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Cardioprotective Effect of *C. cassia* Bark in the Doxorubicin-Induced Cardiotoxicity Rats

Nithyatharani Ramalingam[1], Manikandan Ramasamy[2*]Srinath Arjunan[3], Prasun Kumar[4], Ushasri Raghavendran[5] and Thenmozhi Sambandam[6]

[1]Department of Microbiology, Shrimati Indira Gandhi College, Tiruchirappalli, Tamilnadu, India
[2]Department of Biochemistry, Shrimati Indira Gandhi College, Tiruchirappalli, Tamilnadu, India*
[3]Department of Biomedical Engineering, Sri ManakulaVinayagar Engineering College, Puducherry, India
[4]Department of Biotechnology, School of Bioscience and Technology, Sharda University, Greater Noida, India
[5]Department of Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai, Tamilnadu, India.
[6]Department of Microbiology, Valliammal College for Women, Chennai, Tamilnadu, India.

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ABSTRACTThis study investigates the therapeutic benefits of *Cinnamon cassia* bark on rats with doxorubicin-induced cardiotoxicity. The ethanolic extract underwent Gas Chromatography-Mass Spectrometry analysis to identify phytocompounds. Five groups of rats were formed, and their cardiac were damaged by doxorubicin, and treated with a daily oral dosage of *C. cassia* extract. Post-treatment, tissue and serum samples were analysed. Comparing the extract to the negative control group propranolol serving as the standard showed that the extract significantly altered biochemical parameters. In silico analysis indicated hexadecanoic acid's protective role against cardiac damage. The extracts exhibited protective effects on cardiac markers and lysosomal enzymes, suggesting the potential use of *C. cassia* bark in combination therapy for cardiac issues.

INTRODUCTION

Myocardial disease, commonly referred to as heart attack, is the most significant type of ischemic heart disease, accounting for 35 percent of all deaths and 80 percent of all cardiac deaths. Ischemic heart disease is a cardiac impairment that can be both acute and chronic. It is caused by a decrease in or stoppage of the blood supply to the myocardium in conjunction with coronary artery disease processes. Only coronary atherosclerosis's late manifestation is ischemic heart disease (Schoen and Cotran1999; Stierman et al. 2021; Martin et al. 2025). The primary contributors to atherosclerosis are elevated cholesterol levels in the blood plasma, specifically in the form of low-density lipoprotein (LDL), as well as familial hypercholesterolemia. Male sex hormones, smoking, diabetes mellitus, hypertension, and an increase in low density lipoprotein in the plasma are additional factors that contribute to atherosclerosis. Due to decreased cellular metabolism, the tissue edematises, the blood arteries become extremely porous, and the heart muscle cells start to expand. After a few hours with little to no blood flow, the cell perishes. India is thought to be the cause of one-fifth of all fatalities worldwide, according to WHO estimates, especially among younger people (Vale et al. 2014).The Global Burden of Disease report indicates that India's age-standardised death rate from cardiovascular diseases (CVD) is 272 per 1,00,000 individuals, which considerably exceeds the global average of 235. According to Prabhakaran et al. (2016), Indians experience the impact of CVDs a decade earlier than Western individuals. Indians are very worried about the high death rate, rapid progression, and early onset of cardiovascular disease. CVD prevalence varies significantly across India, with Tamil Nadu, Kerala, and Punjab exhibit-

*Address for correspondence:
Manikandan Ramasamy

ing the highest rates (Kumar and Sinha 2020). Aspirin, nitrates, glycoprotein Iib/IIIa receptor treatment, anti-thrombin drugs, and calcium agonists are used to treat patients with non-ST-segment elevation myocardial infarction.Patients with elevated ST segment levels are often treated with aspirin, β-blockers, antithrombin, glycoprotein IIb/IIIa receptor inhibitors, nitrates, and angiotensin converting enzyme inhibitors. Despite their effectiveness, these drugs have negative side effects. Because of this, researchers are looking for safe, plant-based drugs. Numerous compounds with a range of biological activity are known as phytoconstituents, and they are found in plants.

Among the Lauraceae family's significant plants is *Cinnamon Cassia (C. cassia).* One of the most significant plants that people use is cinnamon. It has extensive application in the fragrance and essence sectors and finds its way into a variety of food items and pharmaceuticals (Hang et al. 2014). Previous reports proved that the *C. cassia* bark contains several biologically active phytochemicals (Vangalapati et al., 2012). The present study was undertaken to evaluate the cardioprotective potential of the extract derived from *C. cassia* bark.

MATERIAL AND METHODS

Collection of Plants and Extract Preparation

Fresh barks of *C. Cassia* were gathered, and authentication was secured at the Rapinet Herbarium St. Joseph's College in Tiruchirappalli, Tamil Nadu. The dried barks of *C. cassia* were mechanically ground into powder and kept in a sealed container. A Soxhlet apparatus and a hot percolation method were used for the extraction. The solvent in this case is ethanol. 600 millilitres of ethanol were used to extract a powder that weighed about 100 grams. At a regulated temperature of 40–50°C, the extract was evaporated until it was completely dry. Until it was utilized again, the extract was stored in the refrigerator.

GC-MS Analysis

For 24 hours, a sample of 30 g of powdered *C. cassia* bark is steeped and dissolved in 75 ml of ethanol. Next, the filtrates are gathered by letting them evaporate in liquid nitrogen. A Elite-1, 300 m x 0.25 mm x 1 ìm df capillary column is used in the GC-MS analysis, which is performed using a Clarus 500 Perkin-Elmer Gas Chromatograph that is equipped and linked to a mass detector Turbo mass Gold-Perking Elmer Turbomas 5.2 spectrometer.

Ethical Issue, Animal Use, Housing and Experimental Design

150-180 g male albino rats that are 6-8 weeks old are employed. The animals are provided with water and a normal pellet meal, and they are housed in dry, clean plastic cages. This study has been approved by the Institutional Ethical Committee and is being conducted in the animal house of SrimadAndavan Arts and Science College, Trichy.

Rats were randomly divided into 5 groups of 6 rats each.

- G1: Normal rats (normal saline water)
- G2: Negative control (Doxorubicin treated (1.5ml/kg b.w)
- G3: Normal rats treated with ethanolic extract of *C. cassia* bark (500 mg/kg b.w)
- G4: Negative control rats treated with ethanolic extract of *C. cassia* bark (500 mg/kg b.w)
- G5: Negative control rats treated with standard drug Propranolol (25 mg/kg b.w)

After the study period, the samples were collected for further analysis. The samples were subjected to the analysis of various parameters such as lipid profiles, cardiac marker enzymes (Apple et al. 1988;Okinaka et al. 1961; Burtis and Ashwood 1996), lysosomal enzymes (King 1965; Kawai and Anno 1971; Moore and Morris 1982; Sapolsky et al. 1973) and mitochondrial enzymes (Bell and Baron 1960;Slater and Bonner 1952;Mehler et al. 1948;Tsoo et al. 1967; Pearl et al. 1963).

In silico Analysis

The molecular docking of the ligand and proteins was studied using Auto Dock software. Similarly, the ligand molecule hexadecanoic acid in .sdf format and protein structures were downloaded from the Protein Data Bank. Heteroatoms and water molecules were eliminated from the protein structure before docking. The protein structure was then supplemented with partial atomic Kollman charges and polar hydrogen atoms.The grid boxes were created for PDB protein structures using AutoDock software. The Molecular Docking analy-

sis was done using the Lamarckian genetic algorithm. The molecular interaction of protein-ligand complexes were visualised using Discovery Studio Visualizer 20.1 software.

Statistical Analysis

The data show the number of experiments and their mean ± standard deviation (S.D.). Given that the current study has examined more than two treatment groups. In statistical analysis with the statistical package of social science (SPSS) version 14.0 for Windows, one-way analysis of variance is employed. A significance level is indicated by P values < 0.05.

RESULT AND DISCUSSION

Bioactive Compounds from *C. cassia* Bark Extract

The investigation of phytocompounds found in various plant extracts has made extensive use of GC-MS studies. Figure 1 and Table 1 display the findings of the GC-MS analysis of *C. cassia* barks. The results shows nearly 20 phytoconstituents that have been found in the *C. cassia* bark extract of ethanol.

The compounds, including heptadecanoic acid and ethyl ester, exhibit pharmacological activities such as antioxidant effects. Hexadecenoic acid has been reported to possess antioxidant activity and cholesterol-lowering effects, while tetra methyl-2-hexadecen-1-ol is known for its antimicrobial and anti-inflammatory properties. In addition, 9,12,15-octadecatrienoic acid has been associated with hypocholesterolemic, hepatoprotective, and cancer-preventive activities (Dr. Duke's Phytochemical and Ethnobotanical Database).This study's results demonstrate that the pharmacological activity of *C. cassia* is attributed to the presence of phytochemicals and supports the synergetic effect.

The diversity of molecular formulas and molecular weights observed in the chromatographic table demonstrates the chemical heterogeneity of the bark extract. Molecular weights range from relatively small molecules near 100 Da to larger compounds exceeding 400 Da, reflecting the presence of both simple metabolites and complex lipid derivatives. Such heterogeneity is characteristic of medicinal plants that synthesize a wide array of secondary metabolites as adaptive responses to en-

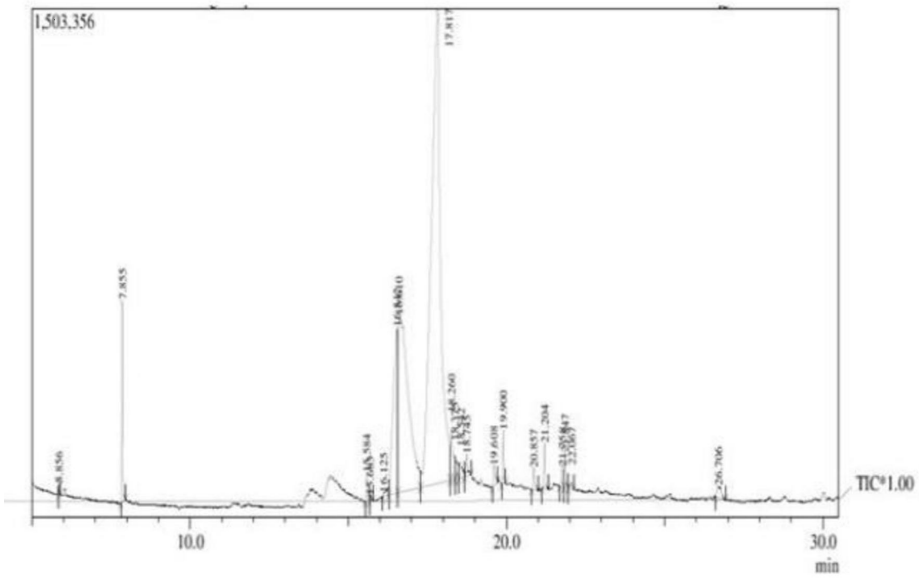

Fig. 1. GC-MS analysis report of ethanolic extract of *C. cassia* Bark

Table 1:Bioactive compounds present in *C. cassia* bark identified by GC-MS

Peak	*Retention Time*	*Molecular formula*	*Name of the compound*
1	5.856	C9H20O2	Butane,1,1-Diethoxy-2-Methyl-
2	7.855	C9H20O3	Propane,1,1,3-Triethoxy-
3	15.584	C20H26O4	1,2-Benzoldicarbonsaeure,Di
4	15.683	C6H14O2	1,2-StageIdiol
5	16.125	C6H12O5	L-Galactose,6-Deoxy-
6	16.542	C7H14O6	Alpha.-D-Mannofuranoside,Meth
7	16.610	C6H12O5	Inositol,1-Deoxy-
8	17.817	C7H14O6	MomeInositol
9	18.375	C20H40O	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol
10	18.542	C6H11DO6	D1-1,3,5/2,4,6-CyclostageIhexol
11	18.745	C9H18O	5-Methyl-5-Octen-1-Ol
12	19.608	C12H24O	Oxirane,Tetradecyl-
13	19.608	C28H44O4	9-Octadecenoic Acid, (2-Phenyl 1,3-Dioxolan-4-Yl)MethylEster, Cis
14	19.900	C19H38O2	HeptadecanoicAcid,EthylEster
15	20.857	C16H34O	1-Hexadecanol
16	21.204	C20H40O	2-Hexadecen-1-Ol
17	21.758	C18H30O2	9,12-OctadecadienoicAcid(Z,Z)-
18	21.847	C18H30O2	9,12,15-OctadecatrienoicAcid
19	22.067	C18H36O2	HexadecanoicAcid,EthylEster
20	26.706	C11H20O	CyclopropanemStageIII,2-Methyl

vironmental stress, pathogens, and herbivores. These secondary metabolites frequently possess therapeutic properties beneficial to humans, including antioxidant, anticancer, hepatoprotective, antimicrobial, and anti-inflammatory activities. Consequently, the GC–MS profile not only provides chemical identification but also offers insight into potential pharmacodynamic mechanisms.

Retention time data also provide valuable analytical information because each compound elutes at a characteristic time depending on its volatility and interaction with the chromatographic column. Consistent retention times across replicate analyses confirm method reproducibility and analytical reliability. When combined with spectral library matching, retention time comparison enhances confidence in compound identification. Modern GC-MS systems integrate automated matching algorithms that compare fragmentation patterns with thousands of reference spectra, thereby reducing manual interpretation errors and improving analytical accuracy (Kim et al. 2023).

Overall, the phytochemical spectrum obtained from GC-MS analysis strongly supports the conclusion that the biological activities attributed to *C. cassia* bark arise from a complex interplay of multiple bioactive constituents rather than a single dominant compound. The coexistence of sugars, fatty acids, esters, alcohols, and cyclic derivatives suggests a multifaceted pharmacological profile involving antioxidant defense, lipid regulation, antimicrobial action, and anti-inflammatory mechanisms. Such compositional diversity provides scientific validation for traditional medicinal uses of the plant and highlights its potential as a source of natural therapeutic agents. The findings therefore reinforce the concept that comprehensive chromatographic profiling is essential for understanding plant-based medicines, as it reveals both major and minor metabolites that collectively determine biological efficacy. In conclusion, GC-MS characterization demonstrates that the pharmacological potential of *C. cassia* bark is closely linked to its chemically diverse phytoconstituent matrix, supporting the theory of synergistic interaction among plant metabolites and underscoring the importance of advanced analytical techniques in modern phytochemical research.

Effect of *C. cassia* Bark Extract on Lipid Profile Parameters

The serum concentrations of total cholesterol, triglycerides, and lipoproteins in both experimental and normal rats are shown in Table 2. In rats (Group II) that received doxorubicin, the levels of total cholesterol, triglycerides, low-density lipoprotein, and very low-density lipoprotein were sig-

Table 2: Effect of ethanolic extract of *C. cassia* on total cholesterol, triglycerides and lipoproteins in normal and experimental rats

Groups	*Total Cholesterol(mg/dl)*	*Triglycerides(mg/dl)*	*HDL (mg/dl)*	*LDL (mg/dl)*	*VLDL (mg/dl)*
Group I	174.43 ± 1.07[a]	79.25 ± 1.14 [a]	62.11 ± 0.76 [a]	96.51± 0.32 [a]	15.85 ± 0.53 [a]
Group II	590.13 ± 2.64 [b]	396.19 ± 0.95 [b]	35.24 ± 0.64 [b]	476.10 ± 0.56 [b]	79.24± 0.10 [b]
Group III	169.02 ± 1.84[a]	76.73± 0.91 [a]	63.37 ± 0.17 [a]	90.34 ± 1.01 [a]	15.35 ± 0.71 [a]
Group IV	230.53 ± 0.99 [c]	131.49 ± 1.17 [c]	59.20 ± 0.32 [a]	145.37 ± 0.93 [c]	26.30 ± 0.29 [c]
Group V	201.18 ± 1.72 [d]	101.26 ± 1.02 [d]	60.16 ± 0.91 [a]	120.86 ± 0.74 [d]	20.25 ± 0.18 [d]

Note:
- Values are expressed as means ± SD for six rats in each group
- Values not sharing a common marking (a,b,c…) differ significantly at $p \leq 0.05$ (DMRT)

nificantly elevated compared to those in the control group, whereas the levels of high-density lipoprotein were significantly reduced. When the pretreated rats were compared to those in the doxorubicin group, it was found that the ethanolic extract of *C. cassia* had a significant effect on various lipoprotein levels: it reduced triglycerides, total cholesterol, low-density lipoprotein and very low-density lipoprotein levels while increasing high-density lipoprotein levels. Group V rats treated with propranolol also exhibited levels that were nearly normal.

When the body strikes a balance between the rates of dietary intake and synthesis and the rates of excretion, cholesterol homeostasis is reached. Excess cholesterol can leak out of cells and enter the bloodstream as high density lipoprotein (HDL), which the liver eliminates. One of the risk factors for MI development is hypercholesterolemia. Elevated blood cholesterol levels and its build-up in the heart are strongly linked to cardiac disease (Salter and White 1996). There is compelling evidence to suggest that hypercholesterolemia causes oxidative stress by reducing tissues ability to defend themselves against free radicals, such as superoxide anions, and by generating oxygen free radicals. These metabolic activities speed up peroxidation reactions, which cause damage to cells (Gokkusu and Mostafazadeh 2003). According to Anandan et al. (2007), the enhanced uptake of low density lipoprotein (LDL)-cholesterol from the circulation by myocardial membranes is the cause of the rise in the myocardial cholesterol content in rats treated with doxorubicin.

In fact, having a higher percentage of cholesterol in HDL than LDL is advantageous since it suggests that cholesterol may be moving from the blood arteries and into the liver. According to Imonek and Bartoova (2005), reducing weight and serum low-density lipoprotein cholesterol levels are critical steps in reducing the risk of a cardiovascular attack.

Changes in cardiovascular functioning may be caused by hypertriglyceridemia and elevated serum levels of cholesterol ester. It has been observed that dietary cholesterol causes the rat liver to produce more triacylglycerol (Fungwe et al. 1993). This might also cause the myocardium's triglyceride levels to rise. Cardiovascular problems are linked to elevated triglycerides (TG) levels (Manjula et al. 1992), DOX stimulates lipolysis in the heart (SushamaKumari et al. 1990). An increase in hepatic triglyceride production and the secretion of higher plasma triglyceride concentrations and cholesterol may result from an enhancement in lipolysis (Rayssiguier 1990). Accumulation of acyl coA and enhanced glycerol formation through higher glycolytic flow may be the cause of increased triglyceride synthesis in cardiac tissue (Subramanian et al. 2003). Triglyceride buildup is one of the CVD risk factors. The mechanism underlying the observed rise in triglycerides following MI could be attributed to heightened fatty acid flow and compromised elimination of very lowdensity lipoprotein from the blood. According to SushamaKumari et al. (1990), there could be a decrease in lipoprotein lipase activity causing a decrease in the absorption of triglycerides from the circulation, which would explain the reported increase in triglycerides. In the current investigation, the levels returned to normal following the administration of *C. cassia* bark. This could be because the extract contains a variety of phytoconstituents. Similar results were seen in *Peltoporumpterocarpum* treated in doxorubicin induced cardiotoxicity rats (Nithyatharani et al. 2024).

Effect of Ethanolic Extract of *C. cassia* Bark on the Lysosomal Enzymes Level in Toxin Treated and Untreated Rats

The results of lysosomal enzymes on both normal and experimental rats, including acid phosphatase, cathepsin-D, β-D-glucuronidase and β-N-acetyl glucosaminidase are displayed in Table 3. When doxorubicin is administered to rats, the experimental animals the levels of lysosomal enzymes rise relative to the control rats. Relative to the disease-control group, rats receiving the ethanolic extract of *C. cassia* bark exhibited a statistically significant decline in the measured parameters. Comparing the plant extract treatment group to the control group, no appreciable differences were seen.

After myocardial ischemia, the intracellular release of lysosomal enzymes may cause cell damage and death either directly (Hoffstein et al. 1975) or indirectly through activation of the complement pathway. This could be the result of produced enzymes leaking into the serum, which could be caused by the heart's cellular compartments being damaged in necrotic circumstances.

An enzyme that is lysosomal is acid phosphatase. For regulated intracellular digestion of cellular constituents by several routes, including autophagy, heterophagy, and endocytosis, lysosomes are necessary. Acid hydrolases are found in the lysosomes of cardiac myocytes, and when these enzymes are released into the cytosol, the heart becomes ischemic, causing damage to the heart's cellular structure and eventual death (Riccutti 1992; Decker and Wildenthal1978).

As lactic acid and metabolic acids build up, adenosine triphosphate is reduced and fatty acid accumulation is increased, leading to ischemia. The cell membrane becomes less strong as a result of this process, which facilitates the release of lysosomal enzymes from confined sacs, including β-D-glucuronidase and β-N-acetyl glucosaminidase. Lysosomal proteases called cathepsins may be implicated in the autophagic digestion of specific cytoplasmic regions as well as myofibrillary and mitochondrial proteins. All animal cells contain the lysosomal aspartic protease known as cathepsin-D (Sudharsan et al. 2006). Following treatment, lysosomal enzyme activity was dramatically reduced by ethanolic extracts of *C. cassia*.

The present findings demonstrate that administration of doxorubicin markedly elevated lysosomal enzyme activities, including acid phosphatase, β-D-glucuronidase, β-N-acetyl glucosaminidase, and cathepsin-D, whereas treatment with the ethanolic extract of *C. cassia* bark significantly restored these parameters toward normal values, indicating a protective effect on lysosomal integrity. Doxorubicin is well known to induce cardiotoxicity primarily through oxidative stress–mediated membrane injury, mitochondrial dysfunction, and lipid peroxidation, which destabilize lysosomal membranes and promote leakage of hydrolytic enzymes into the cytosol and extracellular milieu (Wallace2003; Valko et al. 2007). The elevation of lysosomal hydrolases observed in toxin-treated animals therefore reflects structural disruption of subcellular organelles and is considered a biochemical hallmark of myocardial injury and cellular necrosis. Stabilization of lysosomal membranes is thus an important cytoprotective mechanism, as it prevents uncontrolled proteolysis, preserves intracellular compartmentalization, and maintains metabolic homeostasis.

Research by Karthikeyan et al. (2007) demonstrated that rats given doxorubicin had significantly

Table 3: The effect of *C. cassia* bark extract on the lysosomal enzymes in the doxorubicin toxin treated and control rats

Parameters/ Groups	*Acid phosphatase (U/L)*	*β-D-glucuronidase (μ mol/h/100 mg protein)*	*β-N-acetyl glucosaminidase (μ mol/h/100 mg protein)*	*Cathepsin D (μ mol/h/100 mg protein)*
Group I	31.56 ± 0.26^{a}	21.52 ± 0.35^{a}	48.61 ± 1.04^{a}	21.36 ± 0.43^{a}
Group II	51.34 ± 0.63^{b}	40.18 ± 0.26^{b}	79.15 ± 1.01^{b}	52.82 ± 0.72^{b}
Group III	30.04 ± 0.49^{a}	21.40 ± 0.15^{a}	48.23 ± 0.65^{a}	20.64 ± 0.36^{a}
Group IV	40.69 ± 0.21^{c}	26.34 ± 0.28^{c}	55.33 ± 1.09^{c}	25.31 ± 0.31^{c}
Group V	$37.43 \pm 0.85^{c,d}$	24.06 ± 0.18^{c}	52.14 ± 0.96^{d}	24.45 ± 0.62^{c}

Note:
- Values are expressed as means ± SD for six rats in each group.
- Values not sharing a common marking (a,b,c…) differ significantly at $p \leq 0.05$ (DMRT)

higher serum and cardiac levels of β-D-glucuronidase, β -N-acetyl glucosaminidase and cathepsin-D, with these levels returning to normal in rats given grape seed proanthocyanidins. The normalization of lysosomal enzyme activities in the present extract-treated groups suggests that *C. cassia* bark possesses comparable membrane-stabilizing and antioxidant potential. Cinnamon species are rich in bioactive phytochemicals such as cinnamaldehyde, cinnamic acid derivatives, and polyphenolic compounds, which are documented to scavenge reactive oxygen species, inhibit lipid peroxidation, and enhance endogenous antioxidant defenses (Jayaprakasha et al. 2003; Mathew and Abraham 2006). These properties may underlie the observed reduction in enzyme leakage by protecting phospholipid bilayers from oxidative degradation and preserving lysosomal structural integrity. Furthermore, polyphenols are known to modulate signaling pathways related to inflammation and apoptosis, thereby limiting secondary cellular damage associated with toxin exposure and ischemic stress (Valko et al. 2007).

Another important interpretation is that lysosomal enzymes such as cathepsin-D participate in proteolytic degradation of contractile proteins during pathological stress, and their excessive activation contributes to myocardial remodeling and functional impairment. Therefore, attenuation of cathepsin-D activity by the plant extract suggests not only membrane stabilization but also inhibition of pathological protease activation. The absence of significant differences between extract-treated normal animals and untreated controls further indicates that the bark extract does not adversely alter basal lysosomal metabolism, supporting its safety and physiological compatibility. Collectively, these observations imply that the cardioprotective effect of *C. cassia* may result from a multifactorial mechanism involving antioxidant defense, membrane stabilization, and suppression of lysosomal enzyme release, ultimately preventing toxin-induced cellular degeneration. Such findings align with the broader concept that natural phytochemicals can serve as adjunct therapeutic agents to mitigate chemotherapeutic toxicity without compromising normal cellular function.

Effect of *C. cassia* Extract on Cardiac Marker Enzymes

The activities of creatine kinase (CK), creatine kinase MB (CK-MB), and troponin in experimental and control rats are shown in Table 4. The degree of necrotic damage to the cardiac membrane is shown by the considerable ($P<0.05$) increase in the activity of diagnostic marker enzymes, CK, CK-MB, and troponin, in the serum of rats given doxorubicin compared to rats given control. When ethanolic extracts of *C. cassia* were administered to rats, the activity of the marker enzymes were significantly lower than when the rats were treated with doxorubicin alone.

Due to the muscle-specific nature of creatine kinase, which is mostly found in the heart and brain, myocarditis, cardiac insufficiency, arrhythmias, and myocardial infarction are the causes of its elevation in serum levels (Okinaka et al. 1961). DOX is a well-known cardiotoxic chemical that can destroy heart cells because of this. This led to the release of the cytosolic enzyme creatine kinase into the bloodstream, which is used as a diagnostic indicator for injury to the cardiac tissue. The quantity of this cellular enzyme in blood is indicative of changes in the permeability and/or integrity of the plasma membrane (Vijayakumar et al., 2020).

CK-MB serves as the diagnostic marker for MI. This enzyme is released from the heart into the

Table 4: Level of cardiac marker enzymes in the experimental rats treated with *C. cassia* extract

Parameters/Groups	*CK-MB (IU/L)*	*CK (IU/L)*	*Troponin (ng/L)*
Group I	248.67 ± 2.26 [a]	42.02 ± 1.02 [a]	0.43 ± 0.05 [a]
Group II	503.86 ± 2.10 [b]	106.17 ± 0.89 [b]	2.16 ± 0.11 [b]
Group III	248.15 ± 2.97 [a]	42.19 ± 1.10 [a]	0.40 ± 0.09 [a]
Group IV	286.06 ± 1.93 [c]	53.92 ± 0.85 [c]	0.83 ± 0.12 [c]
Group V	262.96 ± 2.07[a,c]	51.14 ± 0.73 [c]	0.72 ± 0.14 [c]

Note:
Ï%Values are expressed as means ± SD for six rats in each group.
Ï%Values not sharing a common marking (a,b,c…) differ significantly at *p* d" 0.05 (DMRT)

bloodstream when the myocardium becomes damaged due to DOX-induced necrosis. When myocardial cells that have CK-MB are harmed or obliterated, their membranes become permeable or may burst, causing these enzymes to leak out. This explains why rats given doxorubicin had higher serum CK-MB activity (Punithavathi and Prince 2009). Serum CK and CK-MB activity in DOX-induced rats was decreased after treatment with extracts of *C. cassia.* Punithavathi and Prince (2009) reported similar findings in their investigation of the protective impact of naringin on cardiac markers, electrocardiographic patterns, and lysosomal hydrolases in Wistar rats with normal and doxorubicin-induced myocardial infarction. The similar results were noted in the *Psidium guajava* leaves also in the doxorubicin induced cardiotoxicity rats (Manikandan et al. 2023).

It has been demonstrated that cardiac troponin is a very sensitive and specific marker for determining myocardial cell damage. According to O'Brien et al. (1997), troponin is an effective biomarker in lab animals that can be used to identify cardiac injury from a variety of sources in a sensitive and targeted manner (Rajadurai and Prince 2007). In the current investigation, the researchers found that the rats given doxorubicin had higher serum troponin levels. The risk of both subsequent myocardial infarction and cardiac mortality is predicted by elevated troponin levels. Rats given doxorubicin dramatically reduced their serum levels of troponin after pretreatment with ethanolic extracts of *C. cassia*. This might be because *C. cassia* ethanolic extracts lessened the extent of myocardial injury.

The phytoconstituents in the extract, especially the polyphenols, flavonoids, and compounds of the cinnamonaldehyde type, which are known to have potent antioxidant and anti-inflammatory properties, may be mechanistically responsible for the cardioprotective effect seen. These molecules can neutralize free radicals, inhibit lipid peroxidation, and enhance endogenous antioxidant defenses such as superoxide dismutase and catalase, thereby preventing oxidative damage to cardiomyocyte membranes. Additionally, phytochemicals may modulate signaling pathways involved in apoptosis, mitochondrial permeability transition, and calcium homeostasis, which are critical processes in doxorubicin-induced cardiotoxicity (Minotti et al. 2004; Octavia et al. 2012). The near-normalization of cardiac marker enzymes in extract-treated groups indicates that the intervention not only limited enzyme leakage but also preserved myocardial functional integrity.

Overall, the biochemical profile presented in Table 4 strongly supports the hypothesis that the ethanolic extract confers significant cardioprotection against doxorubicin-induced myocardial damage. The restoration of CK, CK-MB, and troponin toward control values demonstrates stabilization of cardiac cell membranes, reduction of necrotic injury, and preservation of myocardial architecture. These findings align with growing pharmacological evidence that plant-derived antioxidants can mitigate drug-induced cardiotoxicity and highlight the therapeutic potential of botanical interventions as adjuncts in chemotherapy-associated cardiac complications. Future investigations integrating histopathological, molecular, and electrophysiological analyses would further clarify the mechanistic basis of this protection and validate its translational relevance.

Molecular Docking Analysis

Through Computer Aided medication Design (CADD), a computational method called a molecular docking research is utilized to find both a new medication and a possible target protein. CADD has been widely used in recent years to evaluate the pharmacological characteristics of novel medications, saving a substantial amount of time, money, and energy. The hexadecanoic acid molecular docking scores for 3DRE (Creatine Kinase-Brain), 1I0E (Creatine Kinase-Muscle), and 1A2X (Troponin) are -6.25, -6.02, and -5.56 kcal/mol, respectively, as shown in Table 5.

The binding mode for hexadecanoic acid with creatine kinase-brain, creatine kinase-muscle and troponin complex is analysed and shown in Figures 2, 3, 4, 5, 6 and 7. Figures2 and 3 show the

Table 5: Molecular docking results of the protein-ligand complex

S. No.	*Protein names*	*Binding affinity (kcal/mol)*
1	Creatine kinase Brain (3DRE)	-6.25
2	Creatine kinase Muscle (1I0E)	-6.02
3	Troponin (1A2X)	-5.56

build of the interaction between the hexadecanoic acid and the creatine kinase-brain in the cys A283, val A75, leu A201, val A72 and leu A202 amino acids. Figures4 and 5 show that the compound hexadecanoicacid interacts between the leu A226, trp A228, ile A238, ILE A188, leu A193 and asp A195 in the creatine kinase-muscle. Figures6 and 7 show that the compound hexadecanoic acid is linked to the ala A106, phe A99, ala A96, arg A100, phe A148 and asp A149 amino acids in the troponin. The binding affinity of hexadecanoic acid of creatine kinase-brain, creatine kinase-muscle and troponin are -6.25, -6.02 and -5.56, respectively.

Overall, the docking analysis demonstrates that hexadecanoic acid exhibits consistent binding interactions with multiple protein targets associated with energy metabolism and muscle function. The ranking of affinities (brain creatine kinase > muscle creatine kinase > troponin) suggests selective yet multi-target binding potential. Such properties are often desirable for natural compounds that may exert therapeutic effects through synergistic modulation of related pathways rather than single-target inhibition. The results therefore support the hypothesis that phytochemicals containing fatty acid moieties can contribute to biological activity through direct protein interactions.

In conclusion, molecular docking analysis indicates that hexadecanoic acid forms stable ligand–protein complexes with key metabolic and contractile proteins, with binding affinities ranging from "6.25 to "5.56 kcal/mol. These values signify moderate yet meaningful interactions that may underlie pharmacological effects. Combined with advances in CADD methodologies and the increasing reliability of computational predictions, docking studies provide a valuable platform for identifying bioactive compounds and guiding rational drug design. However, comprehensive validation through experimental and dynamic simulation approaches remains necessary to confirm biological efficacy and therapeutic potential.

Overall, docking results revealed that protein-ligand complexes formed a number of hydrophobic interactions, which mainly participated in charge transfer that aided in intercalating the ligand in the active site of the protein. The ligand exhibited strong hydrogen bonds, and hydrophobic interactions which stabilised chemical bonding between ligands and the active site of the protein.

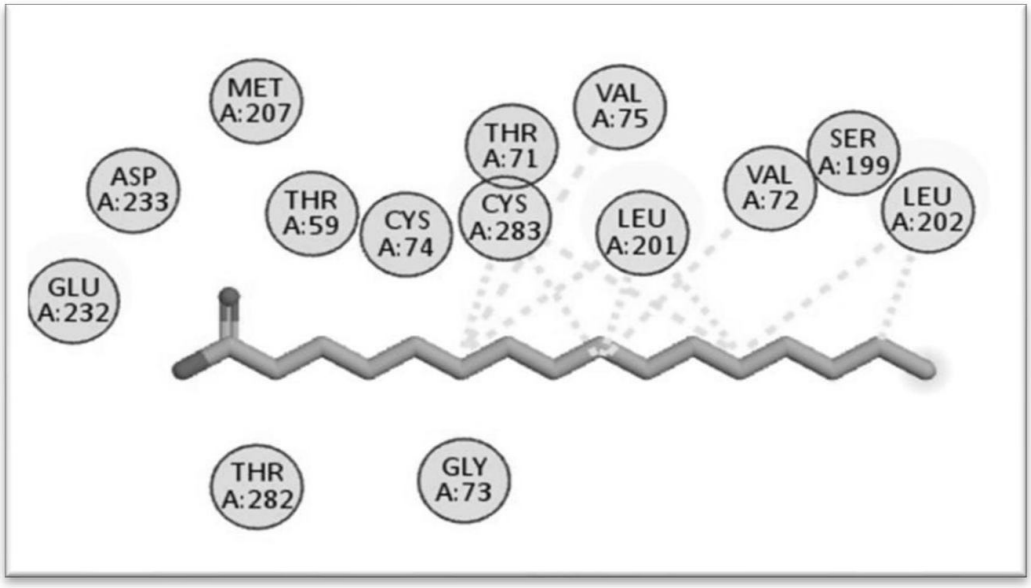

Fig.2. 2D interaction diagram of creatine kinase brain

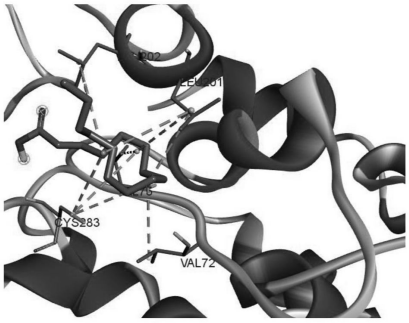

Fig. 3. 3D interaction diagram of creatine kinase brain

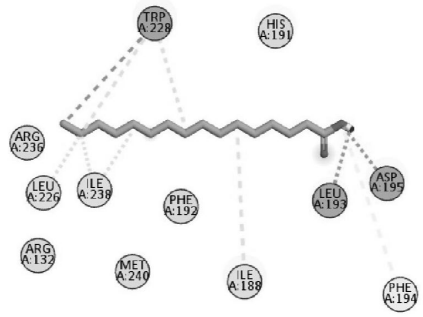

Fig. 4. 2D interaction diagram of creatine kinase muscle

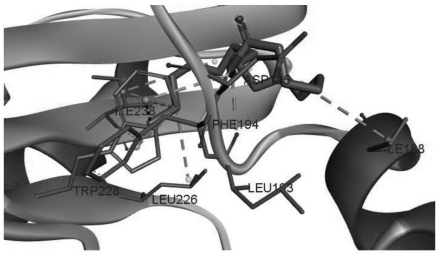

Fig. 5. 3D interaction diagram of creatine kinase muscle

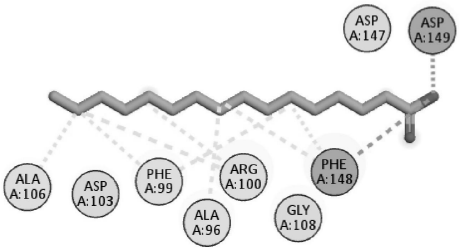

Fig. 6. 2D interaction diagram of troponin

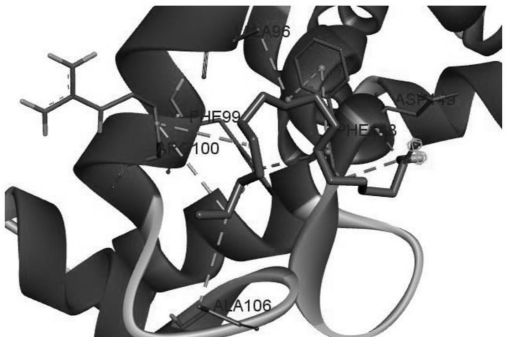

Fig.7. 3D interaction diagram of troponin

CONCLUSION

The protective effect of *C. cassia* barks on the lysosomal enzymes and cardiac marker enzymes of rats that were induced to experience cardiotoxicity due to doxorubin is now amply demonstrated by *in vivo* and *in silico* study. It might be used as a possible medication for heart problems in the near future.

RECOMMENDATIONS

In the near future, it might be used as a potent drug for the heart and its related problems.

Author Contribution

NR & MR-Conceptualization, Methodology, Writing, SA & PK-Paper Writing & Revision, UR & TS-Docking studies, statistical interpretation

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