Original Article

Role of *Chlorophytum Borivilianum* extract against Doxorubicin- induced Myocardial Toxicity in Albino Rats: *Insilico* and *Invivo* studies

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ABSTRACT

The doxorubicin, an anthracycline derivative, is a cytotoxic agent with proven efficacy in various malignancies. The clinical utility has been limited due to its dose - dependent cardiac toxicity. To evaluate the role of *Chlorophytum borivilianum* L. on doxorubicin-induced cardiotoxicity in rats and to predict the role of *Chlorophytum borivilianum* L. by insilico and in vivo methods. Invitro studies were conducted on *Chlorophytum borivilianum* L. Cardiotoxicity was produced by administration of doxorubicin (Dox-15 mg/kg *ip*. for two weeks). Ethanolic extract and fractions of *Chlorophytum borivilianum* L. (250 and 500 mg/kg, p.o.) were administered as pretreatment for 15 days followed by Doxorubicin 2.5 mg/kg i.p. on alternate day for two weeks. The parameters like body weight, food and water consumption, cardiac specific markers like Creatine Kinase (CK-MB), Lactate Dehydrogenase (LDH) and Cardiac Troponin-I (cTnl), ECG changes, antioxidant parameters like superoxide dismutase (SOD), glutathione (GSH), catalase (CAT) and lipid peroxidation (MDA) were monitored. Histopathological studies of the heart were also performed to evaluate myocardial toxicity. Dox treatment results in cardiomyopathy characterised by elevated cardiac biomarkers and deficiency of antioxidant enzymes. By reducing the elevated levels of biomarker enzymes like LDH and CK-MB and the absence of cTnI, pretreatment with the EECB (500mg/kg) significantly protected the myocardium from the toxic effects of Dox. In addition, the EECB increased the reduced levels of GSH, SOD, and CAT while decreasing the elevated levels of malondialdehyde (MDA) in cardiac tissue.

Keywords: *Chlorophytum borivilianum*. L, Doxorubicin, Ethanolic extract, Rats

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1. Introduction

Cancer is the leading cause of morbidity and mortality in developed nations, accounting for approximately Ten million deaths in the year 2020. Cancers include breast, lung, colon, rectum, prostate, skin, and stomach cancer. The most common cancer is cervical cancer, which is the most common one in 23 countries (1,2). Depending on the type and stage of the disease, treatments for cancer might vary in intensity and have adverse consequences. Heart failure and left ventricular dysfunction are more likely to occur as a result of traditional chemotherapy and treatment methods (3). The Anthracycline Antibiotic Doxorubicin (DOX) is the most frequently prescribed drug for treatment of various malignancies. However,Its harmful effect on cardiomyocytes is dose-dependent, which is of great concern (3). Antioxidant treatment has been reported to be helpful in the management of cardiotoxicity in previous investigations as a defence mechanism against DOX - mediated cardiotoxicity (4). *Chlorophytum borivilianum* (Liliaceae) is a geophyte with a strong traditional history and has a wide range of pharmacological properties, including antimicrobial, analgesic, anti-inflammatory, antipyretic, hepatoprotective, antioxidant, hypolipidemic, antistress, antiarthritic, antidiabetic, aphrodisiac, immunomodulatory, antiulcer, anticancer, anthelmintic, and larvicidal activities. Network Pharmacology (NP) is an emerging field that combines genomic approaches with systems biology using computational biology tools to support drug discovery (5). It aims to understand the complex relationships between biological systems, drugs, and diseases and to identify potential mechanisms of complex biomaterials through the analysis of massive dataset (6). NP analysis aims to uncover novel therapeutic options while improving the safety and efficacy of existing drugs (7). Molecular docking is an important tool in drug development, as it assesses the affinity of small molecule to bind to protein targets, such as receptors, enzymes, and transporters (8). *Chlorophytum borivilianum* L. tubers have been shown to have antioxidant activity and there is a paucity of information on their cardioprotective effects, making them suitable for *insilico* and *invivo* studies.

2. Materials and Methods

2.1 Network Pharmacology Analysis

2.1.1 Mining of phytoconstituents and proteins involved in CVS

The phytoconstituents of *Chlorophytum borivilianum* have been discovered in the scientific literature and traditional medicine books. The database has been created taking into account the phytoconstituents, their types, SMILES, and PubChem ID. Duplicate phytoconstituents were eliminated when the database was created. The PubChem database provided the canonical SMILES and PubChem ID for each phytoconstituent. In the Binding DB, SMILES were consulted to predict the target. (with 70% similarity to recognized ligand compounds). Using the known targets of CVSlisted in the Therapeutic Target Database (TTD), the proteins associated with diabetes were found. (Each protein molecule was identified as the gene ID of the CVSs target and was obtained from the UniProt database (9).

2.1.2. Prediction of drug likeness and ADMET Profile

MolSoft (http://www.molsoft.com/) was used to forecast phytoconstituents for the drug likeness score using the "Lipinski's Rule of Five" model. AdmetSAR 2.0 is comparable.

2.1.3. Pathway and Network Analysis

Using STRING, a set of proteins associated with DM was examined and gene enrichment analysis was performed to determine the pathways affected byphytoconstituents. To find the pathways involved in CVSs, the KEGG pathway analysis was also performed (https://www.genome.jp/kegg/). Cytoscape 3.5.1 was used to build the network connecting protein molecules, identified pathways, and phytoconstituents (10). The whole network, which depends on the number of edges (edge count), was interpreted using the colour and node size scale. The huge node symbol denoted the node with the highest edge count.

2.1.4. Docking Analyses

Using the MMFF94 force field, the three-dimensional structure of stigmasterol was extracted from the PubChem database. The alpha fold protein database and RCSB (https://www.rcsb.org) were used to find the target molecule, the PRKCA receptor. Discovery Studio was used to remove heteroatoms and water molecules from the protein molecule by using AutoDock4.0 was used to predict the binding affinity of stigmasterol with the PRKCA (Protein Kinase C Alpha) receptor. After docking, the pose with the highest binding energy was selected to visualize the ligand-protein interaction.

2.2. In vitro experimental validation

2.2.1. Plant material

The whole plant of *Chlorophytum borivilianum* L were procured and authenticated in the month of November from KLE College of Pharmacy, Hubballi, Dharwad district in Karnataka, India. Identified and authenticated by S. N. Emmi H.O.D of Botany Department. of H.S. Kotambari Science Institute Vidyanagar, Hubballi. (Ref No. KLECOPH/2022-23).

2.2.2. Preparation ethanolic extract.

The dried roots have been crushed into small fragments and powdered. The solid extract was prepared by cold maceration the root powder (1000 g) in 2 L of sterile

distilled water for 48 hours at room temperature. The mixture was then filtered into a clean round bottom flask using Whatmann Millipore No. 1 filter paper, concentrated to dry using a rotary evaporator at 50±5°C, and lyophilized using a freeze-drying system (11).

2.2.3. Chemicals and drugs

Doxorubicin was purchased from a local dealer and other analytical grade chemicals and enzyme assay kits were purchased from Sigma and enzyme assay kits were purchased from ERBA.

2.2.4. Animals

Wistar rats weighing between 150-200 g were used for the study after obtaining the ethical clearance from the Institutional Animal Ethical Committee (Application No:Mph/NC0221003/KLECoPH/22). All the rats were separately housed separately in polyethylene cages, maintained under standard conditions, and fed with standard rat pellet diet and water *adlibitum*. Before the initiation of the experiment, the rats were acclimatized to the laboratory conditions for seven days.

2.2.5. Preliminary Phytochemical Screening

The ethanolic extract of *Chlorophytum borivilianum* L was subjected to preliminary phytochemical investigations such as alkaloids, calcium, vitamins, proteins, phenol, resins, magnesium, mucilage, polysaccharides and also contains a high quantity of simple sugars, mainly sucrose, glucose, fructose, galactose, mannose, xylose, stigma sterol and saponin.

2.2.6. Acute Oral Toxicity

Acute oral toxicity studies were selected based on previous literature (11).

2.2.7. Experimental design

Group I received vehicle 5 ml/kg body weight *p.o.*

The group II was treated with doxorubicin 2.5 mg/kg body weight *i.p.* in 6 equal injections on alternate days for two weeks.

Group III and group IV received a low dose (250 mg/kg body weight p.o.) of EECB (LEECB) and a high dose (500 mg/kg body weight p.o.) EECB (HEECB), respectively, for two weeks as a pre-treatment followed by doxorubicin as in group II.

Group V received vitamin E as a standard drug (Std) 100 mg/kg body weight *p.o.* for two weeks as a pretreatment followed by doxorubicin as in group II.

2.2.8. Food, water and ECG

Food and water consumption were monitored regularly ,and an ECG (Biopac M35) was performed before and after the treatment.

2.2.9. Enzyme assays

Blood was collected from the retro-orbital plexus under mild ether anaesthesia 36 hours after the final dose of trearment, for measurement of biomarkers such as lactate dehydrogenase (LDH), creatinine phosphokinase-MB(CK-MB), and cardiac troponin-I (cTnI) by a one-step Troponin I test. Animals were killed by isoflurane and a midline incision was made, followed by the dissection of the heart tissue. The cardiac tissue was washed in ice-cold saline, dried by filter paper and weighed immediately. Hearts were removed from all the rats and a 10% w/v homogenate was prepared in 0.9% buffered KCl at pH 7.4 for the estimation of endogenous antioxidants such as glutathione (GSH), melonldehyde (MDA), superoxide dismutase (SOD) and catalase (CAT). The remaining part of the heart was used for histopathological studies.

2.2.10. Histopathological studies

Heart tissue slices were fixed in 10% formalin, and specimens were processed and embedded in paraffin according to protocol. The ventricular area was sectioned and stained using the hematoxylin and eosin technique.

2.2.11. Statistical analysis

One-way analysis of variance (ANOVA) was used to statistically analyze the experimental data, and Dunnett's multiple comparison test was performed using Graphpad Prism 5.0. Data were presented asmean, SEM and P.

3. Results

3.1. Identification of phytoconstituents and proteins involved in CVSs

Eight different phytoconstituents were identified in *Chlorophytum borivilianum* L according to + DLS; through edge count 5 of them were predicted to modulate the CVSs protein molecules (Table 1). These phytoconstituents were identified as steroids, saponin, ester, similarly, the majority of the targeted CVSs protein molecules were surface proteins and enzymes (figure 1).

3.2. Drug Likeness of Compounds

All the eight phytoconstituents were predicted for their drug likeness score; the highest score was obtained by stigma sterol (Table 2).

3.2.1. Pathway and network analysis:

Gene set enrichment analysis revealed182 different pathways regulated by CVS proteins. Peer interpretation of protein interactions using the KEGG pathway analysis revealed ten distinct pathways that are directly involved in the pathophysiology of CVS. The calcium signalling pathway interaction was found to have the largest number of gene sets and the lowest false discovery rate (Table 3). The twenty-five targets include six phytoconstituents. Stigma Sterol had the highest number of edges ; contact was detected with twenty-three protein molecules. i.e., CACNA1B, PRKCD, RXFP1, PDGFRB, MTOR, GIRA2, CFTR, AXL, TLR4, STAT3, PLAT, NFE2LE, GRIN1, CAMK2D, PRKCB, PIK3CA, PDGFRA,

Table 1. Types of compounds and their targets

Compound	Compound Type	PubChem ID	Target Protein
Stigmasterol	Steroid	5280794	CACNA1B, PRKCD, RXFP1, PDGFRB, MTOR, GIRA2, CFTR, AXL, TLR4, STAT3, PLAT, NFE2LE, GRIN1, CAMK2D, PRKCB, PIK3CA, PDGFRA, PIK3CD, PIK3CB, NOS3, NFKB1, PRKCA, PIK3R1
Butyl isopropyl ester	Saponin	66984153	CACNA1B, PRKCD, RXFP1, PDGFRB, MTOR, GIRA2, CFTR, AXL, TLR4, STAT3, PLAT, NFE2LE, GRIN1, CAMK2D, PIK3CA, PDGFRA, PIK3CD, PIK3CB, NFKB1, PRKCA, PIK3R1, ADRB1,
Pthalic acid.	Ester	6423815	GRIN1, NFE2L2, PLAT, STAT3, TLR4, AXL, CFTR, GRIA2, PRKCD, RXFP1, CACNA1B, PIK3R1, PRKCA, NFKB1, PIK3CD, PDGFRA,
Camphor	Terpenoid ketones	2537	CACNA1B, PRKCD, MTOR, CFTR, TLR4, STAT3, PLAT, NFE2LE, CAMK2D, PRKCB, PDGFRA, NOS3, NFKB1, PRKCA, PIK3R1,
Eicosapentaenoic acid	FATTY ACID	151156	RXFP1, GIRA2, TLR4, PLAT, NFE2LE, GRIN1, PDGFRA, PIK3CD, PIK3CB, NOS3NFKB1, PIK3R1, MAP2K2

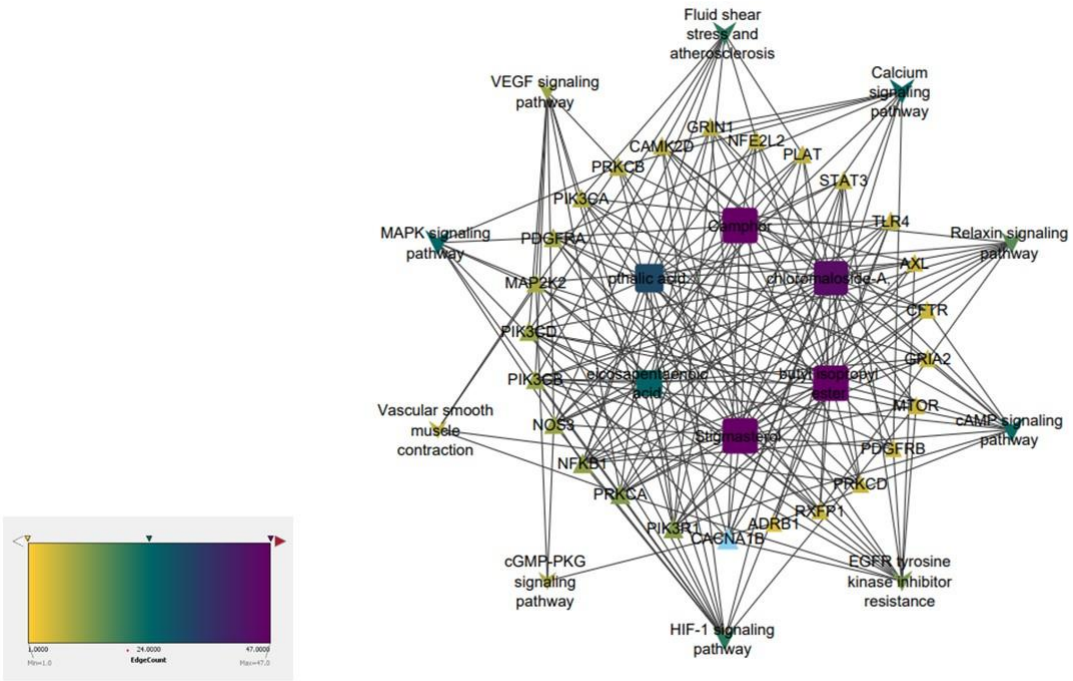

Figure 1. Network representation of the interaction between phytoconstituents, targets and pathways

Table 2. Drug likeness properties of phytoconstituents

PHYTOCHEMICALS	PUBCHEM ID	MF	MW	HBA	HBD	LOG P	DLS
Stigmasterol	5280794	C29 H48 O	412.37	1	1	7.74 (> 5)	0.62
Chloromaloside-A,	151156	C50 H80 O23	1048.51 (> 500)	23 (> 10)	12 (> 5)	-0.61	0.36
Camphor	2537	C10 H16 O	152.12	1	0	2.34	0.11
Eicosapentaenoic acid	446284	C20 H30 O2	302.22	2	1	6.01 (> 5)	0.11
Linolenic acid	5280934	C18 H30 O2	278.22	2	1	5.88 (> 5)	0.09
Pthalic acid.	6423815	C20 H30 O4	334.21	4	0	6.21 (> 5)	0.05
Hecogenin	91453	C27 H42 O4	430.31	4	1	5.31 (> 5)	0.04
Butyl isopropyl ester	66984153	C15 H26 O4	270.18	4	0	4.14	0.01

MW- Molecular weight, NHBA- Number of Hydrogen Bond acceptor number, NHBD-Hydrogen Bond Donor numbers, DLS- Drug likeness Score

Table 3. Gene Set Enrichment Analysis of Proteins Involved in CVSs

Description	Observed Gene Count	False Discovery Rate	matching proteins in your network (labels)
HIF-1 signaling pathway	20	2.08E-13	SERPINE1, NFKB1, MAP2K2, PIK3CA, STAT3, FLT1, PIK3CB, NOS3, PRKCB, NOS2, CAMK2D, MTOR, TLR4, PIK3CD, LDHB, PRKCA, SLC2A1, PIK3R1, HIF1A, LDHA
Calcium signaling pathway	24	1.34E-12	CD38, PDGFRA, PDGFRB, HTR2C, CYSLTR2, PTGER1, NOS3, ADORA2B, PRKCB, CHRM1, SPHK1, NOS2, P2RX7, CACNA1H, HTR7, CAMK2D, ADRB1, CACNA1B, GRIN1, TACR2, CHRM5, PTK2B, CXCR4, PRKCA
Fluid shear stress and atherosclerosis	20	4.08E-12	KEAP1, MMP2, PLAT, NFKB1, PIK3CA, PIK3CB, NOS3, HSP90AA1, CTSL, PRKAA1, MAP3K5, CHUK, HSP90AB1, MMP9, PIK3CD, NFE2L2, GSTP1, MAPK9, PIK3R1, ITGB3
cAMP signaling pathway	23	2.49E-11	CFTR, NFKB1, GHSR, PTGER2, MAP2K2, PIK3CA, SLC9A1, PDE3B, PIK3CB, GRIA2, CHRM1, CAMK2D, PDE3A, DRD2, ADORA1, ADRB1, GRIN1, PIK3CD, HCAR2, PPARA, MAPK9, PIK3R1, FFAR2
Ras signaling pathway	22	4.75E-10	NFKB1, PDGFRA, PDGFRB, MAP2K2, PIK3CA, ZAP70, FLT1, PIK3CB, PRKCB, TBK1, HTR7, PTPN11, CHUK, GRIN1, ABL1, PIK3CD, GRB2, PLA2G2A, MAPK9, PRKCA, PIK3R1, PAK4
MAPK signaling pathway	24	9.68E-10	DUSP3, NFKB1, PDGFRA, TAOK1, PDGFRB, RPS6KA6, MAP2K2, FLT1, PRKCB, CACNA1H, TGFBR2, MAP3K5, CHUK, CACNA1B, TAOK3, GRB2, MAP3K1, MAPK9, PRKCA, STK3, RPS6KA1, NR4A1, RPS6KA5, MAP3K14
Relaxing signaling pathway	16	5.82E-09	MMP2, NFKB1, MAP2K2, PIK3CA, PIK3CB, NOS3, MMP1, NOS2, TGFBR2, MMP9, PIK3CD, GRB2, MAPK9, RXFP1, PRKCA, PIK3R1
EGFR tyrosine kinase inhibitor resistance	13	1.01E-08	PDGFRA, PDGFRB, MAP2K2, PIK3CA, STAT3, PIK3CB, AXL, PRKCB, MTOR, PIK3CD, GRB2, PRKCA, PIK3R1
VEGF signaling pathway	10	3.18E-07	MAP2K2, PIK3CA, PIK3CB, NOS3, PRKCB, SPHK1, PTGS2, PIK3CD, PRKCA, PIK3R1
Renin-angiotensin system	4	0.0018	CTSG, LNPEP, ANPEP, PRCP
cGMP-PKG signaling pathway	8	0.0096	OPRD1, MAP2K2, PDE3B, NOS3, PDE3A, PIK3CG, ADORA1, ADRB1
Vascular smooth muscle contraction	7	0.0117	MAP2K2, PRKCQ, ADORA2B, PRKCB, PRKCD, PLA2G2A, PRKCA

PIK3CD, PIK3CB, NOS3, NFKB1, PRKCA, PIK3R1. Similarly, calcium signaling pathway interaction modulated the highest number of protein molecules i.e, PDGFRB, ADRB1, GRIN1, CAMKD, NOS3, PRKCA, PRKCB, PDGFRA, CNA1B. Stigma Sterol mainly modulated proteins involved in calcium signaling pathway interaction route i.e.,CACNA1B, PRKCD, RXFP1, PDGFRB, MTOR, GIRA2, CFTR, AXL, TLR4, STAT3, PLAT, NFE2LE, GRIN1, CAMK2D, PRKCB, PIK3CA, PDGFRA, PIK3CD, PIK3CB, NOS3, NFKB1, PRKCA, PIK3R1. (figure 1). Proteins from calcium signaling pathway interaction pathway were also modulated by other phytoconstituents to show the synergistic effect for CVSs activity. Five targets were selected based on network pharmacology analysis and edge count. The Ramchandran plot was then used to perform the Ramchandran analysis.

3.3. Docking studies

The binding affinity and inhibitory constant of PRKCA (Protein Kinase C Alpha) receptor were found to be – 8.8 kcal/mol. One hydrogen bond interactions was found in ligand-protein complex i.e., PROA:112 with "H" atom. (figue 2).

3.4. Ramachandran plots

The Ramachandran plot was used to evaluate the accuracy of predicted protein structure. The Ramachandran plot predicts the structural stereo chemical property. The PROCHECK analyzes the overall model geometry with the residue-by-residue geometry and provides the stereo chemical quality of a predicted model. The PROCHECK tool requires a modeled protein file as an input and generates the Ramachandran plot. It shows that for the given protein 93.241% of the residues belong to the most favored regions, additionally allowed regions, generously allowed regions, and disallowed regions, respectively. It is expected that no more than 2% of the residues should belong to the allowed region and no residue should be in the disallowed or outlier region. For this protein, nine residues, that is, ASP 3(A), ASN 8(A), GLU 322(A), ASP323(A), ASP 481 (A), VAL 16(A), GLU157(A), ARG 462(A)ALA 7(A),belong to generously allowed regions and disallowed regions. These residues can be remodeled or the energy be minimized to produce a model with better stereochemical properties. According to the Ramachandran plot, all five proteins (PIK3CB, PIK3R1, PRKCA, NFKB1, NOS3) passed the Ramachandran analysis with protein quality above 90%. In particular PRKCA showed the highest binding affinity compared to the remaining proteins. (figure 3).

3.5. Phytochemical constituents in EECB

The preliminary phytochemical investigation revealed the presence of carbohydrates, steroids, triterpenoids, glycosides, saponins, flavonoids, alkaloids, tannins, and phenolic compounds.

3.6. Acute oral toxicity

The dose of the plant extract and the doses for doxorubicin were selected based on previous literature. Thus, the maximum nonlethal dose was set up at more than 5000 mg/kg. Therefore, 1/10 (higher dose) and 1/20" (lower dose) of 5000 mg/kg dose were selected for experimental study. (i.e. 500 mg/kg and 250 mg/kg, respectively).

3.7. General observations

Animals treated with doxorubicin developed rough fur;these rats also had soft, watery faeces and crimson exudates around the eyes. In addition, necrosis was seen at the site of doxorubicin. At the end of the the study period, these problems were worse. However, animals treated with experienced fewer changes.

3.8. Effect of ethanolic extract of *Chlorophytum borivilianum* L. on body weight

Figure 4 showed that the body weight was gradually decreased in doxorubicin treated group as compared to control (Control vs Dox, $p<0.001$). Treatment with HEECB and Vit-E showed significant increase in body weight and weight gain as compared to doxorubicin treated group body (HEEC+Dox, $p<0.01$; Vit-E+Dox, $p<0.001$). But treatment with LEECB group failed to significantly increase body weight and body weight gain.

3.9. Effect of ethanolic extract of *Chlorophytum borivilianum* L. on food and water consumption

In the doxorubicin group, food and water intake was significantly reduced compared to the control group (Control vs Dox, $p<0.001$). In treatment groups i.e. LEECB, HEECB and Vit-E showed significant improvement in food intake (LEECB+Dox, $p<0.05$; HEECB+Dox, $p<0.01$; Vit-E+Dox, $p<0.01$) and water intake (LEECB+Dox, $p<0.05$; HEECB+Dox, $p<0.01$; Vit-E+Dox, $p<0.001$) as compared to doxorubicin treated group (figure 5).

3.10. Effect of ethanolic extract of *Chlorophytum borivilianum* L. on specific cardiac markers

Doxorubicin Treatment causes an increase in the levels of LDH, CK-MB, and enzymes as compared to the control group (Control vs Dox, $p<0.001$). As the pretreated groups i.e. HEECB and Vit-E showed significantly decreased levels of LDH enzyme (HEECB+Dox, $p<0.01$; Vit-E+Dox, $p<0.001$), CK-MB enzyme (LEECB+Dox, $p<0.05$; HEECB+Dox, $p<0.001$; Vit-E+Dox, $p<0.001$) as compared to doxorubicin treated group respectively. Cardiac troponin I (cTnl) presence was found out by using a one -step rapid test. Doxorubicin -treated rats showed the presence of troponin I, which is cardiac specific biomarker in cardiotoxicity. The pretreated groups i.e. LEECB (moderate), HEECB, and Vit-E showed the absence of troponin as compared to doxorubicin treated group (Figure 6)

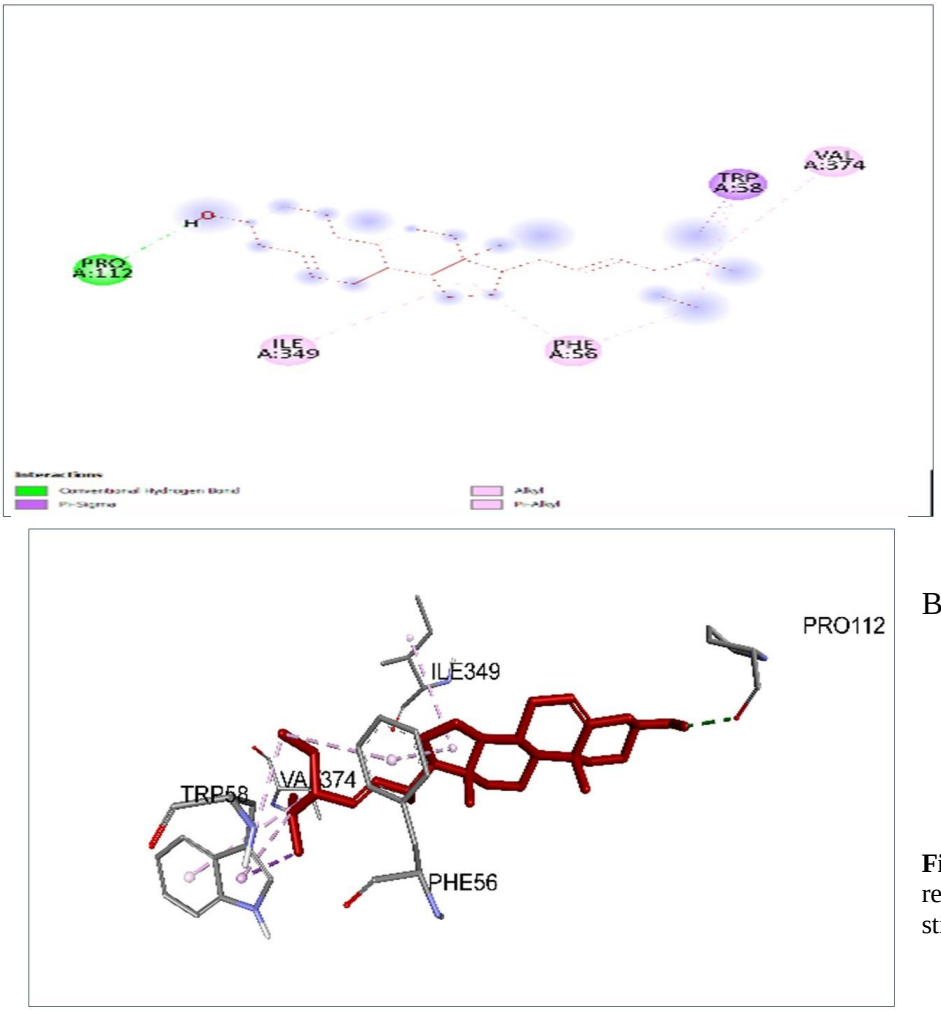

Figure 2: Interaction of stigma sterol with PRKCA receptor. (a) 3D poses of stigmasterol (b) 2D plot of stigmasterol with PRKCA receptor interaction

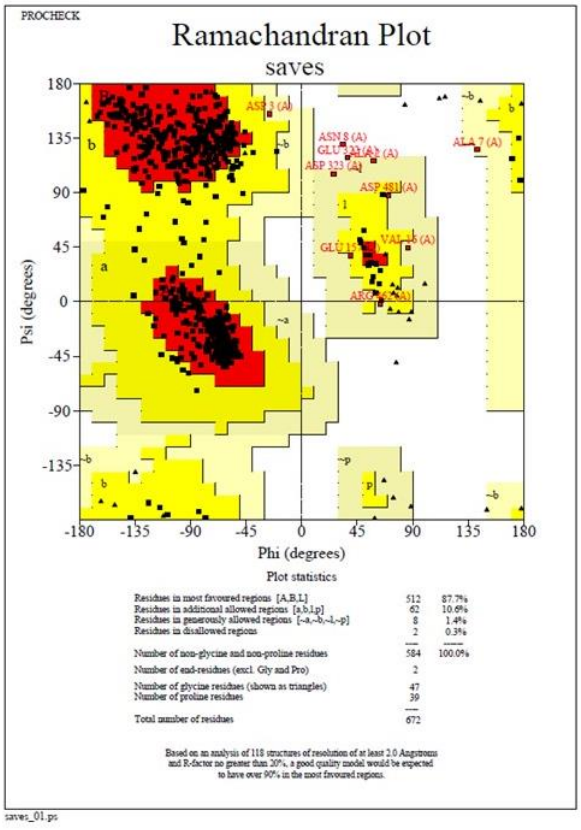

Figure 3: Protein kinase C Alpha Ramchandran plot

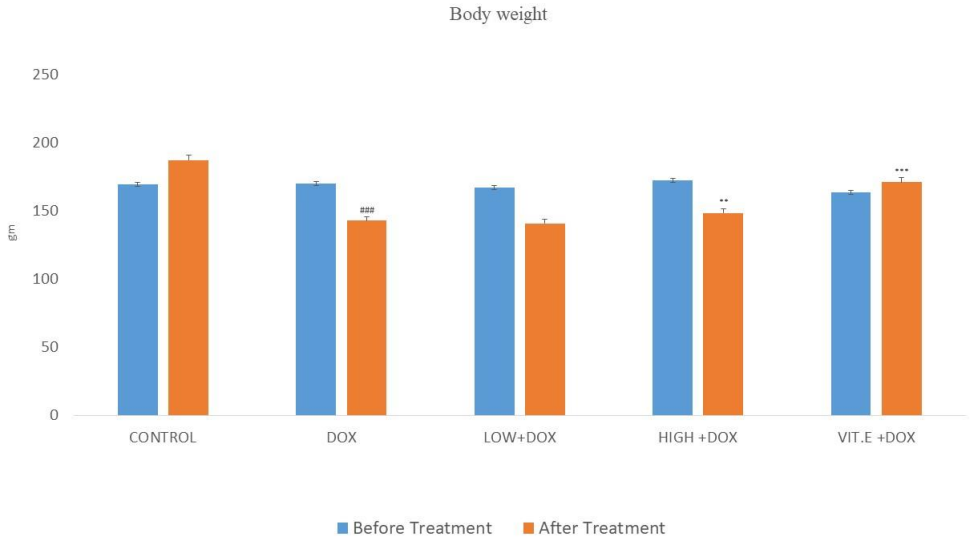

Figure 4. Effect of ethanolic extract of *Chlorophytum borivilianum* L. on body weight

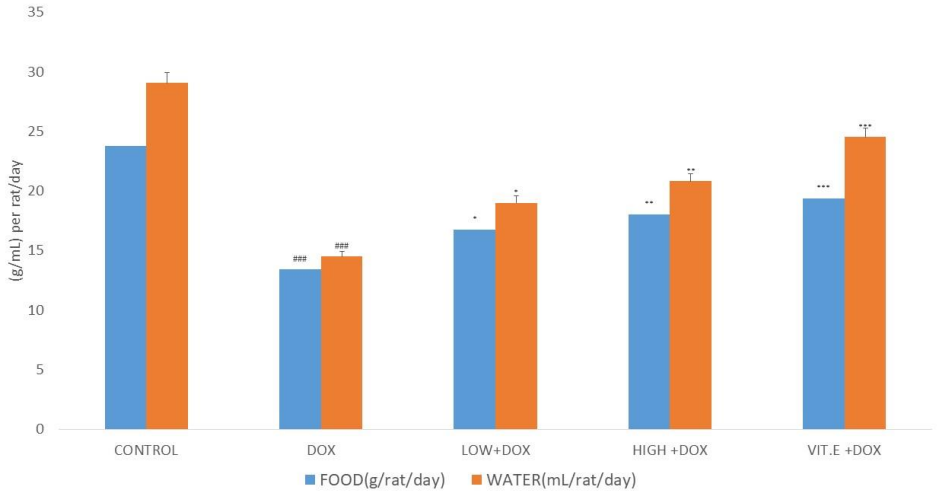

Figure 5. Effect of ethanolic extract of *Chlorophytum borivilianum* L. on food and water intake

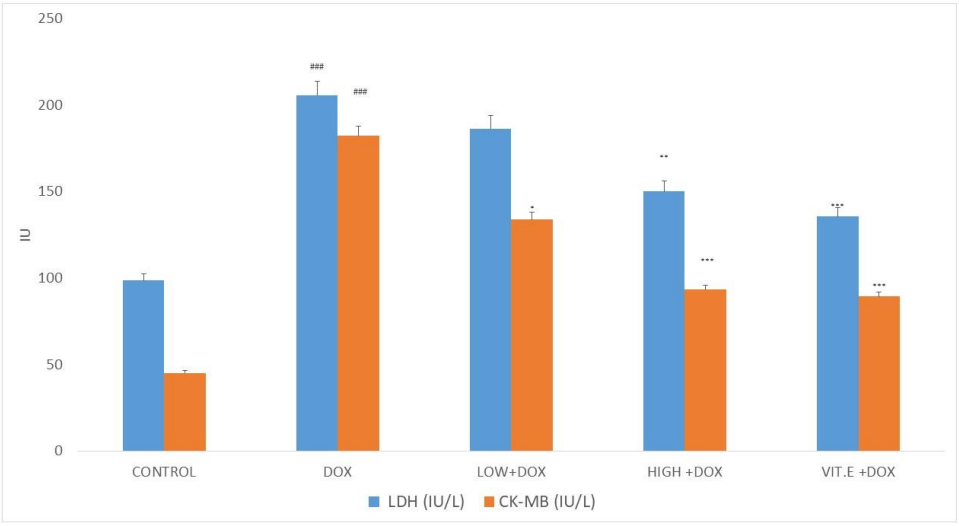

Figure 6. Effect of Ethanolic Extract of *Chlorophytum borivilianum* L. on specific cardiac markers

3.11. Effect of ethanolic extract of *Chlorophytum borivilianum* L on ECG parameters

Doxorubicin induced rats increased the QT intervals and as compared to control rats (p<0.001), and decreased the R-wave amplitude and Heart rate as compared to control rats (p<0.001, p<0.001). Treated groups showed decrease in QT interval as compared to doxorubicin group (p<0.05), and increased the R wave amplitude and Heart rate as compared to control rats (p<0.01, p<0.05) (figure 7).

3.12. Effect of ethanolic extract of *Chlorophytum Borivilianum* on heart to body weight ratio

Heart weight to body weight ratio in doxorubicin treated rats were significantly increased as compared to control group (Control vs Dox, p< 0.001), In pretreated groups i.e. HEECB and Vit-E showed significant decrease in the heart weight to body weight ratio as compared to doxorubicin treated group (HEECB + Dox , p< 0.01 Vit- E + Dox, p< 0.001) (figure7)

3.13. Effect of Ethanolic Extract of *Chlorophytum borivilianum* L.on antioxidant enzyme levels in heart

Doxorubicin treated rats showed decrease in GSH, SOD and CAT levels as compared to control group (Control vs Dox, p<0.001). Whereas pre-treatment with LEECB, HEERC and Vit-E increased the GSH (HEECB+Dox, p<0.01; Vit-E+Dox, p<0.001), SOD (LEERB+Dox, p<0.05; HEERB+Dox, p<0.01; Vit-E+Dox, p<0.001) and CAT (HEERB+Dox, p<0.01; Vit-E+Dox, p<0.001) levels as compared to doxorubicin respectively. Doxorubicin treated rats showed an increase in MDA levels as compared to control group (Control vs Dox, p<0.001). Whereas pre-treatment with HEECB and Vit-E decreased the MDA level (HEECB+Dox, p<0.01; Vit-E+Dox, p<0.001) as compared to Dox (figure 8).

3.14. Histopathological Studies

Figure 9 showed that both Doxorubicin and low-dose treated rat heart tissue showed severe cardiomyocyte degeneration, intermuscular edema, inflammatory cell infiltration, and vacuolization. In contrast, the normal group showed normal morphological appearance, while the high-dose group showed mild cardiomyocyte degeneration, intermuscular edema, inflammatory cell infiltration, and vacuolization. In addition, the Vit.E group showed mild cardiomyocyte degeneration, intermuscular edema, and inflammatory cell infiltration, with normal vacuolization.

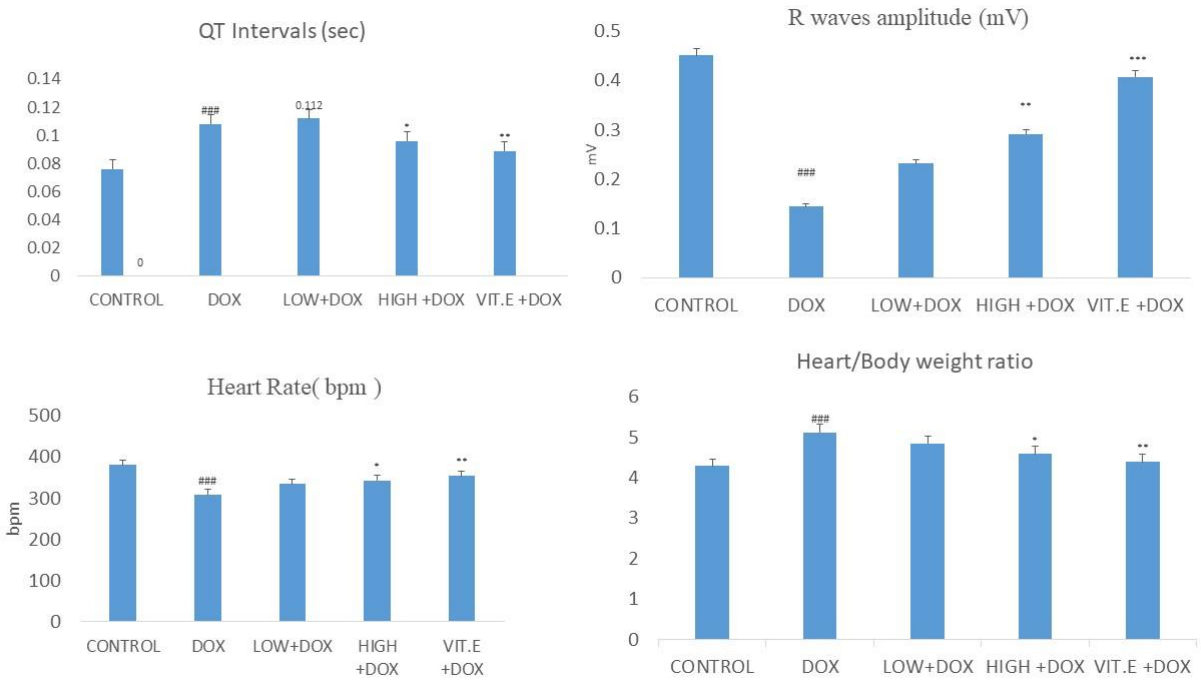

Figure 7. Effect of Ethanolic Extract of *Chlorophytum borivilianum* L on ECG parameters

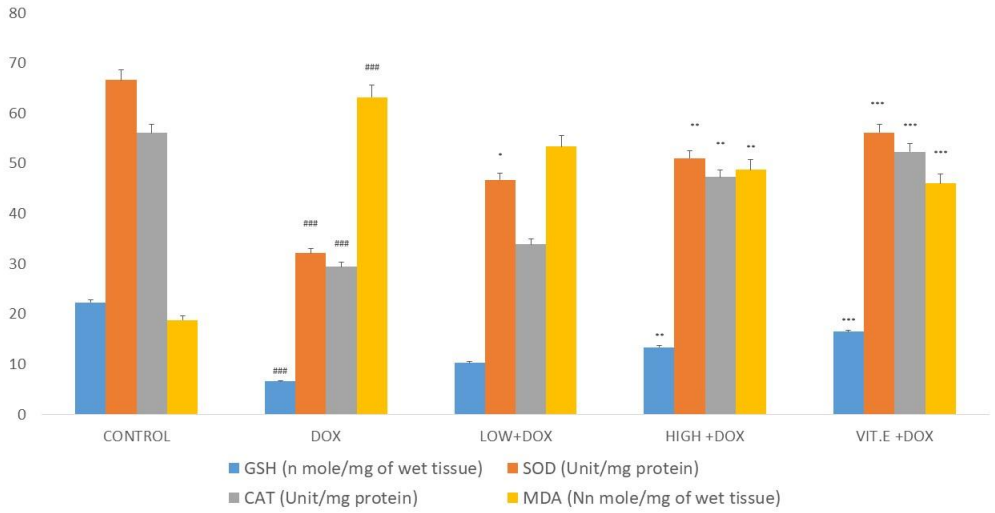

Figure 8. Effect of Ethanolic Extract of *Chlorophytum Borivilianum* on Heart to Body Weight Ratio

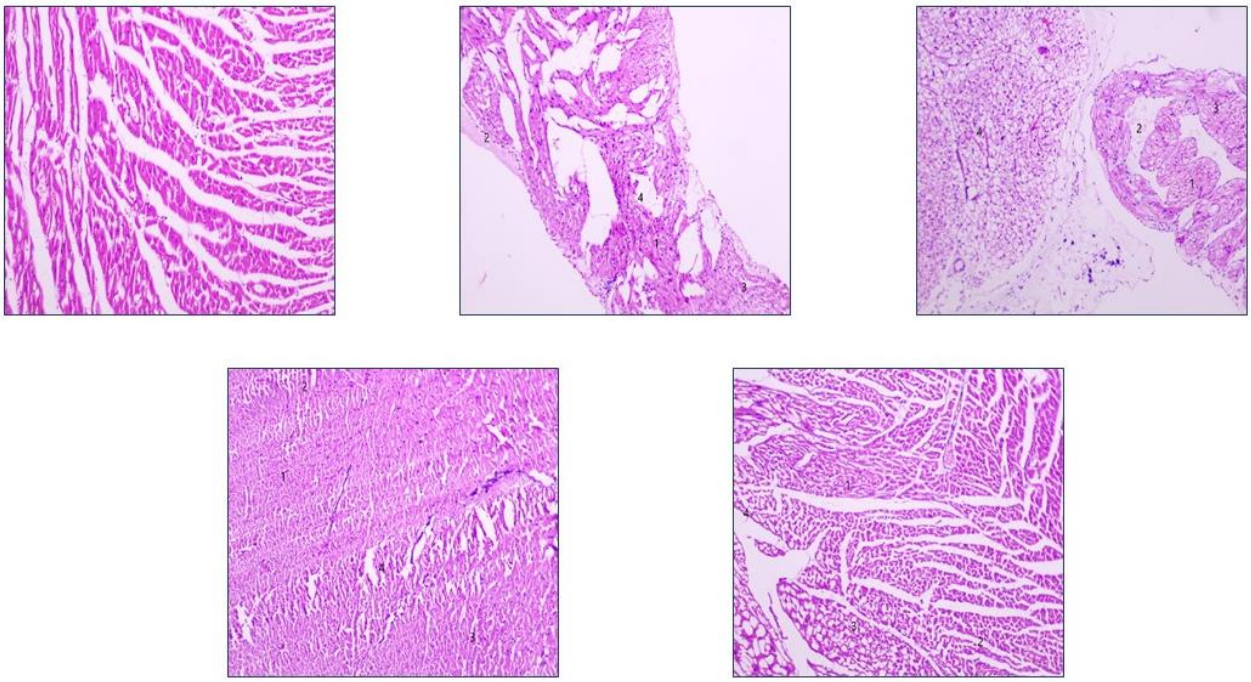

Fig 4:Histopathological studies by hematoxylin and eosin staining. (All the figures were captured under 40 x magnifications) (a) Photomicrograph of normal heart (b) Doxorubicin treated group showing sever cardiomyocyte degeneration, intermuscular edema, inflammatory cell infiltration, and vacuolization. (c) LEEBD+Dox treated group sever cardiomyocyte degeneration, and moderate intermuscular edema, inflammatory cell infiltration, and vacuolization. (d) HEEBD+Dox treated group shoes the mild cardiomyocyte degeneration, intermuscular edema, inflammatory cell infiltration, and vacuolization and(e) Std+Dox group respectively showing mild cardiomyocyte degeneration, intermuscular edema, inflammatory cell infiltration, and normal vacuolization

Figure 9. Histopathological Studies of the Heart

4. Discussion

An accepted strategy is to investigate traditional drugs for the treatment of complicated disorders such as CVS by using network pharmacology. Numerous attempts have been made to understand the potential molecular mechanisms of *Chlorophytum borivilianum* L in the treatment of CVS by using a pharmacological approach to study the molecular processes of traditional remedies. We built a network that included the interactions between potential routes, targets, and phytoconstituents. The findings suggest that steroids, saponins, esters, and carbohydrates may interact with several protein molecules involved in the etiology of CVS as possible phytoconstituents. One of these, stigmasterol, a steroid, may play a role in the pharmacotherapy of CVS by focusing on the network's many protein molecules. The steroid was investigated as a prominent phytoconstituent in the *L.* by Gulab Singh Thaku et al (12). Were involved in regulating the various pathogenic protein molecules associated with CVS. KEGG pathway analysis indicates the important role of *Chlorophytum borivilianum* L. in management of CVS by modulating 10 major pathways, i.e., cAMP signaling pathway, MAPK signalling pathway, Calcium signaling pathway, vascular smooth muscle contraction Signaling pathway, cGMP-PKG Signaling pathway, HIF-1 Signaling pathway, EGFR tyrosine kinase inhibitor resistance Signalling pathway, Relaxin signaling pathway, VEGF signaling pathway, fluid shear stress and atherosclerosis. We have identified 6 compounds that modulate 5 protein targets associated with CVS, i.e., PIK3R1, PRKCA, NFKB1, NOS3, PIK3CB. The PI3K pathway, including PIK3R1, is involved in various biological activities, including cell growth, proliferation, survival, and metabolism. It is associated with cardiovascular processes and disorders, such as cardiac hypertrophy and pathological ventricular hypertrophy, which are associated with various cardiovascular diseases (13-15). PRKCA, also known as Protein Kinase C Alpha, is critical for signal transduction, cell division, proliferation, and apoptosis, and is being studied in the cardiovascular system for physiological and pathological functions. PRKCA plays a critical role in cardiac hypertrophy, which is the expansion and thickening of the heart muscles. It controls excitation-contraction coupling and contractile properties and changesion channels and proteins. PRKCA also mediates ischemic injury by limiting blood supplyand plays a role in smooth muscle contraction (16). NFK-B1, a transcription factor, controls inflammatory and immunological responses and is involved in cell proliferation, differentiation, apoptosis, and immunological responses (17). Endothelial nitric oxide synthase (NOS3) is a critical component of the cardiovascular system, producing nitric oxide (NO) for various physiological processes in blood vessels. Endocrine cellsin blood arteries express NOS3, which stimulates vasodilation, prevents clotting, has anti-inflammatory properties, promotes angiogenesis, and controls blood pressure. Deficiency or low production of NOS3 can aggravate cardiovascular disorders such as endothelial dysfunction, atherosclerosis, hypertension, and decreased blood flow (18, 19). PIK3CB is a critical protein in the cardiovascular system that controls vascular tone, angiogenesis, and cardiac contractility. It is expressed in smooth muscle cells, cardiomyocytes, and endothelial cells and mediates hormones and growth factors. Abnormal PIK3CB signaling can lead to heart failure, and proper control is essential for normal function (20). Doxorubicin, one of the most potent cytotoxic agents, has demonstrated efficacy in a number of malignant cancers. Due to its acute and long-term cardiotoxicity, its therapeutic use is limited. This has been attributed to free radical production and lipid peroxidation. Over time and in a dose-dependent manner, doxorubicin accumulates in cardiac tissue and eventually leads to ventricular dysfunction (21). The current study investigated the effect of the ethanolic extract of *Chlorophytum borivilianum* L on doxorubicin-induced cardiotoxicity. The current study showed that after receiving doxorubicin, rats developed alopecia, scruffy fur, a pink tint, crimson exudates around the eyes and nose, and necrosis at the injection site. These changes were less pronounced in the vitamin E-treated groups, explaining the substance's potent cell-protective properties with an anti-inflammatory, antioxidant, and anti-fibrotic effects (22, 23). The doxorubicin-treated group had decreased food and drink intake, resulting in decreased body weight and an increase in heart weight, as measured by the heart weight to body weight ratio. This could be caused by swollen mitochondria or dilated, enlarged, and

hypertrophied atrium and ventricles (24). However, none of these changes were observed in the pre-treated groups. The research also demonstrated the biochemical changes and oxidative damage that occurred in the heart tissue as a result of the cumulative injection of 15 mg/kg body weight of doxorubicin. Myocardial cells were destroyed, releasing CK-MB, LDH, and cTnI into the bloodstream, which served as diagnostic indicators of cardiac injury (25). The levels of these enzymes in the blood reflect any changes in the integrity and/or permeability of the plasma. The diagnostic markers, which indicate the degree of doxorubicin-induced cardiac injury and are consistent with other observations, were significantly elevated in the doxorubicin-treated rats (26). Pre-treatment with EECB showed a significant decrease in the doxorubicin-induced elevation of blood diagnostic markers. This clearly demonstrates that EECB is responsible for of maintaining the architectural integrity of myocytes and normal structural integrity, which limits the leakage of these enzymes and may be explained by its membrane-stabilizing property.The primary indicator of myocardial injury is an abnormal electrocardiogram. The characteristic observations in doxorubicin-treated rats were a prolongation of the QT interval and a reduction in the amplitude of the R wave. These changes are evidence of doxorubicin-induced myocardial injury, consistent with a previous report (27). Pre-treated groups had a protective effect against the altered ECG patterns induced by doxorubicin. In the current study, doxorubicin-treated rats also showed an increase in MDA levels, indicating increased lipid peroxidation, and a decrease in GSH, SOD, and CAT levels. SOD is known to protect against damage by converting O_2- radicals to H_2O_2 and preventing the production of OH radicals by the O_2- driven Fenton reaction (28). H_2O_2 can also be eliminated by CAT. Primarily due to antioxidant capacity, pretreatment with EECB the antioxidant state and prevented cardiac injury. Doxorubicin administration caused severe changes in the heart, including cardiomyocyte degeneration, intermuscular edema, inflammatory cell infiltration, and vacuolization, according to histopathologic studies. The vacuolar changes in myocardial fibers can lead to myocardial tissue deterioration, myofibrillar loss, myocardial hypertrophy, and nuclear fragmentation. . Therefore, Histopathological data revealed that EECB reduced myofibrillar loss and mitochondrial enlargement of mitochondria in doxorubicin-induced cardiac damage. Recent research revealed that *Chlorophytum borivilianum*.L may be able to prevent and cure CVSs through a regulatory network with multiple links, targets, and signaling pathways. Our results should also provide new views and theoretical foundations for the research of the active compounds in *Chlorophytum borivilianum*.L and likely routes in the prevention and treatment of CVSs through the application of pharmacology and molecular docking networks. The results on the tests were conducted in vivo and in vitro were mutually supportive. It is possible to conclude that *Chlorophytum borivilianum* L. showed cardio protective action in doxorubicin-induced cardiotoxicity based on the findings of general appearance, specific cardiac markers, ECG studies, biochemical markers, antioxidant activity, and histopathology research. The presence of stigmasterol, fatty acids, and linolic acid in thr plant might be the source of the cardio protective action.

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Authors' Contribution

Study concept and design: SKN, KN
Acquisition of data: SKN, KN
Analysis and interpretation of data: SKN, KN, NMJ
Drafting of the manuscript: SKN, SBP
Critical revision of the manuscript for important
Intellectual content: SBP, NMJ, NMM
Statistical analysis: SKN, KN
Administrative, technical, and material support: NMJ, NMM.

Ethics

Institutional Animal Ethical Committee (proposal No:Mph/NC0221003/KLECoPH/22).

Conflict of Interest

The authors declare that they have no conflict of interests.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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