

Article

Unveiling the Cardioprotective Power: Liquid Chromatography–Mass Spectrometry (LC–MS)-Analyzed *Neolamarckia cadamba* (Roxb.) Bosser Leaf Ethanolic Extract against Myocardial Infarction in Rats and In Silico Support Analysis

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Abstract: *Neolamarckia cadamba* (Roxb.) Bosser, a member of the Rubiaceae family, is a botanical species with recognized therapeutic properties. It is commonly used in traditional medicine to treat cardiac ailments and other disorders. However, the precise active constituents and the potential mechanisms by which they manage cardiovascular disorders remain unclear. Therefore, this study aimed to ascertain the bioactive components and investigate their underlying mechanisms of action. *N. cadamba* is used to treat cardiovascular disorders using the integrated metabolomic methodology. An HPLC-QTOF-MS/MS analysis determined the potential chemicals in the *N. cadamba* leaf ethanol extract (NCEE). A thorough investigation of the NCEE samples used in this study led to the identification of 32 phytoconstituents. Of the 32 compounds, 19 obeyed Lipinski's rule of five (RO5). A molecular docking study directed towards HMG-CoA reductase used 19 molecules. The reference drug atorvastatin indicated a binding energy of -3.9 kcal/mol, while the other substances, Cinchonain Ib and Dukunolide B, revealed binding energies of -5.7 and -5.3 kcal/mol, respectively. Both phytoconstituents showed no toxicity and exhibited favorable pharmacokinetic properties. In vivo study results concluded that treatment with NCEE significantly reduced the cardiac myocardial infarction (MI) marker CK-MB and atherogenic risk indices, such as the atherogenic index plasma (AIP), cardiac risk ratio (CRR), and atherogenic coefficient (AC) in isoproterenol-induced MI rats. In MI rats, NCEE therapy significantly improved the antioxidant system of the heart tissue, as evidenced by the increased levels of GSH and SOD, lower levels of the oxidative stress marker MDA, and significantly decreased HMG-CoA activity. Additionally, electrocardiogram (ECG) signals from rats treated with NCEE resembled those treated with traditional atorvastatin to treat myocardial infarction. This study used H&E staining to show that administering NCEE before treatment reduced cardiac myocyte degeneration in rats with myocardial infarction, increased the presence of intact nuclei, and increased myocardial fiber strength. The potential cardioprotective effect observed in

myocardial infarction (MI) rats treated with NCEE can be extrapolated from computational data to be caused by Cinchonain Ib.

Keywords: *Neolamarckia cadamba*; HR-LC-MS; molecular docking; myocardial infarction; oxidative stress; HMG-CoA reductase

1. Introduction

Myocardial infarction (MI) is a prominent global health concern, with increased morbidity and mortality rates. Myocardial damage is influenced by increased levels of inflammation, cellular proliferation, and the build-up of fibrous and adipose tissues within arterial walls [1]. Moreover, the heightened occurrence of myocardial infarction might be attributed to augmented levels of oxidative and nitrosative stress, aided by multiple well-established mechanisms [2]. Furthermore, it is essential to acknowledge that hypercholesterolemia and hypertriglyceridemia, whether occurring separately or together, substantially affect the initiation and progression of atherosclerotic plaques [3]. Previous studies have shown that lipid accumulation in arterial blood vessels results in inflammation and establishes a distinct type of atherosclerosis [4]. The primary event in atherosclerosis development is the deposition of lipid-containing cells within the arterial wall. Recent investigations have indicated that the progression of atherosclerosis is notably affected by the activity of these three pivotal enzymes. The inhibition of HMG-CoA reductase, an enzyme that regulates cholesterol biosynthesis, is crucial for cardioprotection [5]. Statins, the HMG-CoA reductase inhibitors, lower the risk of atherosclerosis and related cardiovascular events by reducing LDL cholesterol levels. In addition to cholesterol control, statins exhibit anti-inflammatory effects, improve endothelial function, stabilize atherosclerotic plaques, and possess antioxidant properties, making them a comprehensive strategy for protecting the heart [6,7].

Isoproterenol (ISO) is a potent synthetic sympathomimetic catecholamine that exerts its effects by binding β -adrenoceptors. The injection of ISO into animals results in the rapid development of infarction-like lesions. These lesions bear a striking resemblance to the morphological characteristics of coagulative myocytolysis, which is an indicator of acute myocardial infarction in humans [1,2]. Hence, scholars have employed ISO-induced myocardial damage as a paradigm to investigate the beneficial effects of various phytochemicals and synthetic drugs [3]. In recent years, the primary focus of research on cardiovascular disease therapeutics has been developing and evaluating anti-atherogenic drugs, particularly those derived from herbal sources. Owing to the minimal or negligible occurrence of adverse effects associated with herbal drugs, individuals have increasingly directed their attention toward this form of treatment [4].

Neolamarckia cadamba, is a medicinal plant that belongs to the Rubiaceae family. The medicinal properties and therapeutic benefits of *N. cadamba* have been extensively documented in ancient medicine. Charka Samhita is a classical Ayurvedic text widely regarded as one of the most authoritative and comprehensive [8]. The phytochemical analysis of *N. cadamba* has revealed the presence of several bioactive compounds. Some of the reported phytoconstituents include cadambagenic acid, cadamine, quinovic acid, β -sitosterol, cadambine, ursolic acid, prunetinoside, glucogenkwanin, prunasetin, sakuranetin, and puddumetin [9]. Reports have revealed that *N. cadamba* has diuretic [10], larvicidal [11,12], antidiabetic [13], antioxidant [14], anticancer, immunomodulatory [15], antiarthritic [16], anti-inflammatory, and analgesic effects [17]. Although *N. cadamba* is frequently used medically, insufficient data support its use in treating myocardial infarction (MI). Additionally, In silico molecular docking techniques have provided important insights into how different phytochemicals and enzymes interact. This will facilitate the development of new therapeutic strategies for treating cardiovascular diseases. The present study used In silico molecular docking analyses to investigate the potential interactions between the phytochemicals discovered in NCEE and HMG-CoA reductase, as well as to evaluate the cardioprotective effects of NCEE against

ISO-induced myocardial infarction in rats, compared with those of atorvastatin, a typical inhibitor of HMG-CoA reductase in clinical use.

2. Results and Discussion

2.1. HR-LC–MS Analysis of *Neolamarckia cadamba* Ethanol Extract (NCEE)

The yield of NCEE was 8.53% *w/w*. The NCEE samples were examined using HR-LC/MS analysis to identify the phytoconstituents. The retention period, experimental *m/z* values, MS/MS fragments, discrepancies in the database (library), metabolite class, and suggested chemicals were used as bases for identification. Positive ionization was used to obtain mass spectrometry results. The majority of mass-to-charge (*m/z*) values in NCEE fall within the range of 109 to 995. The chromatogram of NCEE is shown in Figure 1. Table 1 illustrates the 32 possible phytochemical compounds identified using high-resolution liquid chromatography–mass spectrometry (HR-LC–MS) of NCEE (Figures 2 and 3). The contents of Cinchonain Ib, Dukunolide B, Chlorogenoquinone, Thamnosin, Luteolin, and Catechin in NCEE were determined to be 10.08%, 1.105%, 4.222%, 4.84%, 2.02%, and 1.544%, respectively.

Table 1. Phytochemical compounds identified in NCEE using HR-LC–MS.

| tR (min) | <i>m/z</i> | Error (ppm) | Molecular Formula | Molecular Weight | Height | Base Peak | Identification |
|----------|------------|-------------|--|------------------|-----------|-----------|------------------------------------|
| 1.407 | 239.0782 | −3.36 | C ₇ H ₁₄ O ₆ | 194.0797 | 7259 | 191.0567 | D-Pinitol |
| 1.482 | 191.0558 | 1.62 | C ₇ H ₁₂ O ₆ | 192.0631 | 6,939,261 | 191.0558 | Quinic acid |
| 1.534 | 133.0148 | −4.86 | C ₄ H ₆ O ₅ | 134.0222 | 19,152 | 191.0569 | L-Malic acid |
| 5.805 | 169.0143 | −0.66 | C ₇ H ₆ O ₅ | 170.0216 | 3300 | 197.8084 | Gallic acid |
| 7.643 | 109.0295 | 0.56 | C ₆ H ₆ O ₂ | 110.0367 | 35,876 | 355.104 | Resorcinol |
| 7.643 | 153.0196 | −2.11 | C ₇ H ₆ O ₄ | 154.0269 | 71,074 | 197.8085 | 2,6-dihydroxybenzoic acid |
| 8.622 | 351.0731 | −2.49 | C ₁₆ H ₁₆ O ₉ | 352.0803 | 1,261,779 | 191.0571 | Chlorogenoquinone |
| 8.874 | 593.1546 | −5.48 | C ₂₇ H ₃₀ O ₁₅ | 594.1617 | 1,368,207 | 353.0689 | Biorobin |
| 8.975 | 253.0718 | −0.14 | C ₁₀ H ₁₀ O ₄ | 194.0579 | 6547 | 191.0571 | Vanillin acetate |
| 9.046 | 177.0199 | −3 | C ₉ H ₆ O ₄ | 178.0271 | 28,346 | 191.0571 | 5,7-Dihydroxychromone |
| 9.066 | 399.1312 | −4.71 | C ₁₇ H ₂₂ O ₈ | 354.1331 | 468,368 | 191.0568 | Fusarenone X |
| 9.109 | 179.0357 | −3.82 | C ₉ H ₈ O ₄ | 180.0429 | 290,533 | 191.0571 | 4-Hydroxyphenylpyruvic acid (HPPA) |
| 9.109 | 289.0729 | −3.44 | C ₁₅ H ₁₄ O ₆ | 290.08 | 162,667 | 191.0571 | Catechin |
| 9.569 | 121.0297 | 0.92 | C ₇ H ₆ O ₂ | 122.0367 | 10,887 | 609.15 | Benzoic acid |
| 9.785 | 739.1713 | 1457.58 | C ₃₄ H ₃₃ NO ₁₅ | 694.1732 | 845,632 | 177.0188 | Dexylosylbenanomicin A |
| 9.979 | 463.0913 | −6.47 | C ₂₁ H ₂₀ O ₁₂ | 464.0985 | 486,727 | 300.0288 | Myricitrin |
| 10.032 | 163.0409 | −4.77 | C ₉ H ₈ O ₃ | 164.0481 | 29,719 | 565.2001 | 4-Hydroxycinnamic acid |
| 10.578 | 285.0418 | −4.84 | C ₁₅ H ₁₀ O ₆ | 286.0491 | 7696 | 515.1239 | Luteolin |
| 10.619 | 381.1218 | 5.42 | C ₁₅ H ₂₈ O ₇ P ₂ | 382.129 | 394,523 | 135.0458 | 2-cis,6-trans-farnesyl diphosphate |
| 10.629 | 529.2234 | −7.5 | C ₂₇ H ₃₄ N ₂ O ₉ | 530.2304 | 698,376 | 295.1093 | 3-α(S)-Strictosidine |
| 10.819 | 497.1497 | −9.02 | C ₂₆ H ₂₆ O ₁₀ | 498.1571 | 669,685 | 109.0298 | Dukunolide B |
| 10.848 | 625.125 | −8.1 | C ₃₀ H ₂₆ O ₁₅ | 626.1322 | 564,414 | 300.0297 | 6''-Caffeoylhyperin |
| 10.905 | 447.0937 | −0.74 | C ₂₁ H ₂₀ O ₁₁ | 448.1009 | 50,786 | 451.1075 | Kaempferol 7-O-glucoside |
| 11.235 | 451.1073 | −8.49 | C ₂₄ H ₂₀ O ₉ | 452.1146 | 4,076,514 | 341.0697 | Cinchonain Ib |
| 12.068 | 543.2032 | −1.18 | C ₃₀ H ₂₈ O ₆ | 484.1892 | 1,446,440 | 265.1011 | Thamnosin |
| 12.076 | 995.4003 | −18.71 | C ₄₅ H ₆₁ CoN ₆ O ₁₂ | 936.3855 | 405,084 | 265.1003 | Cob(I)urate a,c diamide |
| 12.35 | 613.1406 | −1.11 | C ₂₀ H ₃₁ N ₄ O ₁₆ P | 614.1479 | 444,048 | 341.0697 | CMP-N-acetylneuraminic acid |
| 15.118 | 503.3421 | −8.52 | C ₃₀ H ₄₈ O ₆ | 504.3494 | 710,989 | 503.3415 | Protobassic acid |
| 15.781 | 501.3266 | −9.08 | C ₃₀ H ₄₆ O ₆ | 502.334 | 388,829 | 435.2933 | Esculentic acid (Phytolacca) |
| 19.378 | 617.3906 | 1809.59 | C ₃₃ H ₅₃ NO ₆ | 558.3769 | 494,114 | 163.0418 | gamma-Chaconine |
| 19.551 | 615.3201 | −4.64 | C ₃₃ H ₄₆ O ₈ | 570.3219 | 1,266,231 | 163.0419 | Avermectin B1b aglycone |
| 20.291 | 647.4022 | −10.26 | C ₄₀ H ₅₆ O ₇ | 648.4093 | 945,095 | 193.0534 | trans-3-Feruloylcorosolic acid |
| 23.745 | 455.3595 | −13.7 | C ₃₀ H ₄₈ O ₃ | 456.3666 | 5,285,974 | 455.3583 | Ursolic acid |

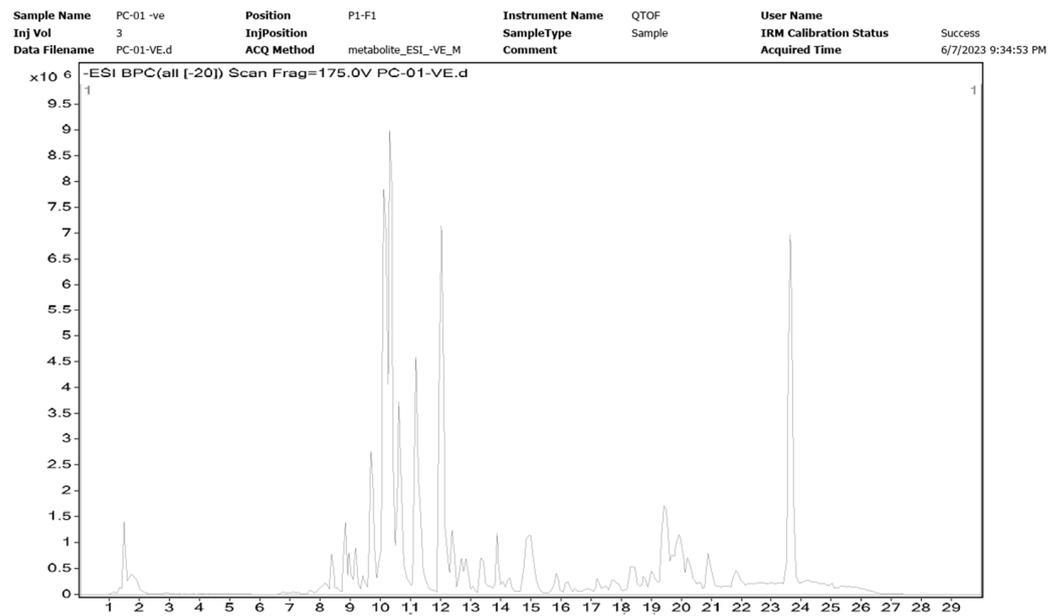


Figure 1. Chromatogram of the identified phytochemical constituent profiles in NCEE using HR-LC-MS.

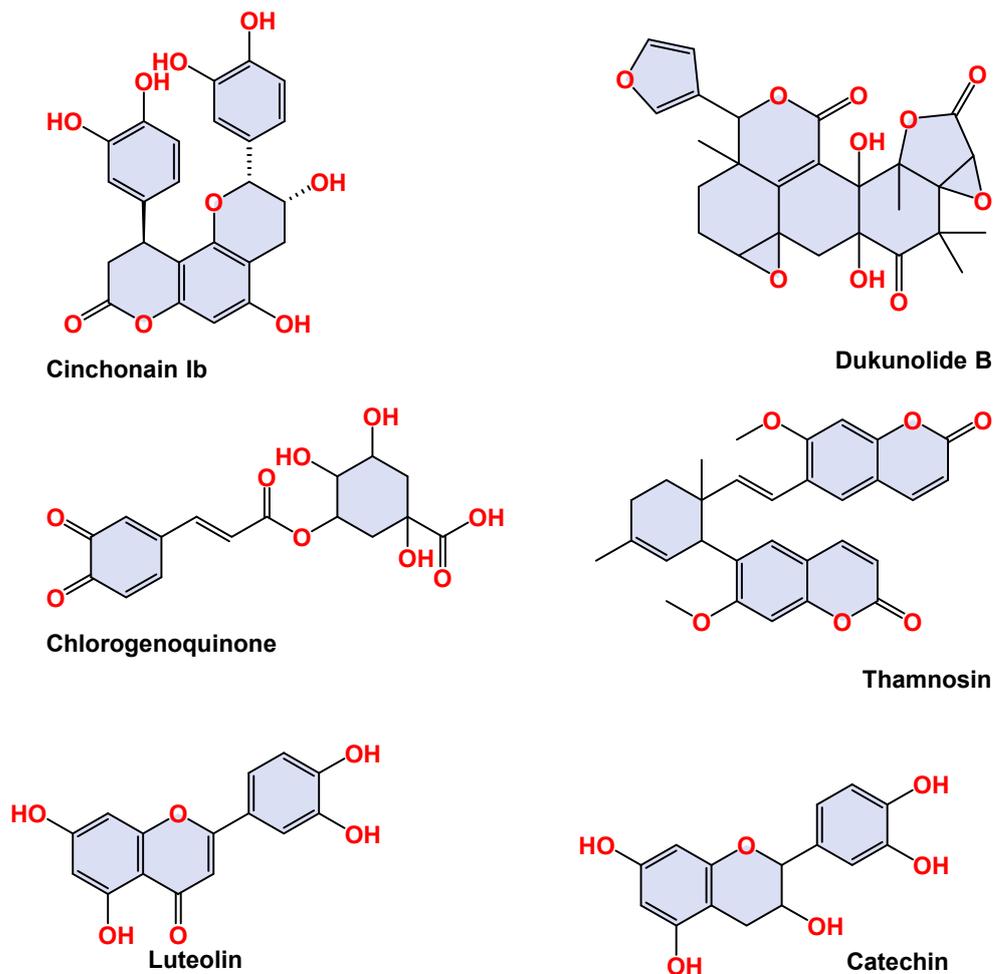


Figure 2. Two-dimensional representation of the top-ranked docked phytoconstituents against HMG-CoA reductase eluted from the HR-LC-MS analysis of NCEE.

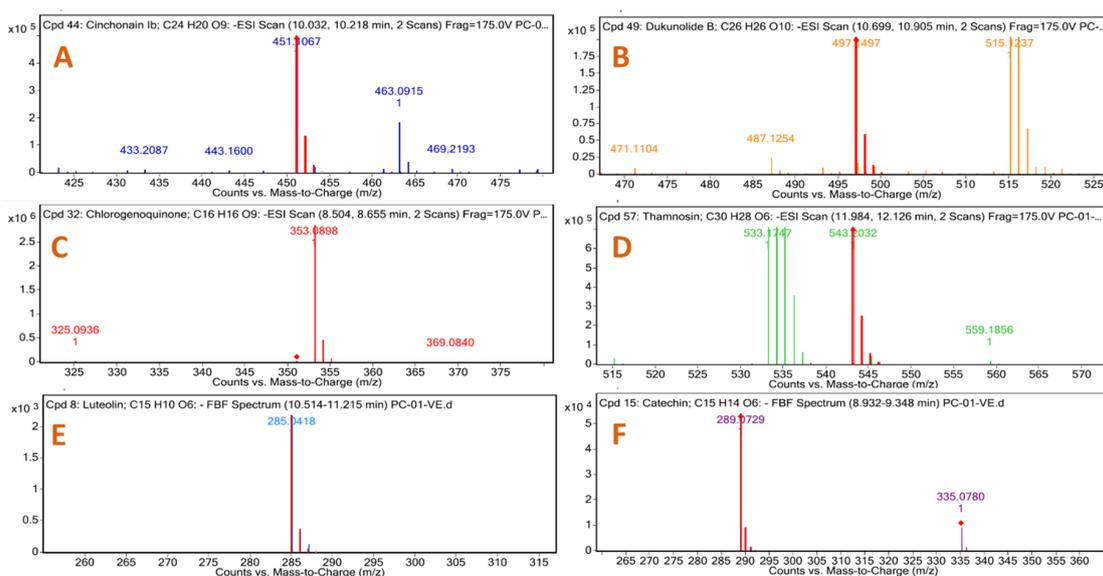


Figure 3. Mass spectral analysis of top-ranked docked compounds against HMG-CoA reductase eluted from NCEE: (A) Cinchonain Ib, (B) Dukunolide B, (C) Chlorogenoquinone, (D) Thamnosin, (E) Luteolin, and (F) Catechin.

2.2. Drug Likelihood

The primary aim of this study is to identify potential therapeutic molecules with desirable drug-like properties. The compounds retrieved from NCEE were analyzed using DruLito, specifically emphasizing their drug-like characteristics. According to the data obtained from the DruLito server, of a total of 32 compounds, 19 compounds adhered to Lipinski's rule, whereas the remaining 13 did not conform to this rule. Information on drug likelihood is presented in Table 2. The phytochemicals were subsequently subjected to molecular docking analysis to compare their outcomes with the standard drug atorvastatin (Table 2).

2.3. Molecular Docking Studies

A re-docking study validated the docking procedure before docking the ligands to the HMG-CoA reductase structure. Atorvastatin, which was present in the crystal structure at 1HWK pdb was docked to the HMGR-binding site. After validation, the RMSD value between the re-docked and co-crystallized poses was 0.9332 Å for 1HWK. These RMSD values indicated the efficiency and validity of the docking protocol (Figure 4).

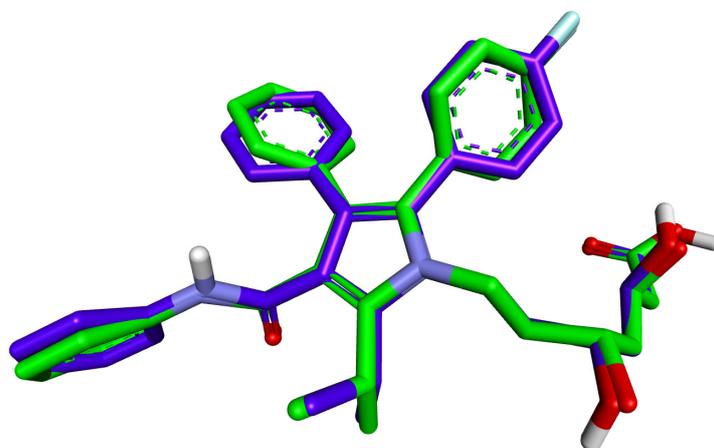


Figure 4. Validation of the docking algorithm by redocking the native inhibitor atorvastatin on the target human HMG-CoA reductase (PDB ID:1HWK). Green: native crystallized pose of atorvastatin; Purple: docked pose of atorvastatin.

Table 2. Drug-likeness properties of various compounds eluted from HR-LC–MS of NCEE.

| Sr. No. | Compound | MW | logp | HBA | HBD | TPSA | AMR | Lipinski's Rule Violated |
|---------|------------------------------------|--------|--------|-----|-----|--------|--------|--------------------------|
| 1 | Benzoic acid | 115.99 | 0.982 | 2 | 0 | 17.07 | 36.96 | No |
| 2 | 5,7-Dihydroxychromone | 171.98 | 0.554 | 4 | 0 | 26.3 | 48.72 | No |
| 3 | 4-Hydroxycinnamic acid | 155.98 | 0.751 | 3 | 0 | 17.07 | 48.8 | No |
| 4 | Luteolin | 275.97 | 1.486 | 6 | 0 | 26.3 | 81.76 | No |
| 5 | Gallic acid | 163.97 | 0.964 | 5 | 0 | 17.07 | 41.77 | No |
| 6 | 4-Hydroxyphenylpyruvic | 171.98 | −0.229 | 4 | 0 | 34.14 | 48.46 | No |
| 7 | Catechin | 275.97 | 0.852 | 6 | 0 | 9.23 | 81.07 | No |
| 8 | Vanillin acetate | 183.98 | 0.78 | 4 | 0 | 52.6 | 53.72 | No |
| 9 | Resorcinol | 103.99 | 0.654 | 2 | 0 | 0 | 34.17 | No |
| 10 | D-Pinitol | 179.97 | −1.797 | 6 | 0 | 9.23 | 40.53 | No |
| 11 | 2,6-dihydroxybenzoic acid | 147.98 | 1.045 | 4 | 0 | 17.07 | 40.17 | No |
| 12 | Ursolic acid | 407.98 | 8.954 | 3 | 0 | 17.07 | 132.26 | Yes |
| 13 | Thamnosin | 455.97 | 4.775 | 6 | 0 | 71.06 | 148.54 | No |
| 14 | Quinic acid | 179.97 | −1.979 | 6 | 0 | 17.07 | 38.88 | No |
| 15 | Kaempferol 7-O-glucoside | 427.94 | 0.18 | 11 | 0 | 44.76 | 114.53 | Yes |
| 16 | Chlorogenoquinone | 335.95 | −2.011 | 9 | 0 | 77.51 | 82.81 | No |
| 17 | Biorobin | 563.92 | −1.083 | 15 | 0 | 63.22 | 145.57 | Yes |
| 18 | Fusarenone X | 331.96 | −0.967 | 8 | 0 | 65.13 | 81.98 | No |
| 19 | Dexylosylbenanomicin A | 661.93 | −0.705 | 16 | 0 | 95.97 | 175.16 | Yes |
| 20 | Myricitrin | 443.94 | 1.15 | 12 | 0 | 44.76 | 116.37 | Yes |
| 21 | 2-cis-6-trans-farnesyl diphosphate | 353.91 | 1.888 | 7 | 0 | 72.22 | 96.1 | No |
| 22 | Dukunolide B | 471.95 | −1.089 | 10 | 0 | 103.96 | 115.45 | No |
| 23 | 6''-Caffeoylhyperin | 599.92 | 1.378 | 15 | 0 | 71.06 | 163.06 | Yes |
| 24 | Cinchonain Ib | 431.95 | 1.18 | 9 | 0 | 35.53 | 125.14 | No |
| 25 | Thamnosin | 455.97 | 4.775 | 6 | 0 | 71.06 | 148.54 | No |
| 26 | CMP-N-acetylneuraminic acid | 582.9 | −6.358 | 20 | 0 | 130.61 | 121.07 | Yes |
| 27 | Protobassic acid | 455.97 | 5.874 | 6 | 0 | 17.07 | 140.37 | Yes |
| 28 | Esculentic acid (Phytolacca) | 455.97 | 5.855 | 6 | 0 | 34.14 | 137.52 | Yes |
| 29 | gamma-Chaconine | 505.97 | 5.035 | 7 | 0 | 21.7 | 147.36 | Yes |
| 30 | Avermectin B1b aglycone | 523.96 | 1.137 | 8 | 0 | 53.99 | 152.72 | Yes |
| 31 | trans-3-Feruloylcorosolic acid | 591.96 | 9.313 | 7 | 0 | 52.6 | 187.26 | Yes |
| 32 | Ursolic acid | 407.98 | 8.954 | 3 | 0 | 17.07 | 132.26 | Yes |

Table 3 displays the binding energies of the 19 ligands derived from NCEE, which obeyed Lipinski's rule of five. Of the 19 compounds, 7 showed better binding affinity than the standard. This indicates that NCEE contains compounds that can combat HMG-CoA reductase activity. The 2D interactions of the ligands with their respective amino acids are presented in Figure 5, and their 3D interactions are depicted in Figures 6–8.

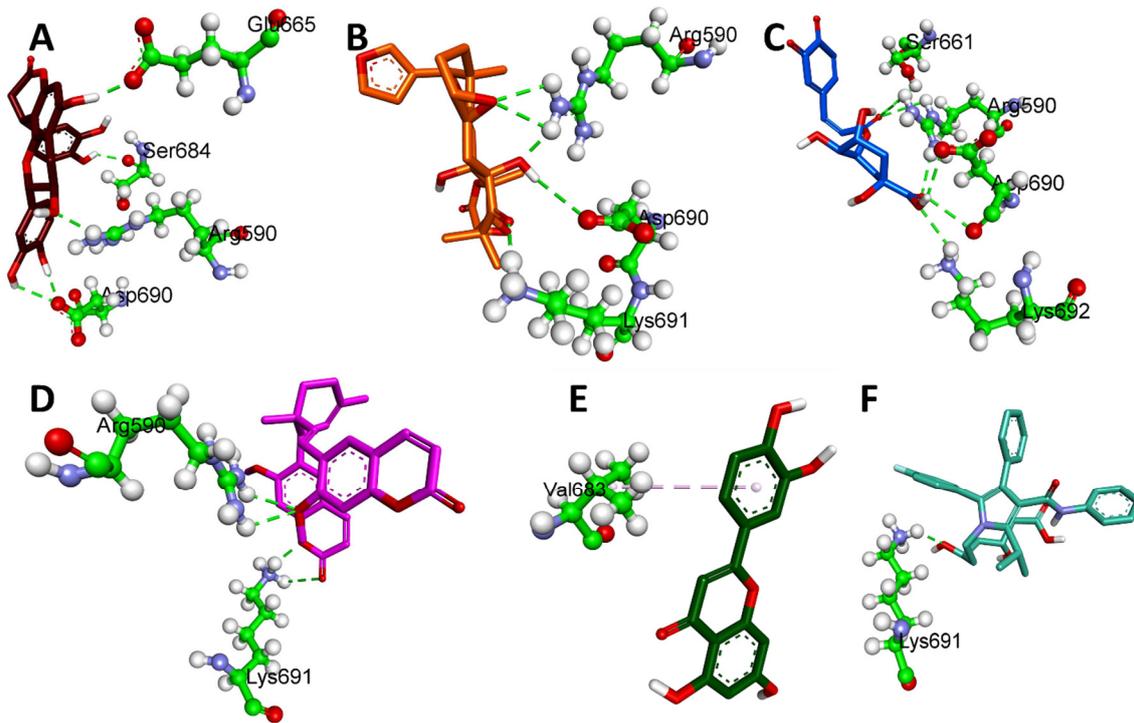


Figure 6. Three-dimensional H-bond interactions of phytochemicals eluted from the HR-LC-MS analysis of NCEE with HMG-CoA reductase: (A) Cinchonain Ib, (B) Dukunolide B, (C) Chlorogenoquinone, (D) Thamnosin, (E) Luteolin, and (F) Atrovastatin.

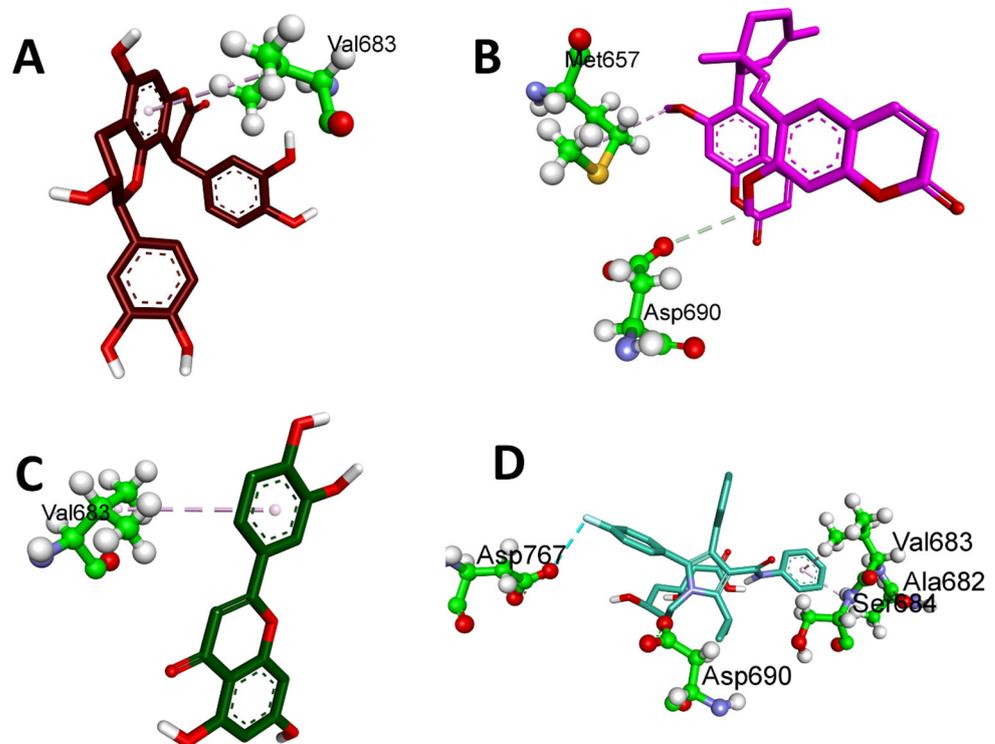


Figure 7. Three-dimensional hydrophobic interactions of phytochemicals eluted from the HR-LC-MS analysis of NCEE with HMG-CoA reductase: (A) Cinchonain Ib, (B) Thamnosin, (C) Luteolin, and (D) Atrovastatin.

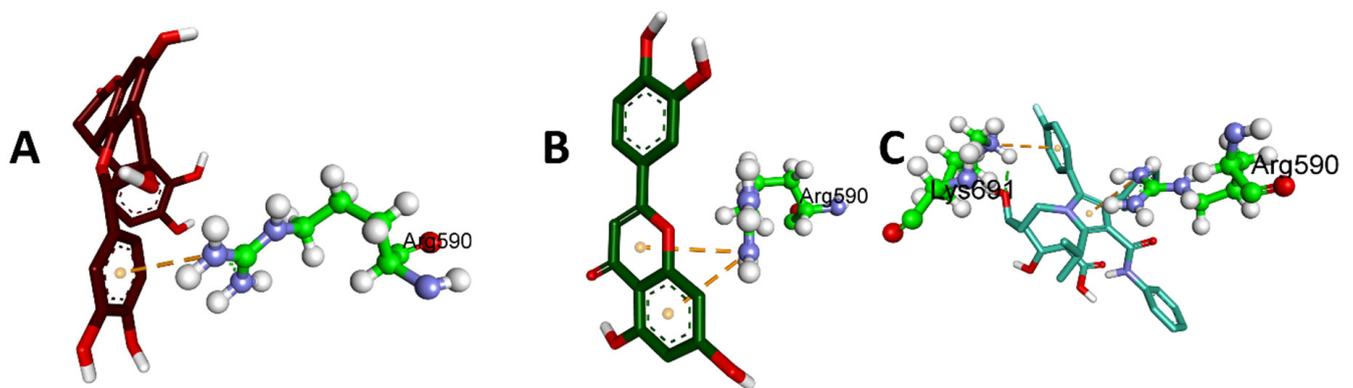


Figure 8. Three-dimensional electrostatic interactions of phytocompounds eluted from the HR-LC-MS analysis of NCEE with HMG-CoA reductase: (A) Cinchonain Ib, (B) Luteolin, and (C) Atrovastatin.

Table 4 shows the binding affinity, ΔG (kcal/mol), of the six ligands to the target protein, the amino acids involved in binding, and the distances between them. The binding affinity measures the degree to which a ligand binds to a protein. The closer the distance between the amino acids involved in binding, the stronger the interaction.

Table 4. Binding energies and interaction details of eluted compounds from HR-LC-MS of NCEE with HMG-CoA reductase (PDB:1HWK).

| Ligands | Binding Affinity, ΔG (kcal/mol) | Amino Acids Involved and Distance (Å) | | |
|-------------------|---|--|---|--|
| | | Hydrogen-Bond Interactions | Hydrophobic Interactions | Electrostatic Interactions |
| Cinchonain Ib | −5.7 | ARG A:590 (6.09), GLU A:665 (5.19), SER A:684 (3.25), ASP A:690 (3.39, 4.47) | VAL A:683 (5.93) | ARG A:590 (6.84) |
| Dukunolide B | −5.3 | ARG A:590 (6.10, 6.50), ASP A:690 (4.66), LYS A:691 (4.47) | - | - |
| Chlorogenoquinone | −4.9 | ARG A:590 (2.34, 4.75), ASP A:690 (2.92), SER A:661 (2.95), LYS A:692 (2.66) | - | - |
| Thamnosin | −4.9 | ARG A:590 (5.93), LYS A:691 (5.17) | MET A:657 (5.17), ASP A:690 (5.12) | - |
| Luteolin | −4.8 | ARG A:590 (6.18), ASP A:690 (2.94), ASN A:658 (3.51) | VAL A:683 (5.61) | ARG A:590 (5.93, 6.78) |
| Catechin | −4.7 | ARG A:590 (4.70), SER A:661 (4.17), ASN A:658 (4.53), ASP A:690 (3.93) | - | ARG A:590 (7.42, 7.64) |
| Artovastatin | −3.9 | LYS A:691 (4.76) | ASP A:767 (5.57), ALA A:682 (6.39), VAL A:683 (5.16), SER A:684 (4.38), SP A:690 (5.10) | ARG A:590 (6.74), MET A:657 (5.99, 6.42) |

The results showed that the binding affinities of the ligands decreased in the following order: Cinchonain Ib > Dukunolide B > Chlorogenoquinone > Thamnosin > Luteolin > Catechin > Atorvastatin. This suggests that the binding affinity of the ligands is correlated with the number and strength of the interactions between the ligand and protein (Table 4).

According to a study by Ercan et al. [18], phytochemicals from NCEE interact well with the active sites of amino acids, including Arg590, Ser661, Val683, Ser684, Asp690, and Lys691. This suggested that these phytochemicals have the potential to inhibit HMG-CoA reductase.

2.4. ADMET Analysis

The ADMET properties of the selected phytochemicals were evaluated using the Swiss ADME (<http://www.swissadme.ch/>), accessed on 22 August 2023, admetSAR (<http://lmmd.ecust.edu.cn/admetSar2/>), accessed on 22 August 2023 and ProTox-II (https://tox-new.charite.de/protox_II/), accessed on 22 August 2023 web servers. The predicted ADMET attributes of the ligands are listed in Table 5.

To ensure the safety and efficacy of a drug, evaluating its absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile is crucial [19]. This evaluation helps to identify potential issues, such as toxicity, that could result in drug withdrawal from the market. By analyzing these characteristics, researchers can determine whether a compound is likely to be absorbed, distributed, metabolized, and excreted, and whether it causes any harmful effects.

Computational methods for predicting ADMET properties, such as *in silico* methods, have become increasingly popular. These methods offer several advantages over traditional *in vitro* methods, including being faster, cheaper, and potentially more life-saving [20]. *In silico* processes use computer models and algorithms to predict a compound's ADMET properties based on its chemical structure and other relevant factors without animal models. This reduces the time and expenses associated with traditional methods [21].

We evaluated the ADMET profiles of the shortlisted compounds, including drug-likeness, partition coefficients, solubility, HIA, BBB permeability, and cytochrome P450 inhibition (Table 2) [22]. Based on SwissADME, ProTox-ii, and admetSAR, we analyzed the acceptable ADME characteristics, paying particular attention to nontoxicity, as ideal drug candidates [22].

An essential property of ADMET is its ability to absorb drugs from the human gut [HIA]. The ability of drugs to be absorbed from the human gut, known as human intestinal absorption (HIA), is a crucial property of ADMET [23]. HIA plays a pivotal role in the transport of drugs to target cells [24]. A higher HIA level improves the intestinal absorption of a compound [20]. In addition to chlorogenoquinone, all compounds showed HIA values greater than 0.9, indicating good membrane permeation.

These compounds have been evaluated for their hepatotoxic, carcinogenic, and mutational potential [25]. The ProTox II results revealed that, except for Dukunolide B and Luteolin, the rest of the compounds were non-carcinogenic. They can also be used as drugs for the treatment of myocardial infarction. As these compounds cannot accumulate in the body, they are less likely to cause cancer if treated for a long time. No hepatotoxicity or cytotoxicity was observed for any of the tested compounds. ADMET studies often use these properties to analyze drug behavior [26].

2.5. Acute Oral Toxicity Test

The administration of 2000 mg/kg NCEE did not cause any observable signs of toxicity. The intervention did not elicit any significant alterations in the respiratory patterns, levels of consciousness, or physical mobility. Restlessness, diarrhea, coma, and convulsions were absent during the two-week follow-up period. The extract was devoid of mortality at 2000 mg/kg.

Table 5. ADMET analysis of selected phytoconstituents from the LC–MS analysis of NCEE based on molecular docking studies.

| Phytocompounds | Swiss ADME | | | | | | ADMET SAR | | | | | | | PROTOX-II | | | | | |
|-------------------|------------|--------------------|---------------|-----------------|--------------|-------------|-----------|-----------------|--------|--------|--------|---------|--------|-----------|------------------|----------------|-----------------|--------------|--------------|
| | log P o/w | Water Solubility | GI Absorption | Lipinski's Rule | Veber's Rule | PAINS Alert | TPSA | Lead Likelihood | HIA | CaCO2 | CYP1A2 | CYP2C19 | CYP2C9 | CYP2D6 | LD50 (mg/kg) | Hepatotoxicity | Carcinogenicity | Mutagenicity | Cytotoxicity |
| Cinchonain Ib | 1.72 | Moderately soluble | Low | Yes | No | 1 | 156.91 | No | 0.9651 | 0.9014 | 0.9311 | 0.9153 | 0.8302 | 0.8996 | 2500 (Class 5) | Inactive | Inactive | Inactive | Inactive |
| Dukunolide B | 2.09 | Soluble | Low | Yes | No | 0 | 148.33 | No | 0.9876 | 0.7312 | 0.7957 | 0.8360 | 0.7483 | 0.9308 | 555 (Class 4) | Inactive | Active | Inactive | Inactive |
| Chlorogenoquinone | 0.22 | Very soluble | Low | Yes | No | 2 | 158.43 | No | 0.6350 | 0.7382 | 0.9477 | 0.8825 | 0.8960 | 0.9002 | 50 (Class 2) | Inactive | Inactive | Inactive | Inactive |
| Thamnosin | 4.63 | Poorly soluble | High | Yes | Yes | 0 | 78.88 | No | 0.9907 | 0.8063 | 0.5228 | 0.6371 | 0.8185 | 0.8590 | 500 (Class 4) | Inactive | Inactive | Inactive | Inactive |
| Luteolin | 1.86 | Moderately soluble | High | Yes | Yes | 1 | 111.13 | Yes | 0.9650 | 0.8957 | 0.9106 | 0.9025 | 0.7898 | 0.9116 | 3919 (Class 5) | Inactive | Active | Active | Inactive |
| Catechin | 1.33 | Soluble | High | Yes | Yes | 1 | 110.38 | Yes | 0.9654 | 0.8956 | 0.9046 | 0.9041 | 0.8227 | 0.8771 | 10,000 (Class 6) | Inactive | Inactive | Inactive | Inactive |

2.6. Effects of NCEE on Serum and Cardiac Biochemical Parameters in Rats

2.6.1. Electrocardiogram Monitoring

The ISO group (85 mg/kg) exhibited a statistically significant decrease in RR intervals ($p < 0.001$ ***), PR intervals ($p < 0.001$ ***), QRs intervals ($p < 0.001$ ***), P amplitude ($p < 0.001$ ***), and QTc amplitude ($p < 0.001$ ***), and an increase in T amplitude ($p < 0.001$ ***), as well as heart rate ($p < 0.001$ ***) and ST amplitude ($p < 0.001$ ***), compared to the normal group. In contrast, rats that received pretreatment with 400 mg/kg NCEE exhibited a statistically significant increase in the RR interval ($p < 0.01$ *), PR interval ($p < 0.01$ **), QTs interval ($p < 0.001$ ***), P amplitude ($p < 0.001$ ***), and QTc amplitude ($p < 0.05$ *), and a decrease in T amplitude ($p < 0.01$ **), as well as a decrease in heart rate ($p < 0.01$ **), when compared to the ISO group. A significant and statistically significant increase in RR intervals ($p < 0.001$ ***), PR intervals ($p < 0.001$ ***), QTs intervals ($p < 0.001$ ***), QTc amplitude ($p < 0.05$ *), and P amplitude ($p < 0.001$ ***), together with a decrease in T amplitude ($p < 0.001$ ***) and heart rate ($p < 0.001$ ***), were found in those receiving ATV treatments compared to those receiving ISO treatment. Electrocardiogram (ECG) abnormalities are frequently utilized as the principal criteria for establishing a definitive myocardial infarction (MI) diagnosis. ST-segment elevation is a significant marker of cardiomyocyte membrane injury, suggesting a potential distinction between ischemic and non-ischemic regions and the consequent impairment of cell membrane functionality [27,28]. Considering the inward sodium and calcium currents and outward potassium and chloride currents during the heart's electrical systole, the QT interval offers insight into the heart's functional condition [29,30]. ST elevation can determine the existence of aberrant cardiac disorders such as arrhythmias, cardiac dysfunction, and rapid cardiac collapse [28]. The electrocardiogram (ECG) of the ISO group showed significant ST-segment elevation, a significant drop in R wave amplitude, reduced RR, PR, QRs, and QTc intervals, and a substantial increase in heart rate (HR) and T amplitude. These results demonstrate the presence of isoproterenol-induced myocardial infarction (MI). The changes in electrocardiographic (ECG) patterns induced by ISO are in line with the findings of other studies [31,32]. According to Patel et al. (2010), the significant increase in heart rate (HR) in the ISO group may have been caused by ISO stimulation of the β -adrenoceptor [31]. Previous studies have provided evidence to support the notion that an elevated heart rate (HR) is causally linked to the escalation of cardiac oxygen demand, ultimately resulting in a hastened occurrence of myocardial necrosis. The administration of ISO may reduce R-wave amplitude due to the development of myocardial edema [33]. The present investigation demonstrated that groups pretreated with NCEE exhibited a significant decrease in electrocardiogram (ECG) abnormalities generated by ISO (Figures 9 and 10).

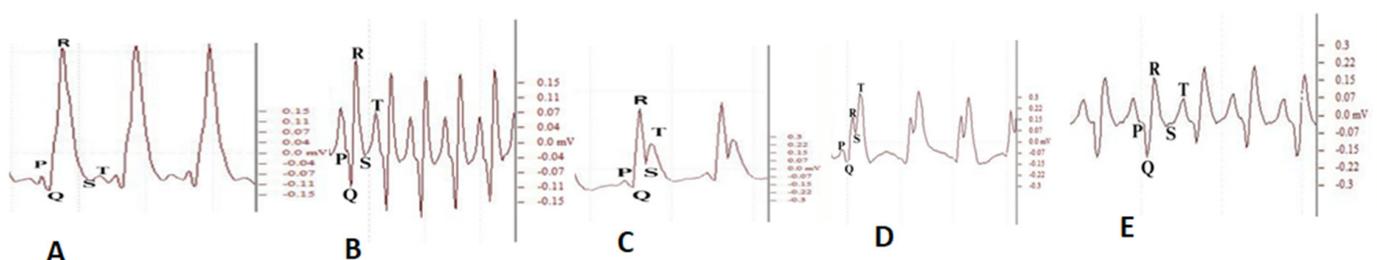


Figure 9. Electrocardiogram: (A) normal rats, (B) ISO, (C) ISO + ATV, (D) ISO + NCEE (200 mg/kg), and (E) ISO + NCEE (400 mg/kg).

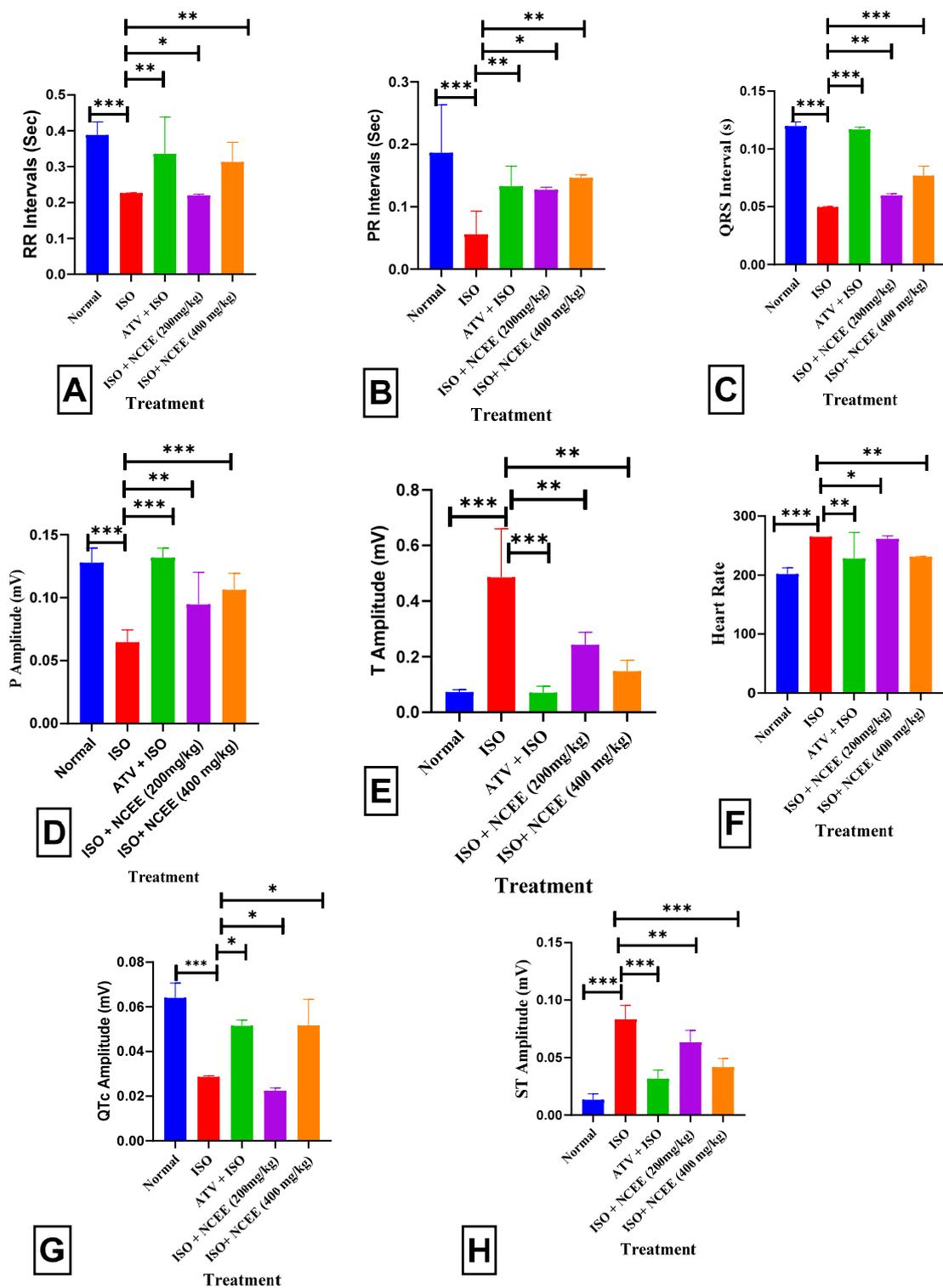


Figure 10. Effect of NCEE on electrocardiogram: (A) RR Intervals, (B) PR Intervals, (C) QRS intervals, (D) P Amplitude, (E) T Amplitude, (F) heart rate, (G) QTc amplitude, and (H) ST Amplitude. All results are expressed as mean \pm SEM and one-way ANOVA with Dunnett's multiple comparison tests (significance at $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***). The results were compared with those of the ISO group.

2.6.2. Effects of NCEE on the Serum Parameters

The ISO group (85 mg/kg) exhibited a statistically significant increase in serum CK-MB ($p < 0.001$ ***), SGOT ($p < 0.001$ ***), TC ($p < 0.001$ ***), TG ($p < 0.001$ ***), LDL

($p < 0.001$ ***), VLDL ($p < 0.001$ ***), AC ($p < 0.001$ ***), AIP ($p < 0.001$ ***), and CCR ($p < 0.001$ ***), and significantly decreased HDL ($p < 0.001$ ***), when compared to the normal group. Pretreatment with 400 mg/kg NCEE resulted in a statistically significant decrease in CK-MB ($p < 0.01$ **), SGOT ($p < 0.01$ **), TC ($p < 0.001$ ***), TG ($p < 0.01$ **), LDL ($p < 0.05$ *), VLDL ($p < 0.001$ ***), AC ($p < 0.05$ *), AIP ($p < 0.01$ **), and CCR ($p < 0.01$ **), and significantly increased HDL ($p < 0.05$ **), compared to the ISO group. Creatine kinase (CK) facilitates the reversible transformation of creatine and adenosine triphosphate (ATP) into creatine phosphate and adenosine diphosphate. The myocardium comprises a dimeric enzyme consisting of two subunits, namely, M and B. Approximately 20% of the overall cardiac creatine kinase (CK) exists in the MB isoform, hence offering diagnostic specificity and sensitivity for detecting MI [34]. The findings of this study align with those of previous studies and demonstrate that ISO administration leads to necrotic myocardial injury [35,36]. The enzyme SGOT is predominantly located in the liver, heart, and muscles, and is secreted into the bloodstream in response to organ damage. According to Lofthus et al., the serum levels of SGOT activity increase within 3–8 h following the initiation of myocardial injury [37]. These levels peaked at 24 h and gradually reverted to normal throughout 3–6 days. By observing a significant increase in serum CK-MB levels in the ISO group compared with the normal group, the current investigation confirmed the incidence of MI caused by ISO. The NCEE-treated groups showed a marked reduction in the cardiac blood marker levels. These results imply that NCEE protects the heart by maintaining the structural and functional integrity of the myocyte plasma membrane and the contractile mechanism, thereby reducing the release of cardiac enzymes into the bloodstream. Lipids are crucial in atherosclerosis and hyperlipidemia and directly impact cardiovascular disease. Lipids can also alter the biological membranes' stability, consistency, and composition. Increased blood lipid levels contribute to the development of arteriosclerosis and are significant risk factors for myocardial infarction. Myocardial infarction patients had higher levels of total cholesterol, triglycerides, LDL cholesterol, VLDL cholesterol, and lower HDL cholesterol concentrations, according to Kumar and Al-Yahya et al. [38] and Meeran, M. N. et al. [39]. In the current study, rats treated with ISO exhibited significant increases in TC, LDL, VLDL, TG, and AI levels. In addition, HDL levels were lower in these rats. However, 400 mg/kg treatment reversed these adverse effects on the lipid profile. These data demonstrated that the modulation of the lipid profile by NCEE is an essential component of its cardioprotective action (Figure 11).

The ISO group exhibited a notable decrease in cardiac antioxidant levels, specifically CAT ($p < 0.001$ ***), GSH ($p < 0.001$ ***), and SOD ($p < 0.001$ ***). Additionally, an elevation in oxidative stress was observed, as indicated by an increase in MDA levels ($p < 0.001$ ***). Pretreatment with NCEE (400 mg/kg) resulted in a significant increase in CAT ($p < 0.001$ ***), GSH ($p < 0.001$ ***), and SOD ($p < 0.001$ ***), as well as a decrease in MDA ($p < 0.001$ ***), compared to the ISO group. Liver HMG-CoA reductase activity was increased as reflected by a decrease in the HMG-CoA/mevalonate ratio ($p < 0.001$ ***) in ISO-treated rats compared to normal rats (Figure 12). The formation of the reactive hydroxyl radical (OH•), which acts as a catalyst for lipid peroxidation and damages and disrupts cellular membranes, is frequently blamed for the incidence of ISO-induced myocardial infarction (MI) [40]. Antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), are critical players in the initial defense mechanisms within cells to combat oxidative stress caused by diverse stressors. Our study showed that the myocardial SOD and CAT activities were significantly lower in the model group than in the control group. SOD and CAT enzyme activities were lower in ISO-induced MI, which may be related to an increase in the generation of reactive oxygen species, specifically superoxide and hydrogen peroxide. Therefore, oxidative stress may suppress SOD and CAT activity [41]. Reactive oxygen species (ROS) accumulate due to GSH inhibition, making cardiomyocytes more susceptible to oxidative injury [42,43]. In contrast, 400 mg/kg NCEE administration resulted in a notable increase in enzymatic activity and GSH levels. These findings confirmed that the antioxidative properties of NCEE play a crucial role in its cardioprotective effects.

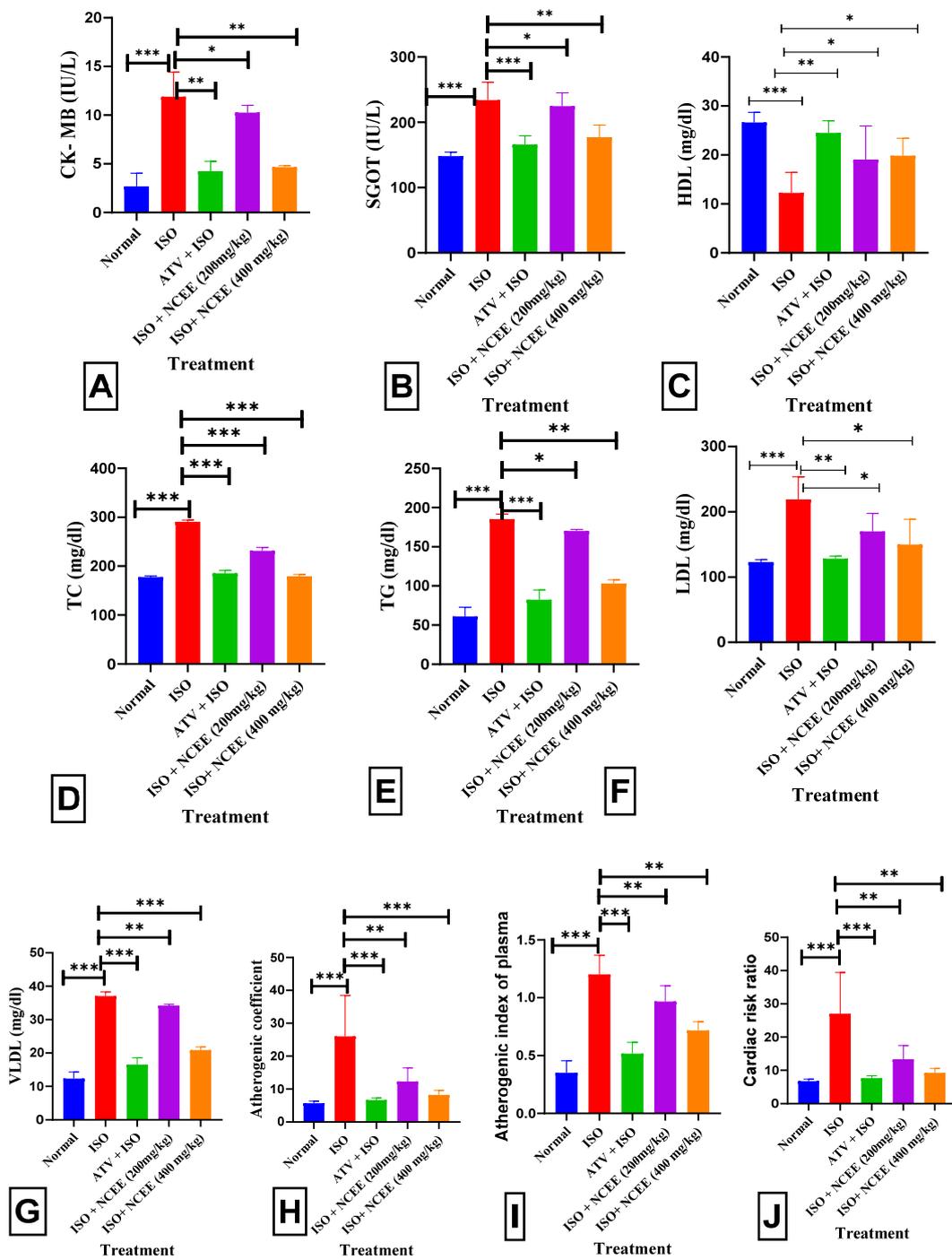


Figure 11. NCEE effects on serum parameters: (A) CK-MB levels, (B) SGOT, (C) HDL levels, (D) TC levels, (E) TG levels, (F) LDL levels, (G) VLDL levels, (H) AC, (I) AIP, and (J) CRR. All results are expressed as mean \pm SEM and one-way ANOVA with Dunnett’s multiple comparison tests (significance at $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***). The results were compared with those of the ISO group.

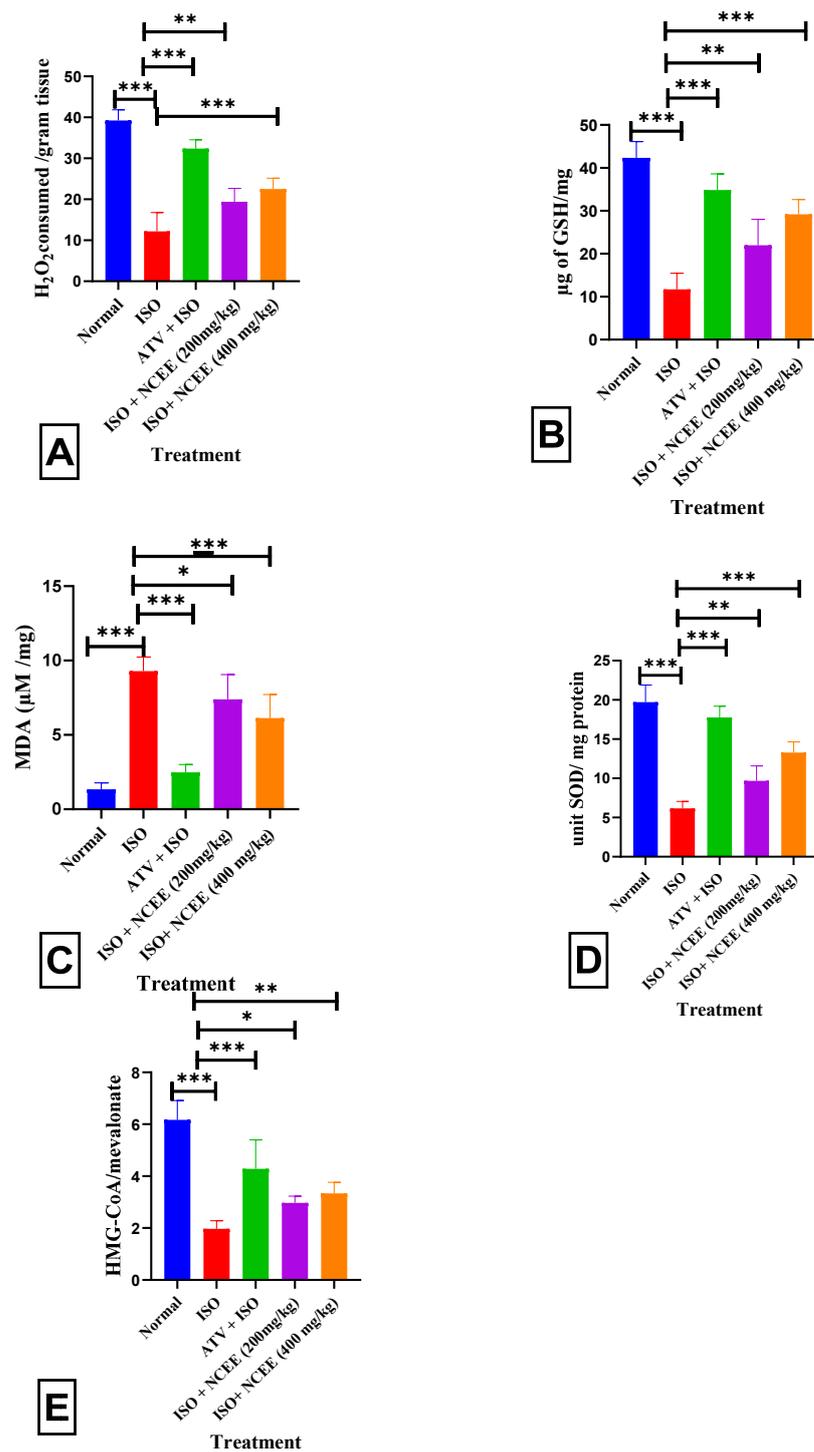


Figure 12. Effects of NCEE on tissue parameters. Heart tissue: (A) CAT activity, (B) GSH level, (C) MDA level, and (D) SOD activity; liver tissue: (E) HMG-CoA activity. All results are expressed as mean \pm SEM and one-way ANOVA with Dunnett's multiple comparison tests (significance at $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***). The results were compared with those of the ISO group.

2.6.3. Liver HMG-CoA Analysis

The assessment of HMG-CoA reductase activity using an indirect approach demonstrated that the ISO-induced disease group exhibited a significant ($p < 0.001$ ***) reduction in the HMG-CoA/mevalonate ratio (Figure 12E). This reduction suggests an elevation in cholesterol synthesis compared to the normal group. In the groups pretreated with 400 mg/kg NCEE, a statistically significant increase ($p < 0.01$ **) in the HMG-CoA/mevalonate ratio was

observed. This finding suggests that NCEE can potentially decrease the overall activity of HMG-CoA reductase compared to the ISO group. Following the administration of ISO, the evaluation of the blood lipid profile revealed substantial dysregulation characterized by a marked elevation in triglyceride, cholesterol, LDL-C, and VLDL-C levels, accompanied by a considerable decrease in HDL-C values. These findings suggest a hyperlipidemic effect possibly resulting from perturbations in lipid metabolism in the treatment of ISO. This finding suggests a potential direct association with myocardial infarction [44]. The higher the ratio, the lesser the mevalonate formed, indicating a reduction in HMG-CoA reductase activity and reduced cholesterol synthesis [6,45]. The hypolipidemic activity of NCEE may result from its HMG-CoA reductase activity, which controls cholesterol synthesis. This result is similar to the hypolipidemic and HMG-CoA reductase activity-reducing capability of sulfated polysaccharides from *Padina tetrastromatica* [6].

2.7. Histopathological Studies

The histopathological analysis of the ISO group demonstrated cardiac muscle fiber degradation and significant damage to cardiac myocytes (Figure 13), which aligns with previous findings reported by Qudus et al. (2021) [46]. The myocardial fibers in the NCEE (400 mg/kg) group exhibited a practically normal architecture, indicating a minimal degree of deterioration. These findings provide further evidence to support the cardioprotective action of NCEE.

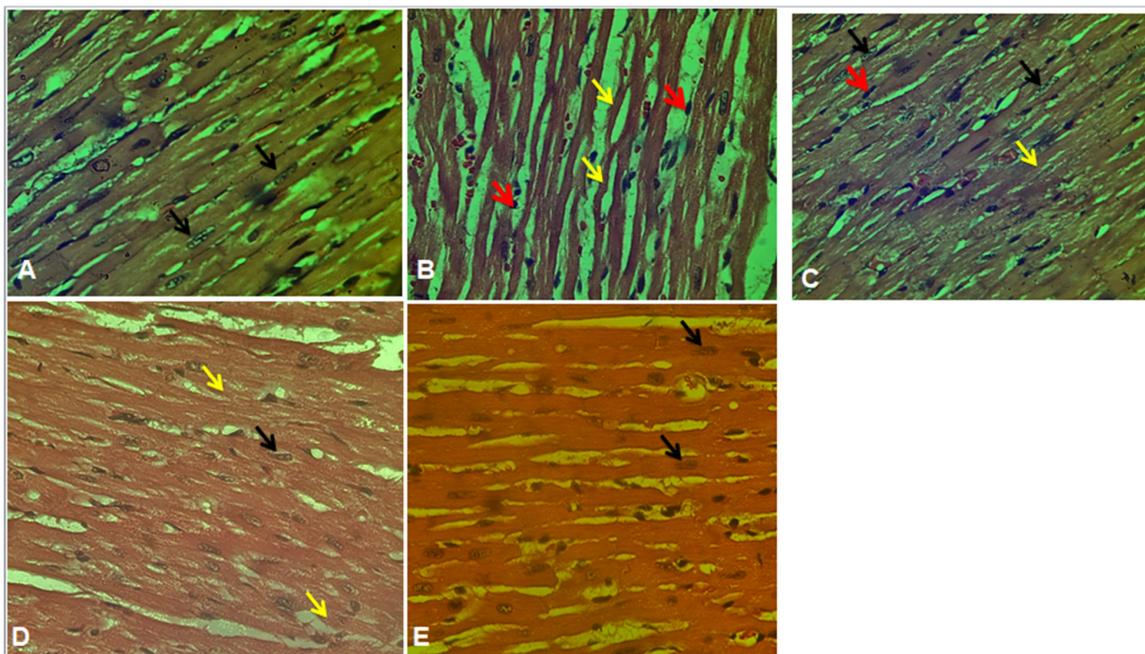


Figure 13. Histopathological examination of the heart in experimental groups of rats. (A) Control group with the standard histological architecture of cardiac myocytes with prominent nuclei (black arrow). (B) The ISO group showed a marked degeneration of cardiac muscle fibers; extensive damage of cardiac myocytes (yellow arrow); loss of cytoplasmic striation of myocytes with shrinkage, elongation, and disarrangement of nuclei; and marked inflammatory cell infiltration, mainly neutrophils; myocytes showed an absence of nuclei (red arrow). (C) The ATV-treated group showed compact, uniformly arranged myocardial fibers, few areas of chronic inflammatory cells (red arrow), myocytes with uniform nuclei, and striations (black arrow). (D) Group IV NCEE (200 mg/kg, per oral) showed an appearance of damage of cardiac myocytes (yellow arrow), few cardiac myocytes with prominent nuclei (black arrow). (E) Group V NCEE (400 mg/kg, per oral) showed compact, uniformly arranged myocardial fibers, with uniformly and prominently placed nuclei (black arrow). A significant loss of wave-like cardiac fibers was observed, and fewer inflammatory cells appeared in some areas than in group IV NCEE (200 mg/kg)-treated rats.

3. Materials and Methods

3.1. Plant Material and Extraction

The *N. cadamba* specimen was collected and later identified in the Department of Botany at the PSC & KVSC Government College in Nandyal, Andhra Pradesh, India. A voucher specimen was deposited at the herbarium of the same faculty and assigned number PSC/KVSC/BOT/0009. The leaves were then shade-dried, ground, powdered, and extracted using ethanol. The extract was subsequently dried under a vacuum in a rotary evaporator.

3.2. Identification of Bioactive Compounds by High-Resolution Liquid Chromatography–Mass Spectrometry (HR-LC–MS)

High-resolution liquid chromatography–mass spectrometry (HR-LC–MS) analysis was performed on the NCEE sample using a ChipCube G6550A iFunnel Q-TOF mass spectrometer equipped with an electrospray ionization source. Phytochemicals were separated using a Hypersil GOLD C-18 column (2.1 × 100 mm, three µm particle size) as the stationary phase. The gradient mobile phases of “solvent A” (100% water) and “solvent B” (100% methanol) were used at a flow rate of 300 µL/min. The injection volume of NCEE was three µL at an injection speed of 100 µL/min with a 5.0 sample flush-out factor. The gradient was started with 95:5 (H₂O/CH₃OH) for 1 min, changed to 0:100 (H₂O/CH₃OH) for 25 min, and returned to 95:5 (H₂O/CH₃OH) for 31 min. The iFunnel MS Q-TOF instrument segment was maintained at a gas flow rate of 13 L/min, temperature of 250 °C, gas flow rate of 11 L/min, sheath gas flow rate of 300 °C, and a nebulizer gas flow pressure of 35 psi. The acquisition method was set to the MS mode with a minimum range of 120 (*m/z*) and a maximum of 1200 (*m/z*), scanning at a rate of 1 spectrum/s. The analysis was conducted at the Sophisticated Analytical Instrument Facility (SAIF) of the Indian Institute of Technology, Bombay (IIT Bombay), India [47,48].

3.3. *In-Silico* Studies

3.3.1. Drug-Likelihood

To assess the drug-like properties of the phytoconstituents identified in NCEE, a drug-likeness tool (DruLito) was employed. DruLito evaluated the chemical compounds based on Lipinski’s rule of five, which examines parameters such as molecular weight, log P, and the number of hydrogen bond donors and acceptors. This analysis provides insights into the likelihood that the identified phytoconstituents possess drug-like properties [49].

3.3.2. Molecular Docking

The docking study was implemented using Auto Dock Vina, and relevant input files for Auto Dock Vina were generated using the Auto Dock program. Human HMG-CoA reductase (PDB ID:1HWK) (Figure 14) [50] was used as a target for this study. The RSCB protein data bank provided crystallized structures, and PubChem provided 3D structural information on the ligands. Incorporating polar hydrogen atoms and gesture charges is necessary to prepare files through Auto Dock. Table 6 presents the coordinates of the grid box and grid box size. Vina was implemented using a shell script supplied by Auto Dock Vina developers. The strength of the bond between the ligand and receptor is represented as a negative score (kcal/mol). The Autodock Vina script produced nine distinct ligand positions with different binding energies for each ligand. A Perl script obtained the ligand with the highest binding affinity for the docked complexes [51].

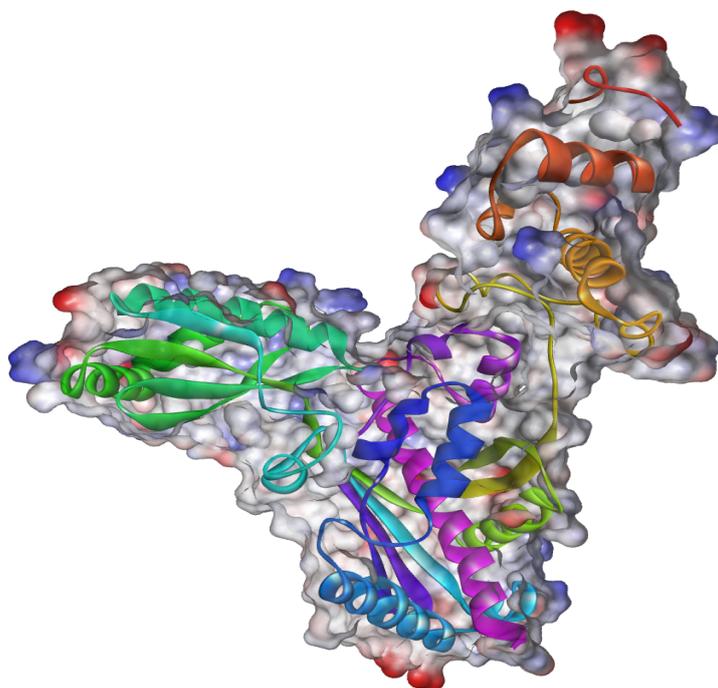


Figure 14. Three-dimensional ribbon-type representation of the complex of the catalytic portion of human HMG-CoA reductase with atorvastatin (PDB: 1HWK).

Table 6. Molecular docking grid box coordinators used by Autodock Vina.

| Centre | x | y | z |
|---------------------------------------|-----------|----------|-----------|
| Human HMG-CoA reductase (PDB ID:1HWK) | 18.313098 | 8.379805 | 15.174463 |
| Size | x | y | z |
| Exhaustiveness | 12 | 12 | 12 |
| | | 8 | |

3.3.3. ADMET Analysis

Furthermore, the absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction tool ADMETSAR was used to evaluate the potential pharmacokinetic properties and toxicity risks associated with the identified phytoconstituents. ADMETSAR utilizes computational models to predict aqueous solubility, blood–brain barrier permeability, cytochrome P₄₅₀ inhibition, hepatotoxicity, and mutagenicity. This analysis aids in assessing the overall drug-like characteristics and potential safety concerns of the phytoconstituents. The ADMETSAR results provide valuable insights into the ADME properties and potential risks associated with the identified compounds, guiding further investigations for developing safe and effective cerebroprotective drugs [52].

3.4. Acute Toxicity

Based on earlier works on acute toxicity studies of NCEE on Wistar rats, according to OECD—423 guidelines, the different doses of NCEE were administered up to 4000 mg/kg b.w. (p.o.) and there is no evidence of toxicity reported. According to the OECD—423 guidelines, in the present study, 1/10th and 1/20th doses are preferred as high and low doses [53].

3.5. Induction of MI

Wistar rats (8 weeks old, both sexes, weight average 220–225 gm) were subjected to subcutaneous injection of isoproterenol at a dose of 85 mg/kg, administered at 24 h intervals over two consecutive days, inducing specific alterations in biochemical markers,

histological observations, and electrocardiogram (ECG) readings that are indicative of myocardial infarction (MI) [54–56].

3.6. Experimental Procedure

Thirty rats were randomly assigned to five groups of six rats each. The treatment regimen was as follows. Experimental protocols were carried out with the approval of Institutional Animal Ethics Committee protocol number (1519/po/Re/S/11/CPCSEA/2022/004)

1. Normal group: rats received two subcutaneous injections of normal saline (vehicle of ISO) in a volume of 1.5 mL/kg, with one injection per day on days 8 and 9.
2. In the ISO group, rats received two injections of ISO (85 mg/kg/day, s.c.) at a volume of 1.5 mL/kg, with one injection per day on days 8 and 9, to induce acute MI.
3. ATV + ISO group: rats received atorvastatin (10 mg/kg, per oral) for nine days, followed by two injections of ISO (85 mg/kg/day, s.c.), with one injection per day on days 8 and 9.
4. NCEE (200 mg/kg) + ISO group: rats were treated with NCEE at a dose of 200 mg/kg/day (p.o.) for nine days, followed by two injections of ISO (85 mg/kg/day, s.c.), with one injection per day on days 8 and 9.
5. NCEE (400 mg/kg) + ISO group: rats were treated with NCEE at a dose of 400 mg/kg/day (p.o.) for nine days, followed by two injections of ISO (85 mg/kg/day, s.c.), with one injection per day on days 8 and 9.

3.7. Electrocardiogram Monitoring

After administering the second dose of ISO, electrocardiograms (ECGs) were measured for all experimental groups after 24 h. The rats were administered anesthesia with Ketamine (60 mg/kg) and Xylazine (9 mg/kg) until complete anesthesia was achieved. Subcutaneous attachment of ECG electrodes was performed on the paws and chest of the rats while they were in a supine position, followed by connection to the Polyrite D (Polygraph Digital, RMS, India). Subsequent electrocardiogram (ECG) parameters were analyzed. The RR, PR, and QRS intervals, as well as the P and T amplitudes, heart rate, and QTc amplitude, are important parameters used in the analysis of cardiac activity.

3.8. Collection of Blood and Tissue Samples

After the ECG recordings, a clean, sterile capillary tube was placed into the eye's inner canthus to draw blood samples from the orbital sinus (retro-orbital plexus) under anesthesia. After cervical dislocation, the rats were slaughtered, and blood was clotted for 20 min. The samples were then centrifuged for 15 min at 4000 rpm. Serum samples were separated to estimate lipid profiles: total cholesterol (TC), triglycerides (TG), HDL, SGOT, and the cardiac marker CK-MB. Atherogenic indices were determined using lipid profile data: atherogenic index of plasma (AIP), cardiac risk ratio (CRR), and atherogenic coefficient (AC) [57]. After sacrifice, the hearts were removed free of connective tissue, cleaned in ice-cold phosphate-buffered saline, and homogenized, and the supernatant was used for the estimation of antioxidant parameters such as catalase (CAT), reduced glutathione (GSH) [58], superoxide dismutase (SOD), and the stress parameter malondialdehyde (MDA). HMG-CoA (β -Hydroxyl β -methylglutaryl-CoA) reductase activity was assessed in the liver tissue homogenate by measuring the ratio of HMG-CoA to mevalonate [59].

3.9. Histopathological Studies

The myocardial tissue from the excised heart was immediately fixed in a 10% buffered neutral formalin solution for histopathological studies. The fixed tissues were embedded in paraffin, and serial sections were cut. Each section was stained with hematoxylin and eosin (H&E stain). The cells were examined under a light microscope, and photomicrographs were obtained [60].

3.10. Statistical Analysis

All data are expressed as the mean \pm S.E.M. Statistical significance between more than two groups was tested using one-way ANOVA, followed by Dunnett's multiple comparison test using a computer-based fitting program (Prism Graph Pad 8.5). *p*-values of less than 0.05 *, 0.01 **, and 0.001 *** were considered statistically significant.

4. Conclusions

In summary, the results of the present study have demonstrated that the analysis of NCEE using HR-LC-MS has revealed the presence of significant groups of phytochemicals that may potentially contribute to the inhibition of HMG-CoA reductase, thereby offering potential therapeutic applications for myocardial infarction. Based on the outcomes of the molecular docking, Cinchonain Ib, Dukunolide B, and Chlorogenoquinone exhibited higher binding affinities compared to atorvastatin. Further experimental investigations are mandatory to establish their effectiveness. The current study's *in vivo* findings indicate that NCEE possesses potential antioxidants that could be accountable for its advantageous impact on cardiovascular illness.

Author Contributions: Conceptualization, P.K.P., and D.P.; methodology, P.K.P., R.N., and S.F.A.; software, R.S., P.G.M., and S.F.A.; validation, P.K.P. and A.V.B.; formal analysis, P.K.P., S.K.K., and P.G.M.; investigation, P.K.P., D.P., and R.N.K.; resources, R.N.K., D.P., and P.K.P.; data curation, R.S., P.G.M., A.V.B., and R.N.; writing—original draft preparation, P.K.P., D.P., and S.K.K.; writing—review and editing, S.F.A.; visualization, D.P. and P.K.P.; supervision, P.K.P. and S.F.A.; project administration, S.F.A.; funding acquisition, S.F.A. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: We analyzed existing, publicly available data. The accession numbers for the datasets are listed in the key resources table. Any additional information required to reanalyze the data reported in this paper is available from the lead author upon request.

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Conflicts of Interest: There are no conflict of interest with other people or organizations that could inappropriately influence or bias the content of the paper.

Abbreviations

| | |
|-------------------------------|---|
| AC | Atherogenic Coefficient |
| ADMET | Absorption, Distribution, Metabolism, Excretion, and Toxicity |
| ATV | Atorvastatin |
| AIP | Atherogenic Index of Plasma |
| AMR | Antimicrobial Resistance |
| ATP | Adenosine Triphosphate |
| CaCO ₂ | Calcium Carbonate |
| CAT | Catalase |
| CK-MB | Creatine Kinase-MB |
| CRR | Cardiac Risk Ratio |
| CYP1A2 | Cytochrome P450 1A2 |
| CYP2C19 | Cytochrome P450 2C19 |
| CYP2C9 | Cytochrome P450 2C9 |
| CYP2D6 | Cytochrome P450 2D6 |
| ECCG | Electrocardiogram |
| GSH | Reduced Glutathione |
| H ₂ O ₂ | Hydrogen Peroxide |
| HBA | Hydrogen Bond Acceptor |

| | |
|------------------|---|
| HBD | Hydrogen Bond Donor |
| HDL | High-Density Lipoprotein |
| HIA | Human Intestinal Absorption |
| HMG-CoA | 3-Hydroxy-3-Methylglutaryl Coenzyme A |
| HR-LC-MS | High-Resolution Liquid Chromatography–Mass Spectrometry |
| iNOS | Inducible Nitric Oxide Synthase |
| ISO | Isoproterenol |
| LD ₅₀ | LD ₅₀ is the abbreviation used for the dose which kills 50% of the test population |
| LDL | Low-Density Lipoprotein |
| logP | Logarithm of the Octanol-Water Partition Coefficient |
| MDA | Malondialdehyde |
| MI | Myocardial Infarction |
| MW | Molecular Weight |
| NCEE | <i>Neolamarckia cadamba</i> Leaf Ethanol Extract |
| PDB | Protein Data Bank |
| ROS | Reactive Oxygen Species |
| SGOT | Serum Glutamic Oxaloacetic Transaminase |
| SOD | Superoxide Dismutase |
| TC | Total Cholesterol |
| TG | Triglycerides |
| TPSA | Topological Polar Surface Area |
| VLDL | Very Low-Density Lipoprotein |

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