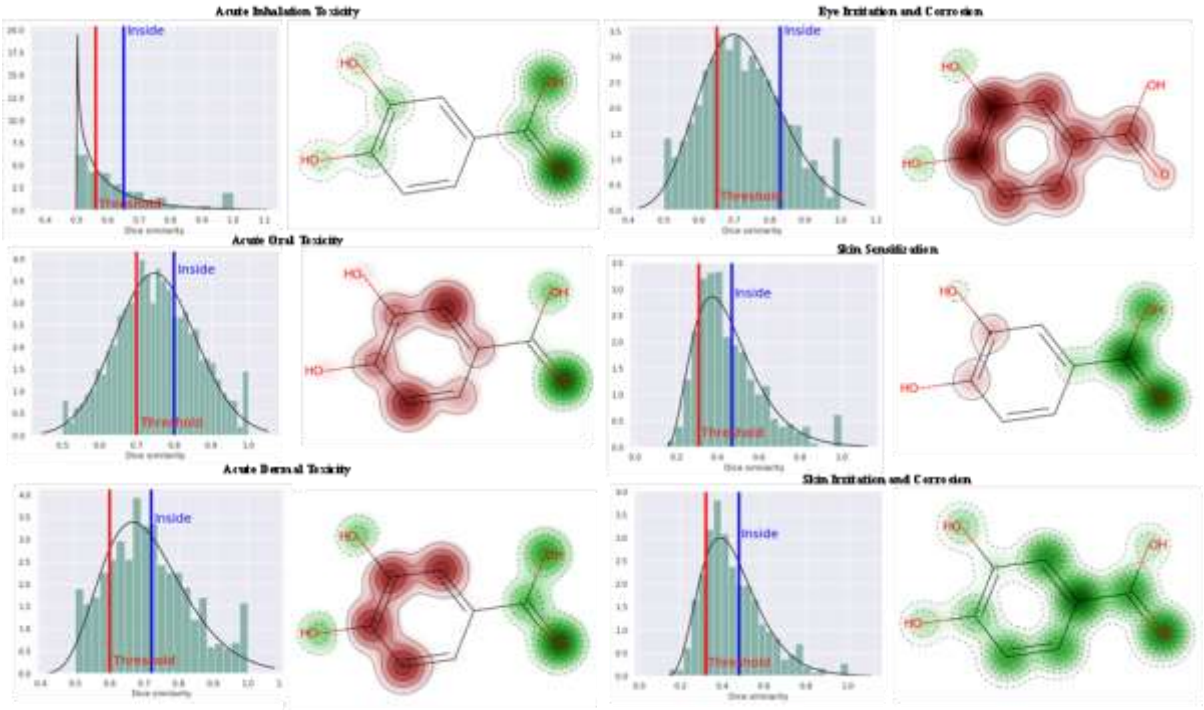

Fig. 2. STopTox model of protocatechuic acid

However, the compound is predicted to cause eye irritation and corrosion, with an 87% confidence, suggesting a moderate risk in that regard. Protocatechuic acid is not expected to be a skin sensitiser, as indicated by an 80% confidence in the non-sensitiser outcome.

Furthermore, the compound is predicted to be negative for skin irritation and corrosion, with a high confidence of 90%. These results suggest that protocatechuic acid poses minimal acute toxicity risks via inhalation, oral, or dermal routes, but caution is advised regarding potential eye irritation.

Antioxidant activity

Ferric reducing power assay

The ferric reducing power assay was performed to evaluate the antioxidant potential of protocatechuic acid. The results demonstrated a clear concentration-dependent increase in reducing activity across the different concentrations (25–400 µM) (Fig. 3). At 25 µM, protocatechuic acid exhibited a reducing power value of 15.62 ± 1.56, which gradually increased to 28.41 ± 3.96 at 50 µM and 41.21 ± 6.18 at 100 µM. A further rise in antioxidant activity was observed at higher concentrations, reaching 59.45 ± 4.05 at 200 µM and 71.09 ± 8.19 at 400 µM. The calculated IC_{50} value for protocatechuic acid was 118.52 µM, and the reference standard ascorbic acid showed an IC_{50} of 87.58 µM, confirming the considerable reducing ability of protocatechuic acid in the ferric reducing power assay.

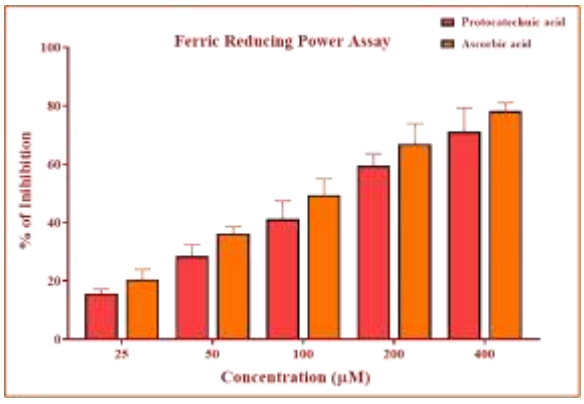

Fig. 3. Antioxidant activity of protocatechuic acid assessed by using ferric reducing power assay
Data represented as mean ± standard deviation (SD) of triplicate experiments

Hydrogen peroxide scavenging activity

The hydrogen peroxide-scavenging potential of protocatechuic acid was assessed to evaluate its capacity to neutralize reactive oxygen species (Fig. 4).

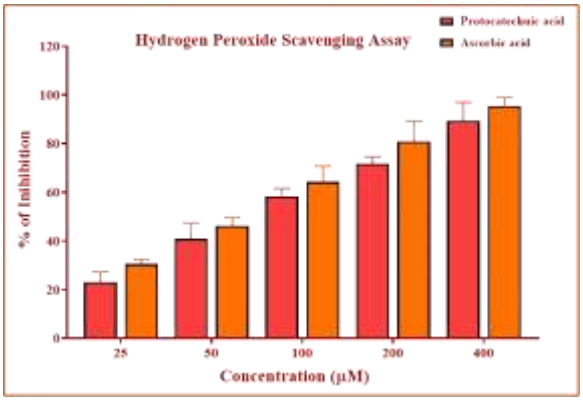

Fig. 4. Antioxidant activity of protocatechuic acid assessed by using hydrogen peroxide-scavenging assay
Data represented as mean ± standard deviation (SD) of triplicate experiments.

The results revealed a concentration-dependent increase in scavenging activity within the 25–400 µM range. At 25 µM, protocatechuic acid demonstrated a scavenging activity of 23.09 ± 4.16%, which rose to 40.95 ± 6.48% at 50 µM and 58.37 ± 3.19% at 100 µM. A further increase in activity was observed with higher concentrations, reaching 71.66 ± 2.96% at 200 µM and 89.43 ± 7.53% at 400 µM. The IC_{50} value for protocatechuic acid was calculated to be 85.47 µM, whereas the reference standard, ascorbic acid, had an IC_{50} of 70.20 µM, highlighting the significant hydrogen peroxide scavenging ability of protocatechuic acid.

Ferrous ion chelating activity

The ability of Protocatechuic acid to chelate ferrous ions was investigated to assess its potential to bind Fe^{2+} ions and prevent metal-induced oxidative reactions (Fig. 5). The results revealed a concentration-dependent increase in chelating activity across the tested range of 25–400 µM. At 25 µM, protocatechuic acid demonstrated a chelating activity of 9.45 ± 0.89%, which increased to 22.64 ± 2.63% at 50 µM and 37.96 ± 1.85% at 100 µM. Higher concentrations led to a further increase in activity, reaching 53.37 ± 5.49% at 200 µM and 67.45 ± 4.69% at 400 µM. The IC_{50} value for protocatechuic acid was calculated to be 163.02 µM, and the reference standard ascorbic acid exhibited an IC_{50} value of 124.33 µM, indicating the significant ferrous ion chelation potential of protocatechuic acid.

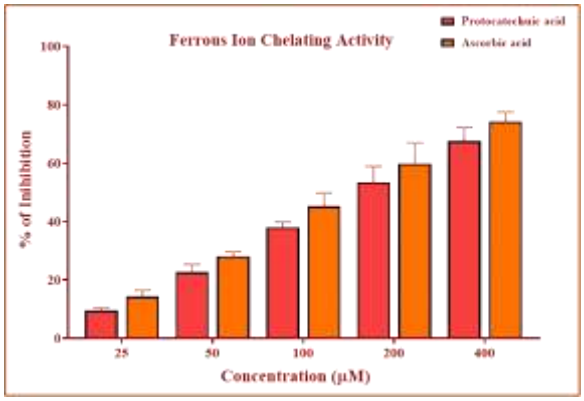

Fig. 5. Antioxidant activity of protocatechuic acid assessed by using ferrous ion chelating assay
Data represented as mean ± standard deviation (SD) of triplicate experiments.

Nitric oxide radical scavenging activity

The nitric oxide radical scavenging potential of protocatechuic acid was examined to assess its ability to counteract nitric oxide-induced oxidative stress (Fig. 6).

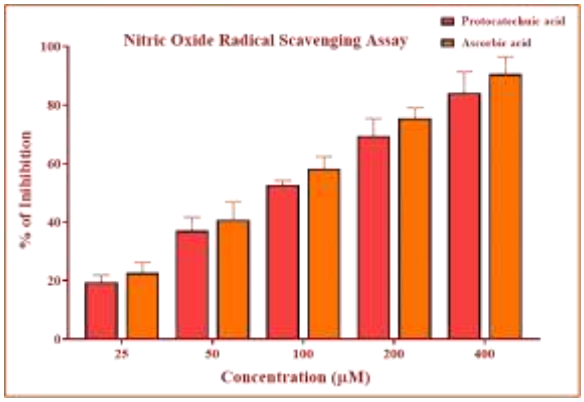

Fig. 6. Antioxidant activity of protocatechuic acid assessed by using the nitric oxide radical scavenging assay
Data represented as mean ± standard deviation (SD) of triplicate experiments.

The results revealed a concentration-dependent increase in scavenging activity across the 25–400 µM range. At 25 µM, protocatechuic acid exhibited a scavenging activity of 19.45 ± 2.36%, which increased to 37.09 ± 4.57% at 50 µM and 52.67 ± 1.48% at 100 µM. A further increase in activity was observed at higher concentrations, reaching 69.37 ± 5.96% at 200 µM and 84.12 ± 7.19% at 400 µM. The IC_{50} value for protocatechuic acid was found to be 97.91 µM and the reference standard, ascorbic acid, demonstrated an IC_{50} value of 90.07 µM, highlighting the prominent nitric oxide scavenging capacity of protocatechuic acid.

Superoxide anion radical scavenging activity

The ability of protocatechuic acid to scavenge superoxide anion radicals was assessed to determine its effectiveness in neutralising superoxide radicals (Fig. 7). The results revealed a concentration-dependent increase in scavenging activity across the 25–400 µM range. At 25 µM, protocatechuic acid demonstrated a scavenging activity of 17.08 ± 3.14%, which increased to 29.16 ± 5.26% at 50 µM and 48.43 ± 1.85% at 100 µM. A further enhancement in activity was observed at higher concentrations, reaching 61.49 ± 3.48% at 200 µM and 78.82 ± 2.49% at 400 µM. The IC_{50} value for protocatechuic acid was calculated to be 120.15 µM, while the reference standard, ascorbic acid, had an IC_{50} value of 97.94 µM, confirming the superoxide radical scavenging potential of protocatechuic acid.

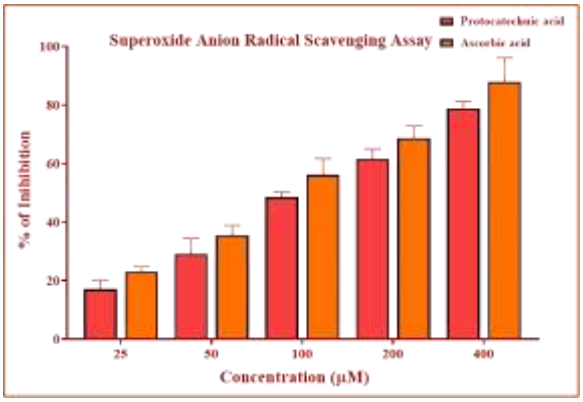

Fig. 7. Antioxidant activity of protocatechuic acid assessed by using the superoxide anion radical scavenging assay
Data represented as mean ± standard deviation (SD) of triplicate experiments.

Anti-inflammation activity: Protein denaturation assay

The protein denaturation assay was conducted to assess the anti-inflammatory potential of protocatechuic acid by examining its ability to prevent heat-induced protein denaturation (Fig. 8). The results revealed a concentration-dependent

increase in the inhibition of protein denaturation across the range of 25–400 µM. At 25 µM, protocatechuic acid showed an inhibition of 13.56 ± 3.87%, which increased to 27.52 ± 1.49% at 50 µM and 42.81 ± 4.68% at 100 µM. At higher concentrations, the inhibition further improved, reaching 65.74 ± 7.43% at 200 µM and 81.43 ± 4.18% at 400 µM. The calculated IC_{50} value for protocatechuic acid was 166.66 µM, whereas the reference drug, aspirin, exhibited an IC_{50} value of 128.04 µM, indicating the potential of protocatechuic acid to inhibit protein denaturation.

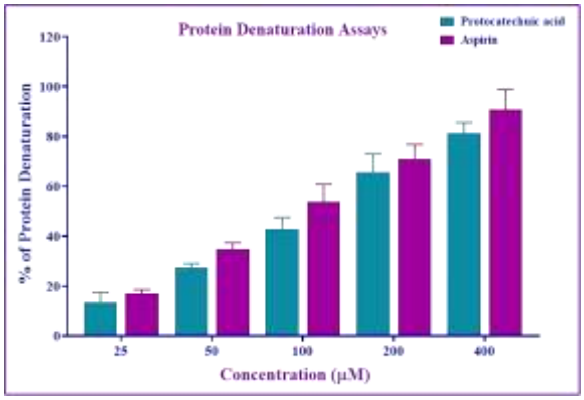

Fig. 8. Anti-inflammatory activity of protocatechuic acid assessed by using the protein denaturation assay Data represented as mean ± SD of triplicate experiments.

DISCUSSION

The physicochemical properties of protocatechuic acid, such as a molecular weight of 154.12 g/mol and a TPSA of 77.76 Å², align with literature values of 77.76 Å² for TPSA and 0.65 for logP (Conserva *et al.*, 2021). A TPSA below 90 Å² typically predicts favourable membrane permeability and drug transport properties (Cotes *et al.*, 2018). The SwissADME prediction of high gastrointestinal absorption is supported by previous research showing that protocatechuic acid is rapidly absorbed in the small intestine and exhibits superior bioavailability compared to other phenolic compounds (Cañas *et al.*, 2022).

However, the prediction that protocatechuic acid is unlikely to cross the BBB contrasts with earlier *in silico* studies and *in vivo* findings, which classify protocatechuic acid as "BBB+" with a high potential to cross the barrier (Senthilvel *et al.*, 2013; Sánchez-Martínez *et al.*, 2022). Moreover, in vivo research has shown that chronic protocatechuic acid administration increases its levels in mouse brain tissue and is recognised as a metabolite that can cross the BBB to exert neuroprotective effects (Gallardo-Fernández *et al.*, 2019; Spagnuolo, 2020).

These discrepancies may arise from differences in computational models, but the experimental literature suggests greater CNS access than predicted by the SwissADME tool.

Regarding toxicity, the STopTox results indicate that protocatechuic acid is safe for systemic administration, consistent with its protective effects against various chemical toxicants, mediated by suppression of oxidative stress and inflammation (Kelidari *et al.*, 2024).

Additionally, protocatechuic acid is categorised as a non-sensitiser and non-irritant for the skin (Borba *et al.*, 2022), but the predicted risk of eye irritation (87% confidence) suggests that ocular exposure should be avoided.

Experimental results indicate that protocatechuic acid exhibits potent, dose-dependent antioxidant and anti-inflammatory activities, with IC_{50} values comparable to those of standard agents such as ascorbic acid and aspirin. In antioxidant assays, protocatechuic acid demonstrated significant radical-scavenging and metal-chelation activities, with IC_{50} values of 85.47 µM for hydrogen peroxide and 97.91 µM for nitric oxide. As a powerful antioxidant, Protocatechuic acid scavenges free radicals and chelates transition metal ions, with its catechol structure playing a crucial role in these functions (Jakovljević *et al.*, 2019; Li *et al.*, 2009). Protocatechuic acid has been shown to be a more effective reductant than standard antioxidants like Trolox, and its ferrous ion chelating activity (IC_{50}: 163.02 µM) is 2.7 times higher than Trolox (Li *et al.*, 2011).

In terms of anti-inflammatory activity, protocatechuic acid demonstrated an IC_{50} of 166.66 μM in the protein denaturation assay, indicating its ability to prevent protein structural loss during inflammation. At the cellular level, Protocatechuic acid's anti-inflammatory effects are mediated by suppressing the NF-κB pathway, thereby reducing pro-inflammatory cytokines and enzymes such as cyclooxygenase-II and inducible nitric oxide synthase (Albrakati, 2021). *In vivo* studies have confirmed protocatechuic acid's anti-inflammatory potential, with doses of 25–50 mg/kg significantly reducing carrageenan-induced paw edema in mice, results comparable to those of the reference drug diclofenac (Albrakati, 2021).

Additionally, protocatechuic acid mitigates oxidative stress in the vasculature, with its ability to scavenge reactive oxygen species playing a crucial role in cardiovascular protection. By neutralising nitric oxide radicals and preventing their reaction with superoxide, protocatechuic acid helps maintain endothelial function and nitric oxide bioavailability (Albrakati, 2021; Chook *et al.*, 2023). Moreover, protocatechuic acid's metal-chelating activity further reduces the risk of lipid peroxidation, a key factor in arterial plaque formation (Kelidari *et al.*, 2024).

The suppression of vascular inflammation via protocatechuic acid's inhibition of the NF-κB pathway also correlates with its effects in preventing atherosclerosis and managing diabetic cardiomyopathy and hyperlipidemia (Bhattacharjee *et al.*, 2017; Li *et al.*, 2025). Research has demonstrated that protocatechuic acid can suppress diabetic cardiomyopathy by stimulating glucose metabolism and reducing myocardial inflammation (Bhattacharjee *et al.*, 2017).

Furthermore, protocatechuic acid has shown antihyperlipidemic effects, helping to manage lipid profiles in diabetic models, which is a major risk factor for coronary heart disease (Harini and Pugalendi, 2010).

CONCLUSION

This comprehensive study highlights the multifaceted therapeutic potential of protocatechuic acid, with promising results from both computational predictions and experimental assays. *In silico* analyses suggest that protocatechuic acid has moderate pharmacokinetic properties and low toxicity, supporting its viability as an oral therapeutic agent. The experimental data demonstrated significant antioxidant activities, particularly in scavenging reactive oxygen species and metal chelation, as well as anti-inflammatory effects through inhibition of protein denaturation. These findings align with protocatechuic acid's role in mitigating oxidative stress and inflammation, key contributors to cardiovascular diseases. Given its robust pharmacological profile, protocatechuic acid presents a strong candidate for further clinical investigation and potential application in the prevention and management of oxidative stress-related cardiovascular conditions.

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