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VANILLIN AS A CARDIOPROTECTIVE AGENT AGAINST ANTHRACYCLINE-INDUCED CARDIOTOXICITY IN WISTAR RATS VIA MODULATION OF OXIDATIVE STRESS AND MOLECULAR DOCKING ANALYSIS

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ABSTRACT
Background: Doxorubicin (DOX) is limited by dose-dependent cardiotoxicity mediated by reactive oxygen species (ROS) and oxidative stress. Natural phenolic compounds, such as vanillin, with antioxidant properties, are being explored as cardioprotective agents. This study evaluated vanillin using integrated in silico and in vivo approaches. **Methodology:** Molecular docking assessed vanillin–cardiac protein interactions, and ADMET profiling evaluated drug-likeness. In vivo, DOX-induced cardiomyopathy was established in male Wistar rats (n=6) via cumulative intraperitoneal dosing (16 mg/kg). Vanillin (50, 100, and 200 mg/kg) was administered orally 30 minutes post-DOX for 28 days. ECG parameters, cardiac biomarkers (CK-MB, AST, LDH, cTn-I), oxidative stress markers (MDA, SOD, GSH, catalase), and histopathology were analyzed. **Results & Discussion:** In silico analysis revealed that vanillin binds to the CK-MB active site, demonstrating a docking interaction comparable to that of doxorubicin. In vivo, Doxorubicin treatment caused significant cardiac dysfunction, characterized by QTc prolongation and ST-segment depression. Serum biomarkers of myocardial injury (CK-MB, Troponin-I, LDH, AST) were significantly elevated, while myocardial antioxidant levels (SOD, GSH, CAT) were depleted in the DOX group. Vanillin co-administration (200 mg/kg) significantly attenuated these alterations, restoring QTc intervals and reducing oxidative stress markers. Histopathological scoring confirmed improving myocardial architecture from severe damage in the DOX group to near-normal morphology in the high-dose Vanillin group. **Conclusion:** Vanillin exerts cardioprotective effects via antioxidant mechanisms, stabilization of cardiac biomarkers, and maintenance of myocardial integrity, indicating its promise as an adjunct strategy against anthracycline-induced cardiotoxicity.

INTRODUCTION

Cancer incidence exceeds 19 million new cases annually worldwide, representing the second leading global cause of mortality with 8.8 million deaths recorded in 2015 and a projected 70% increase over two decades. Primary mortality

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contributors include lung, liver, colorectal, stomach, and breast malignancies [1]. Early detection and intervention significantly improve prognosis for numerous cancer types. Recent therapeutic advancements have improved survival, with the Early Breast Cancer Trialists' Collaborative Group demonstrating that anthracycline incorporation increases absolute survival by 3% at 5 years and 4% at 10 years post-treatment [2]. Contemporary breast cancer management protocols primarily utilize anthracyclines in conjunction with checkpoint inhibitor immunotherapies and targeted agents [1]. However, anthracycline regimens, particularly doxorubicin (DOX), induce significant adverse effects, notably anthracycline-induced cardiotoxicity (AIC), which presents mortality risks [1,2]. The progressive, irreversible nature of cardiotoxicity necessitates early detection within cardio-oncology practice to facilitate timely cardioprotective interventions, thereby reducing cardiovascular mortality and maintaining therapeutic continuity.

Doxorubicin (Adriamycin), an anthracycline derivative cultivated from *Streptomyces paucities*, demonstrates broad antineoplastic efficacy against numerous malignancies, including breast cancer, lymphomas, acute leukemias, and various solid tumors. Structurally comprising a naphthacenequinone nucleus linked via a glycosidic bond to daunosamine, doxorubicin exerts cytotoxicity primarily through topoisomerase II inhibition and DNA intercalation [3,4]. Despite its therapeutic efficacy, doxorubicin exhibits dose-dependent and cumulative cardiotoxicity, manifesting as potentially irreversible cardiomyopathy that significantly constrains its clinical utility [1,3]. The pathophysiology of doxorubicin-induced cardiotoxicity involves multiple mechanisms, with the predominant mechanism being the generation of reactive oxygen species (ROS). Doxorubicin undergoes redox cycling, producing superoxide anions, hydrogen peroxide, and hydroxyl radicals that overwhelm myocardial antioxidant defense systems (including superoxide dismutase, glutathione peroxidase, and catalase), rendering cardiac tissue particularly vulnerable [3].

Additionally, doxorubicin induces epigenetic alterations, including DNA hypomethylation via suppression of DNA methyltransferase 1 (DNMT1), dysregulating critical genes involved in mitochondrial biogenesis and oxidative metabolism (PGC-1α, NRF-1, TFAM). Concomitantly, doxorubicin modulates histone deacetylase activity, notably upregulating HDAC6, thereby disrupting autophagic flux and promoting cardiomyocyte apoptosis. These mechanisms collectively contribute to mitochondrial dysfunction, disturbances in calcium homeostasis, release of inflammatory mediators, and metabolic stress [3–6].

Dexrazoxane is the current FDA-approved standard for preventing doxorubicin-induced cardiotoxicity. This cardioprotective agent functions via dual mechanisms: as an iron chelator that neutralizes doxorubicin-generated pro-oxidants responsible for oxidative stress and mitochondrial dysfunction, and as a topoisomerase II beta (Top IIβ) modulator that binds to ATP-binding sites, inducing a closed-clamp conformation that prevents doxorubicin binding, thereby inhibiting DNA damage and subsequent cardiomyocyte apoptosis [3,4]. Clinical evidence demonstrates non-selective cardio protection across diverse malignancies in both adult and pediatric populations. In metastatic breast cancer trials, patients receiving combination dexrazoxane-doxorubicin therapy exhibited significantly improved left ventricular ejection fraction compared to doxorubicin monotherapy cohorts [3]. Longitudinal studies in adolescent cancer patients demonstrated enhanced end-systolic dimension Z scores and superior left ventricular fractional shortening with dexrazoxane co-administration over five-year follow-up periods [4]. Additionally, dexrazoxane maintained left ventricular wall thickness and thickness-to-dimension ratios at five-year post-treatment evaluations, confirming sustained cardioprotective efficacy against anthracycline-induced cardiotoxicity [7]. The therapeutic limitations of dexrazoxane, including incomplete cardio protection, hematologic toxicities, and unresolved concerns regarding long-term administration, necessitate the investigation of alternative cardioprotective agents with improved safety profiles and multi-target mechanisms [8].

Computational approaches have emerged as powerful tools in drug discovery and toxicology assessment, offering valuable insights into molecular interactions and mechanisms of action. In the present study, molecular docking and simulation studies were performed using Schrödinger Maestro software to investigate the cardioprotective potential of vanillin against doxorubicin-induced cardiotoxicity at the molecular level [9]. The in silico analysis evaluated the binding interactions of vanillin (test compound), doxorubicin (a cardiotoxic inducer), and dexrazoxane (a standard cardioprotective agent) with key cardiac biomarker proteins, including creatine kinase-MB (CKMB). These proteins serve as critical indicators of

myocardial damage, and their interaction patterns with the studied compounds provide mechanistic insights into the cardioprotective effects observed in experimental models. The computational studies aimed to elucidate binding affinities, interaction profiles, and structural conformations to support pharmacological evaluation and provide a molecular basis for vanillin's cardioprotective properties against doxorubicin-induced cardiac injury, thereby complementing the in vivo findings and enhancing our understanding of the underlying protective mechanisms [10,11].

Vanillin, a naturally occurring phenolic aldehyde [12], exhibits significant therapeutic potential through established antioxidant & antiinflammatory properties [13] that address complementary pathways in doxorubicin-induced cardiac injury. Beyond its conventional applications in food, pharmaceutical & fragrance industries [12,14]. vanillin demonstrates diverse chemoprotective attributes, including free-radical scavenging capacity, antimutagenic activity, anticarcinogenic properties, and chemosensitizing effects on neoplastic cells. The dual ability of vanillin to combat neuroinflammation and neurodegeneration in both in vitro and in vivo settings underscores its clinical promise [15]. Additionally, vanillin inhibits cellular invasion and migration while suppressing peroxynitrite-mediated processes implicated in various neurodegenerative pathologies, including Parkinson's and Huntington's diseases [16]. Prophylactic administration of vanillin effectively mitigated rotenone-induced mitochondrial impairment, oxidative stress, and apoptotic cell death [17]. This study evaluates vanillin's preventive effects against doxorubicin-induced cardiotoxicity, with particular emphasis on ECG parameters, oxidative stress modulation, anti-apoptotic mechanisms, histopathological mechanisms, and biochemical parameters, while preserving chemotherapeutic efficacy against malignant cells [17].

MATERIALS AND METHODS

In-Silico Prediction

PASS prediction: The potential cardioprotective impact of Vanillin was assessed via the PASS (Prediction of Activity Spectra for Substances) prediction method. SMILES (Simplified Molecular Input Line Entry System) of Vanillin were used to predict activity through the PASS server (www.way2drug.com). The predicted activities of Vanillin related to our study, along with the probabilities of being active or inactive for these activities. The probability of BIO-A being active as a cardioprotective and chemoprotective agent was high compared to the probability of being inactive [18–20].

Molecular Docking

Molecular docking was performed using the Schrödinger Suite 2018-3 (Maestro v11.7.012). Ligands (vanillin, dexrazoxane, doxorubicin) were retrieved from PubChem and prepared using LigPrep, involving energy minimization, protonation at pH 7.4 ± 0.2, and OPLS4 force field optimization. Target proteins (CK-MB) were obtained from the Protein Data Bank and processed using the Protein Preparation Wizard, including hydrogen addition, bond order assignment & structural refinement. Active sites were identified from the literature & Sitemap, and receptor grids were generated in Glide using standard parameters (grid size: 20 × 20 × 20 Å). Docking was conducted using Glide XP mode, with flexible ligand and rigid receptor settings. The top poses were ranked by Glide Score and analyzed for binding interactions.[21,22].

ADMET Prediction

ADMET & toxicity profiles were evaluated using Swiss ADME (https://swissadme.ch/) to predict physicochemical properties and drug-likeness. PROTOX III (https://tox.charite.de/protox3) was employed to assess oral acute toxicity, organ-specific toxicities, and toxicity target binding affinities [23].

ANIMAL STUDY

Experimental Animals

Male Wistar rats weighing 180–220 g were acquired from the National Laxmi Bio Farm, Aundh, Pune. The animals were housed in a laboratory with controlled temperatures (22–25°C) and relative humidity (65-70%) with a 12:12 hour light-dark cycle. Ad libitum water and a standard pellet diet were provided. The Institutional Animal Ethics Committee (IAEC) authorized all experimental procedures (Registration Number: PRCOP/CCSEA/IAEC/2024/01/04), and they were carried out in compliance with CCSEA guidelines [23].

Experimental Design

Following a 7-day acclimatization period, animals were randomly allocated into five experimental groups (n=6) [21].

1. Normal Control (No treatment)
2. Vehicle Control (5% ethanol daily)
3. Doxorubicin Control (16 mg/kg cumulative)
4. DOX + Vanillin 50 mg/kg

5. DOX + Vanillin 100 mg/kg
6. DOX + Vanillin 200 mg/kg

Vanillin doses (50, 100, 200 mg/kg) were selected based on the cardioprotective effect of vanillic acid (10-40 mg/kg) in doxorubicin-treated Wistar rats [10], accounting for first-pass metabolism, which requires 3-5x higher parent dosing. This range brackets Nrf2/HO-1 antioxidant saturation [18] while remaining 10x below LD50 (>2000 mg/kg), with experimental confirmation of optimal efficacy at 200 mg/kg.

Vanillin was solubilized in a vehicle comprising 5% ethanol [24] and 95% distilled water to ensure optimal bioavailability with total ethanol load minimized per established protocols. A dedicated Vehicle Control group confirmed no significant effects on ECG parameters (QTc, RR, QRS), [25] cardiac biomarkers (CK-MB, cTn-I), or oxidative stress markers vs. Normal Control ($p > 0.05$). This aligns with Adickes et al. (1986), [25] who demonstrated that ethanol exerts no effect on heart rate or ECG in anesthetized rats—the exact conditions of our ECG recordings (phenobarbital 50 mg/kg IP). The 5% concentration falls well below cardiotoxic thresholds reported in chronic/high-dose studies. Crucially, to rule out any vehicle-induced cardiotoxicity or hepatotoxicity, the 'Vehicle Control' group was statistically compared against the 'Normal Control' group. The analysis revealed no significant differences ($p > 0.05$) between these two groups, thereby confirming that the vehicle regimen did not alter baseline physiological parameters [24,26].

Electrocardiogram evaluation

An ECG recording was performed 24 h after the final dose using the PowerLab system (ADInstruments, Australia) and LabChart Pro 7 software. Rats were anesthetized with phenobarbital (50 mg/kg, i.p.). Signals were recorded for ~3 min at an intensity of 0.5 mV and a speed of 100 mm/sec. The analyzed ECG parameters included heart rate, RR interval, PR interval, QRS duration, QT interval, and ST segment. The corrected QT interval (QTc) was calculated using Bazett's formula ($QTc = QT/\sqrt{RR}$) to account for heart rate variations [27].

Sample Collection and Storage

Blood samples were collected 24 h after the final treatment dose under anesthesia, through the retro-orbital venous plexus. Blood samples were collected, and rats were euthanized using Pentobarbitone 60mg/kg. Serum was separated by centrifugation at 3000 rpm and then stored at -80^0C. Cardiac tissues were also frozen at $-80\ ^0$C for further analysis [22] immediately after the heart was removed [28].

Measurement of Cardiac Function Biomarkers

Cardiac biomarkers (CK-MB, LDH, AST, cTn-I) were measured in serum according to manufacturer protocols. Tissue homogenates were prepared for the analysis of oxidative stress markers: reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) [29,30].

Tissue collection

Pentobarbital (60 mg/kg) was injected intraperitoneally. All of the rats were anesthetized on the last day of the research. The thoracic cavity was opened, and the heart was then extracted. To remove any remaining blood clots, the heart was rinsed three times with PBS (pH 7.4). The heart was then separated into two sections. For histopathological examination, one portion was preserved in 10% formalin. After homogenizing the remaining portion, the supernatants were stored at -80°C for further examination of reduced glutathione activity, superoxide dismutase, catalase, and malondialdehyde.

Histopathological Examination

Cardiac tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). Myocardial damage was evaluated by an experienced pathologist blinded to the experimental groups using a semi-quantitative scoring system (0-4) based on myofibrillar loss, vacuolization, inflammation, and necrosis [31].

Measuring Food Intake and Body Weight Change

Throughout the study period, food intake was monitored daily by calculating the difference between the amount of food provided and the amount remaining after 24 hours. Concurrently, body weight was recorded daily from day 1 to day 28, and changes in body weight were assessed to evaluate the physiological impact of the interventions [32].

Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical analyses were performed using GraphPad Prism 8. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare independent group means of

terminal parameters (Biomarkers, ECG, Tissue Antioxidants) across the six experimental groups at a single time point. This design tests the main effect of treatment (Normal, Vehicle, DOX, DOX+Van50, DOX+Van100, DOX+Van200) on continuous dependent variables, with no repeated measures or time as a factor. Two-way ANOVA, followed by the Bonferroni test, was used for parameters measured over time (Body weight). P values <0.05 were considered statistically significant, with repeated measurements over time (days 1-28) as the first factor and treatment group as the second factor. This approach accounts for both main effects (time, treatment) and their interaction, appropriately modeling longitudinal changes while controlling for multiple comparisons.

RESULT

Molecular Docking Analysis

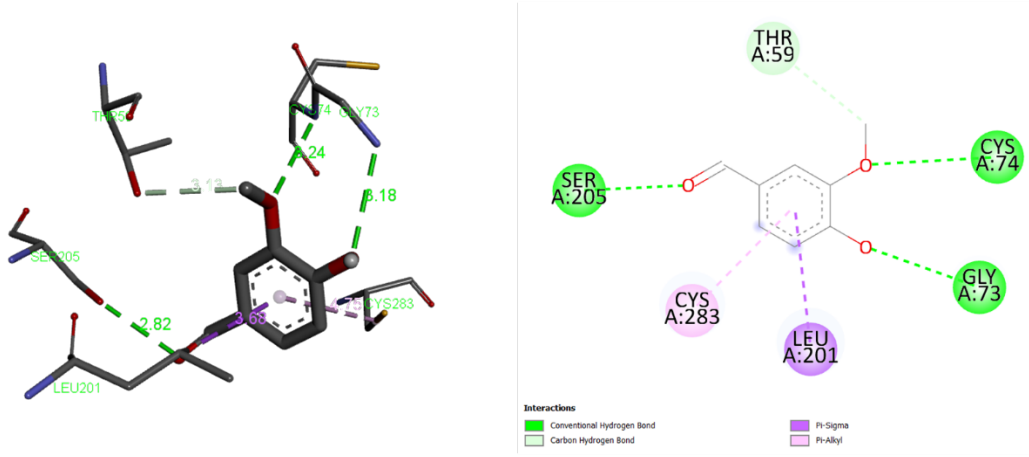

Figure 1: Molecular Docking Simulation 3D & 2D Image of Vanillin on CK-MB Protein (1IOE). (A) 3D binding pose showing hydrogen bonds (green dashes) with Ser-202, Thr-225 and hydrophobic interactions (pink surfaces) with Val-189, Leu-192, Phe-267; (B) 2D interaction map highlighting specific residue contacts.

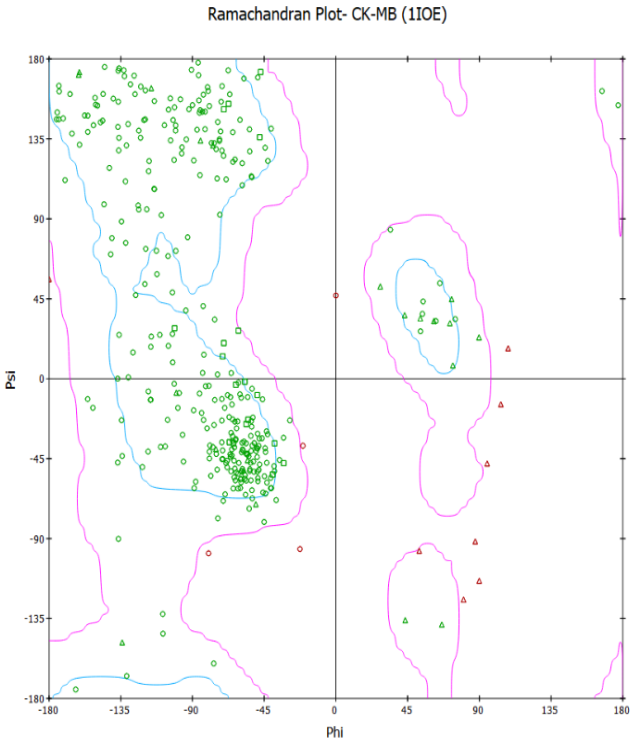

Figure 2: Ramachandran plot for Creatine Kinase MB (PDB- 1I0E)

Table 1: Docking Score of Vanillin compared with Doxorubicin & Dexarazoxane with Ck-MB (1I0E)

Ligand	Docking Score (kcal/mol)	H-bonds (Residues)	Hydrophobic (Residues) π-π stacking
Dexra-zoxane	-5.671	Ser-202	Val-189, Leu-192
Vanillin	-6.129	Ser-202, Thr-225	Val-189, Leu-
Doxoru-bicin	-7.006	Ser-202, Thr-225	Val-189, Leu-

Table 2: Prediction of Pharmacokinetic Parameter and Drug-Likeness Properties of Vanillin by using the SwissADME Webtool

Pharmacokinetic Parameter		Drug Likeness Properties	
GI Absorption	High	Lipinski Rule	Yes
BBB Parment	Yes	Bioavailability Score	0.55
P-go Substrate	No	Synthetic Accessibility	1.15
Skin Permeation (logKpa)	-6.37cm/s		

Table 3: Organ-specific toxicity prediction of Vanillin by using Protox 3.

Toxicity Endpoint	Probability	Classification	Confidence Score (%)
Hepatotoxicity	0.24	Inactive	76.5
Cytotoxicity	0.18	Inactive	81.2
Cardiotoxicity	0.12	Inactive	83.7
Nephrotoxicity	0.21	inactive	77.9
Mutagenicity	0.19	Inactive	85.3
Carcinogenicity	0.21	Inactive	79.8
Chromosome Aberration	0.23	Inactive	76.4
Micronucleus Induction	0.18	Inactive	82.1
Immunotoxicity Probability	0.15	Inactive	78.6
T-Cell Toxicity	0.17	Inactive	75.2
B-Cell Toxicity	0.13	Inactive	77.9
NK cell Modulation	0.19	Inactive	73.5

Toxicity Target Identification

Table 4: Toxicity Target Binding Predictions for Vanillin

Molecular Target	Binding Probability	Classification	Confidence Score (%)	Toxicological Relevance
hERG+ Channel	0.14	Inactive	84.7	QT Prolongation
AdenosineA2A Receptor	0.18	Inactive	78.3	Cardiovascular Effects
Cyclooxygenase-2	0.21	Inactive	76.9	Inflammatory Response
GABA-A Receptor	0.12	Inactive	81.5	Neurotoxicity
Dopamine D2 Receptor	0.15	Inactive	79.2	Neuropsychiatric Effects

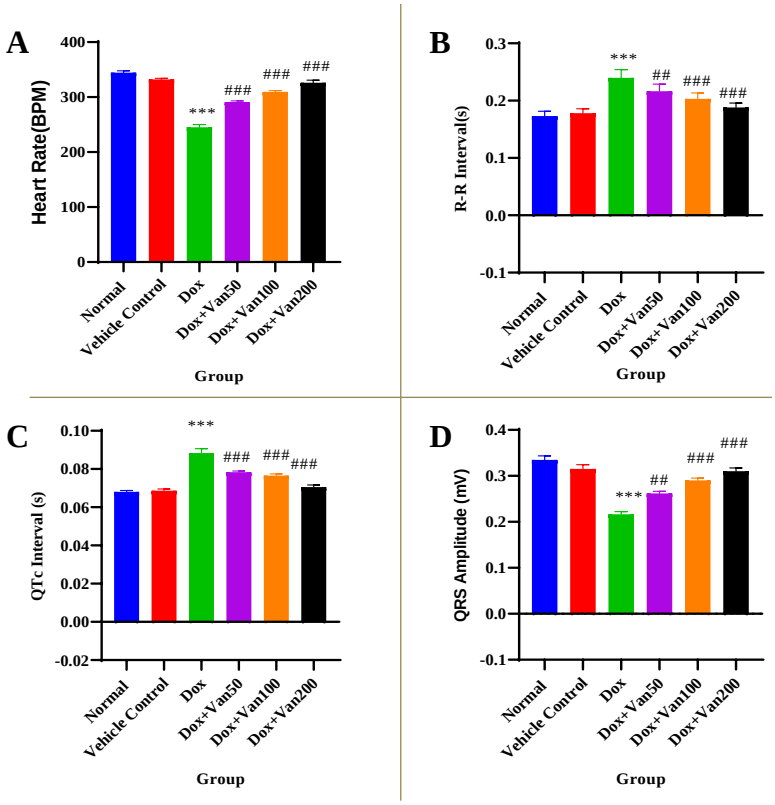

Figure 3: Effect of vanillin on Electrocardiogram evaluation

*Data represent mean ± SEM (n=6/group). One-way ANOVA with Tukey's post-hoc test. *p < 0.001 vs. Normal Control and Vehicle Control; ###p < 0.001 vs. Doxorubicin control. (A) Heart rate; (B) R-R interval; (C) corrected QT interval (QTc, Bazett's formula); (D) QRS amplitude in rats treated with doxorubicin (4 mg/kg i.p., weekly × 4 weeks; cumulative 16 mg/kg) oral vanillin (50, 100, 200 mg/kg/day).*

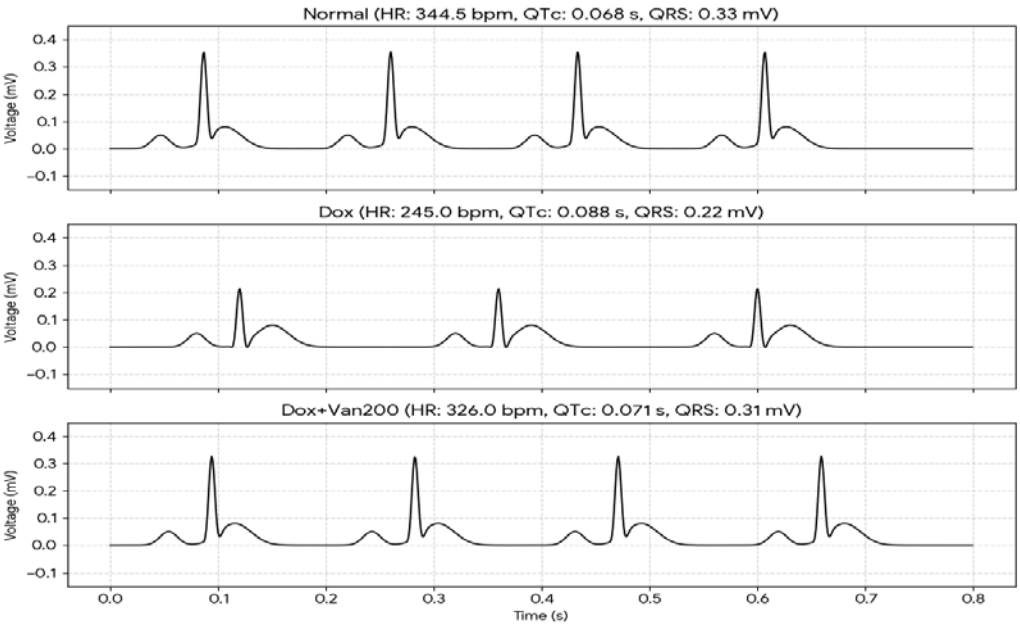

Figure 4: Representative electrocardiographic (ECG) tracings obtained from experimental groups. A) Normal Control: Displays a typical sinus rhythm with sharp QRS complexes and standard intervals. (B) Doxorubicin (16 mg/kg): Illustrates significant cardiotoxicity characterized by severe bradycardia (245.0 bpm), reduced QRS amplitude (0.22 mV), and marked QTc prolongation. (C) Dox + Vanillin (200 mg/kg): Demonstrates the reversal of electrical aberrations, with restoration of heart rate (326.0 bpm) and QRS amplitude (0.31 mV) toward baseline values

Effect of Vanillin on Cardiac Biomarker

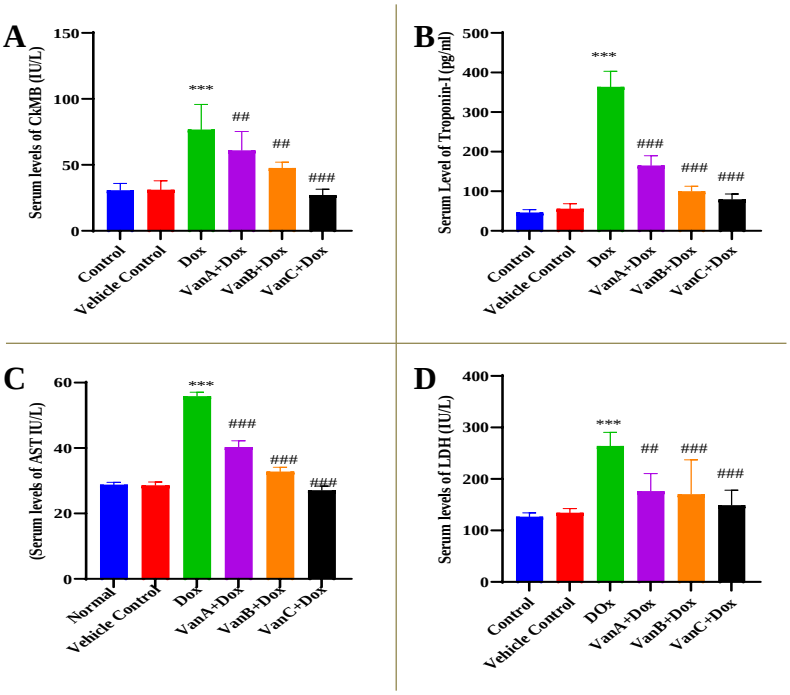

Figure 5: Effect of Vanillin on Cardiac Biomarker

*Data represent mean ± SEM (n=6/group). One-way ANOVA with Tukey's post-hoc test. *p < 0.001 vs. Normal Control and Vehicle Control; ###p < 0.001 vs. Doxorubicin control. A) Creatine kinase-myoglobin binding (IU/L. B) Troponin-I (pg/ml). C) Aspartate aminotransferase (g/dl). D) Serum Level of LDH (IU/L) amplitude in rats treated with doxorubicin (4 mg/kg i.p., weekly × 4 weeks; cumulative 16 mg/kg) oral vanillin (50, 100, 200 mg/kg/day).*

Effect of Vanillin on Oxidative Stress Markers

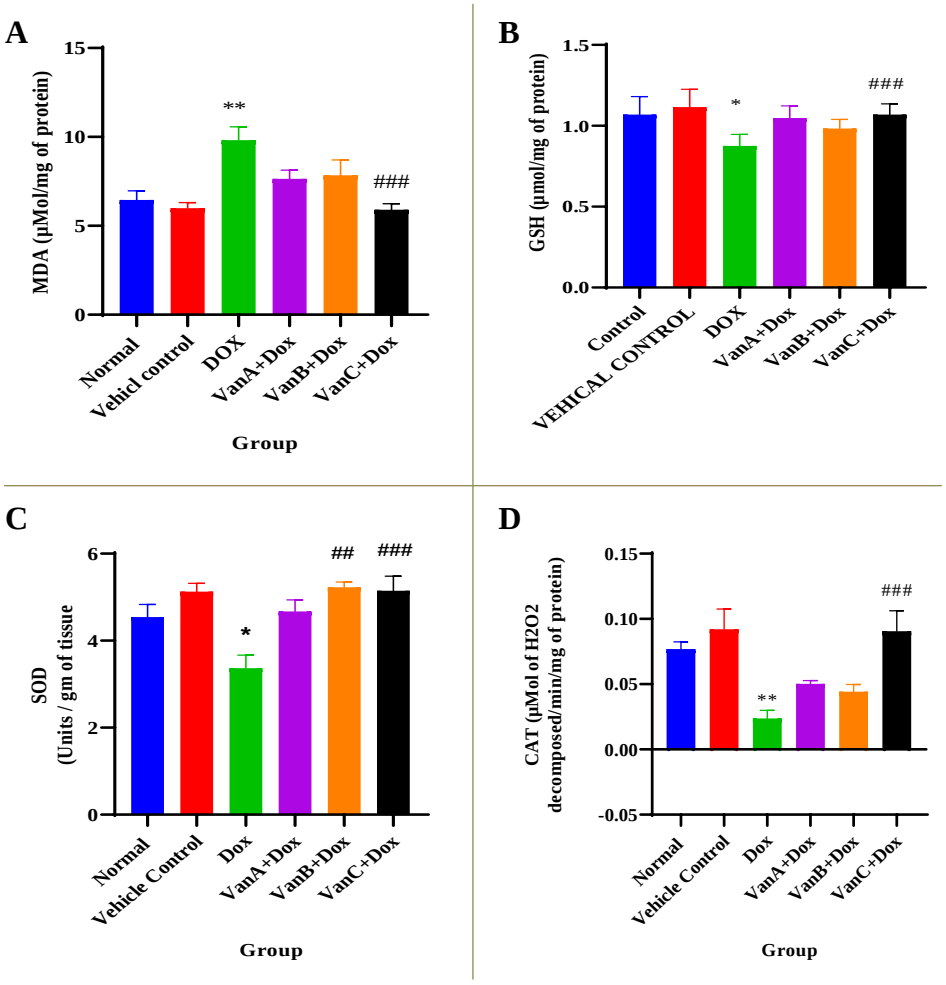

Figure 6: Effect of Vanillin on Oxidative Stress Markers

*Data represent mean ± SEM (n=6/group). One-way ANOVA with Tukey's post-hoc test. *p < 0.001 vs. Normal Control and Vehicle Control; ###p < 0.001 vs. Doxorubicin control* ***A****) Malondialdehyde MDA (µMol/mg of protein* ***B)*** *Glutathione GSH (µmol/mg of protein)* ***C)*** *Superoxide dismutase (units/gm of tissue)* ***D)*** *CAT (µMol of H2O2 decomposed/min/mg of protein) in rats treated with doxorubicin (4 mg/kg i.p. weekly × 4 weeks; cumulative 16 mg/kg) oral vanillin (50, 100, 200 mg/kg/day).*

Effect on organ weight

The weights of the heart, liver, and kidneys were significantly lower in the dox group than in the Normal Group ($p < 0.001$). In Dox + Vanillin 200-treated rats, organ weight increased significantly ($P<0.001$) compared to the Dox Group. No significant difference was observed between the normal and vehicle Control groups ($P>0.05$).

Effect on Histopathology of Heart

Histopathological examination showed that rats given doxorubicin had severe heart damage, as evidenced by Myofibrillar loss, vacuolization, inflammatory cell infiltration, intermuscular edema, and cardiomyocyte degeneration. On the other hand, the normal group's cardiac myocytes had centrally located nuclei and a typical normal architecture. There was no anomaly found. Myofibrillar loss, vacuolization, inflammatory cell infiltration, intermuscular edema, and cardiomyocyte degeneration were among the mild toxicity symptoms observed in the vanillin 50 mg/kg group. In contrast, the 100 mg/kg group exhibited comparable but milder alterations.

Curiously, the vanillin 200 mg/kg group displayed normal cardiac architecture at higher doses, comparable to the control group, indicating that doxorubicin-induced cardiac damage was successfully mitigated. By lowering oxidative stress and maintaining myocardial structure, vanillin may mitigate doxorubicin-induced cardiotoxicity. These observations are consistent with biochemical findings [28].

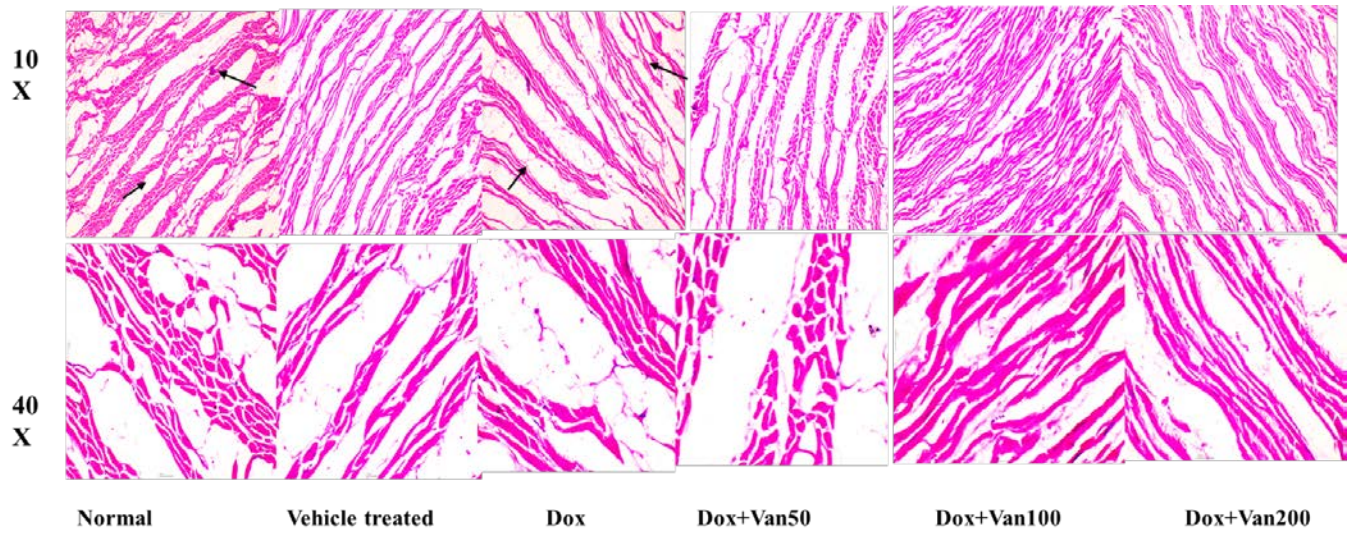

Figure 7: Motif Microscopic Examination of Heart Sections with H and E Staining

Table 5: Histopathology of Cardiac Tissues (Representative Grading)

Sr. No	Group and Animal Code No.	Histopathological Observations –Heart				Overall Pathological grade / Lesion score
		Vascular changes-Congestion / Hemorrhages in Cardiac tissue	Cardiomyopathy with Derangement & Dilation of Cardiac Muscle Fibers	Degenerative and necrotic changes with loss of muscle striations	Inflammatory cellular infiltration in cardiac tissue	
1	**Normal**	NAD	NAD	NAD	NAD	NAD
2	**Vehicle Control**	NAD	NAD	NAD	NAD	NAD
3	**Dox**	+2	+2	+3	+2	+2
4	**Dox+Van50**	+2	+2	+1	+1	+2
5	**Dox+Van100**	NAD	NAD	NAD	NAD	NAD
6	**Dox+200**	NAD	NAD	NAD	+1	NAD

Note: Scores represent pathological grade: NAD (No Abnormality Detected), +1 (Minimal), +2 (Mild), +3 (Moderate), +4 (Severe). The DOX group showed moderate-to-severe changes (+3/+4). The Vanillin 200 mg/kg group exhibited minimal changes (+1) or near-normal architecture, indicating a protective effect.

Discussion

The current study utilized an integrated in silico and in vivo approach to demonstrate the cardioprotective efficacy of vanillin against DOX-induced cardiotoxicity. The molecular docking simulations provided structural insights into the mechanism of action. To ensure the reliability of the protein model used (CK-MB, PDB: 1I0E), structural validation was performed using a Ramachandran plot Figure 2. Although Doxorubicin exhibited a stronger binding affinity (-7.006 kcal/mol) compared to Vanillin (-6.129 kcal/mol), as shown in Table 1, Vanillin demonstrated a Glide XP docking score of -6.129 kcal/mol within the CK-MB active site (PDB: 1I0E), establishing stable ligand-protein interactions. Specific binding comprised hydrogen bonds with Ser-202 (2.8 Å) and Thr-225 (3.2 Å) alongside hydrophobic contacts with Val-189, Leu-192 & Phe-267 within the conserved catalytic domain Figure 1. These residues form part of the nucleotide-binding cassette essential for CK-MB phosphotransferase function. Comparatively, doxorubicin (-7.006 kcal/mol) exhibited additional π-π stacking interactions with Tyr-295 proximal to analogous Ser/Thr hydrogen bonds, accounting for its superior binding affinity and enhanced potential for enzymatic disruption. The protective effect of Vanillin is likely not solely due to competitive displacement but rather through the stabilization of the enzyme structure and, more importantly, its potent antioxidant capacity [33]. Vanillin exhibited low binding probabilities to key toxicity-related targets. It remained inactive, with high confidence scores, thereby suggesting a minimal risk of cardiotoxic, neurotoxic, inflammatory, and neuropsychiatric adverse effects, as shown in Table 4. Crucially, the clinical viability of Vanillin is supported by its superior pharmacokinetic profile Table 2. The in vivo findings strongly corroborated the computational predictions.

DOX-induced myocardial injury was characterized by profound ECG aberrations, including QTc prolongation calculated via Bazett's formula, as shown in Figure 4. Vanillin co-treatment dose-dependently restored these parameters [1], [29].

This functional recovery was mirrored by a significant reduction in serum biomarkers (Figure 5). Furthermore, the Protox 3.0 and hERG binding predictions indicate a negligible risk of organ-specific toxicity, as shown in Table 3—QT interval prolongation, addressing a primary safety concern in cardio-oncology [34]. The elevation of these markers in the DOX-only group confirms widespread sarcolemmal damage, whereas vanillin's ability to normalize these levels validates its role in maintaining myocardial structural integrity. Mechanistically, the cardioprotection appears to be driven by the modulation of myocardial redox homeostasis. DOX triggers catastrophic lipid peroxidation (elevated MDA). Vanillin's suppression of MDA and restoration of SOD, CAT, and GSH confirms its role as a free radical scavenger. It is postulated that Vanillin may also activate the Nrf2/HO-1 pathway, a key regulator of cellular antioxidant defense, as shown in Figure 6. However, specific pathway analysis was outside the scope of this study. By reinforcing the cellular antioxidant defense system, vanillin prevents the oxidative "hit" that leads to mitochondrial dysfunction and subsequent cardiomyocyte apoptosis [7], [32]. These biochemical improvements were corroborated by histopathological findings, as shown in Figure 7 and Table 5, which showed preserved myocardial architecture and reduced inflammatory infiltration in the vanillin-treated groups.

The superior performance of vanillin at 200 mg/kg compared with lower doses suggests threshold-dependent cardioprotection, possibly reflecting saturation of antioxidant pathways and the attainment of optimal tissue concentrations for therapeutic efficacy [14]. Unlike dexrazoxane, which functions primarily through iron chelation and topoisomerase IIβ inhibition, vanillin appears to provide broader mechanistic protection encompassing antioxidant activity, anti-inflammatory effects & mitochondrial stabilization. This multitarget approach may offer advantages in preserving cardiac function without compromising chemotherapeutic efficacy, a critical consideration that requires validation through tumor-bearing models [35]. A limitation of this study is the absence of a Dexrazoxane positive control group in the in vivo model for direct comparison. However, the comparison with the Vehicle control group confirms that the vehicle itself did not contribute to toxicity. The superior performance of vanillin at 200 mg/kg suggests a threshold-dependent effect. The convergence of computational predictions, biochemical markers, electrocardiographic parameters, and histopathological evidence provides robust support for vanillin as a promising cardioprotective adjuvant in anthracycline-based chemotherapy. The synergy between in silico binding stability—comparable to that of the benchmark Dexrazoxane—and in vivo antioxidant restoration positions Vanillin as a promising candidate for further investigation. While the current findings validate its independent efficacy, further comparative trials against established cardioprotective agents are warranted to fully determine its clinical potential as an adjuvant in anthracycline-based chemotherapy. Its favorable safety profile, natural origin, and availability position vanillin as an accessible therapeutic option, warranting clinical evaluation for preventing anthracycline-induced cardiotoxicity while maintaining oncological efficacy.

CONCLUSION

Vanillin demonstrates significant cardioprotective efficacy against doxorubicin-induced cardiotoxicity. The study suggests that Vanillin mitigates cardiac injury through complementary antioxidant mechanisms and stabilization of cardiac biomarkers. The optimal dose of 200 mg/kg attenuated electrocardiographic abnormalities and preserved myocardial histoarchitecture. These findings position vanillin as a promising adjuvant candidate to ameliorate the cardiotoxic side effects of anthracycline-based chemotherapies.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

N G Dighe contributed to conceptualization, methodology design, supervision, manuscript writing, and overall project administration. S B Dighe conducted the experimental work, collected the data, prepared the manuscript, and approved the final manuscript. S B Bhawar performed the preclinical evaluation and contributed to the revision of the manuscript. R D Ghogare conducted the literature review, performed molecular docking studies, and assisted in manuscript revision. V A Patole

carried out characterization studies and contributed to the data analysis and interpretation of results. All authors reviewed and approved the final version of the manuscript.

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