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EVALUATION OF FRACTIONS OF CHLOROPHYTUM BORIVILIANUM L. ROOT EXTRACT AGAINST DOXORUBICIN-INDUCED CARDIOTOXICITY IN ALBINO RATS.

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ABSTRACT:

Objective: To Assess the protective effects of various fractions of Chlorophytum borivilianum L. root extract against doxorubicin-induced cardiotoxicity in albino rats. **Methods**: Adult albino rats were divided into seven groups: control, doxorubicin (DOX), DOX + Vit.E, DOX + Petroleum ether CB fraction, ethyl acetate CB fraction, Chloroform CB fraction & aqueous CB fraction. Cardiotoxicity was induced by administering DOX (15 mg/kg, i.p) over a 2-week period. Post-treatment, biochemical markers are (CK-MB, LDH, and cardiac troponin I) &oxidative stress parameters (MDA, SOD, and catalase) & ECG were measured. Histopathological examinations of heart tissues were conducted to assess structural changes. **Results**: Doxorubicin significantly increased serum cardiac markers and oxidative stress levels, indicating cardiotoxicity. Treatment with Chlorophytum borivilianum fractions, particularly the ethyl acetate CB fraction, significantly mitigated these alterations. The ethyl acetate Chlorophytum borivilianum (CB) group exhibited the most notable cardioprotective effects, evidenced by the normalization of biochemical parameters and improved histopathological profiles, compared to DOX group only. **Conclusion:** Chlorophytum borivilianum root fractions, especially its ethyl acetate fraction, demonstrates significant cardioprotective effects against DOX-induced cardiotoxicity. This protective effect is likely mediated through its antioxidant properties, suggesting its potential as a therapeutic agent for managing chemotherapy-induced cardiac damage.

Keywords: Chlorophytum borivilianum (CB), Doxorubicin, Cardiotoxicity, oxidative stress, cardioprotective effects, Vitamin. E.

1. INTRODUCTION

Cardiac condition (CVD) is a broad category that encompasses several kinds of diseases. It is the main cause for mortality in both developed and developing countries. Deep vein thrombosis, rheumatic heart disease, peripheral arterial disease, coronary heart disease (CHD), and pulmonary embolism are a few of them.(1)

The treatment of solid and hematologic malignancies has advanced significantly with the help of chemotherapy, giving many patients hope that their disease may be cured. But there are drawbacks to these treatments as well. the anthracycline class of chemotherapy drugs cardiac toxicity, with an emphasis on the condition's prevalence, diagnosis, management, and prevention .(2)

Doxorubicin is a class of the anthracyclines family and is a secondary metabolite of a mutant strain of Streptomyces paucities var. caesius. For a variety of cancer types, dox is an efficient antineoplastic agent. However, its widely used in cancer treatment is limited by a number of systemic adverse reactions. It is one of the well-known and widely used antineoplastic drugs for treating different cancers such as breast cancer, leukaemia, and paediatric cancer. Dox that damages DNA because it inhibits the DNA topoisomerase II enzyme. (3)

Doxorubicin-induced cardiotoxicity encompasses various biological processes, including oxidative stress, lipid peroxidation, DNA damage, mitochondrial injury, apoptosis, and autophagy. Oxidative stress stands out as a pivotal mechanism in the myocardial damage

caused by DOX. This pertains to the imbalance between reactive oxygen species (ROS), reactive nitrogen species (RNS), and the body's intrinsic antioxidant defences. Cardiomyocytes generate ROS and RNS, which, when not effectively cleared or neutralized by endogenous antioxidant systems, contribute to oxidative stress. Additionally, factors such as mitochondrial dysfunction, activation of the ubiquitin protease system, release of nitric oxide (NO), inhibition of cardiac progenitor cells, calcium dysregulation, decreased adenosine triphosphate (ATP) levels, inflammatory mediators, endothelial dysfunction, and iron regulatory protein dysfunction also contribute to ROS production and subsequent oxidative stress. (2)

Chlorophytum borivilianum Sant and Fernandes belongs to the family Liliaceae. It is an endangered geophyte with a strong traditional history and therapeutic significance, In Hindi, it is commonly called as Safed musli that means “White tubers”, safed musli that grows extensively, and essential part of the Ayurvedic, Unani, Homoeopathic, and Allopathic medical systems, where the plant's root plays a major role. Safed musli is a traditional remedy for a number of male sexual diseases and is regarded as a general health-promoting tonic.(2)

Essential fatty acids (EFA) include linoleic acid and its isomers because linoleate, a derivative of linoleic acid, reduces the synthesis of prostaglandins and can be used to treat a variety of ailments including cardiovascular disease. The prevention of cancer, dermatitis, cystic fibrosis, and hair loss has all been linked to linoleic acid. because of isolated fatty acids may cause inflammation, rheumatism, and cardiovascular disorders in Chlorophytum borivilianum L. tubers.(4)

Ancient Indian study Bhavaprakash Nighantu, Rasendra Sarsangrah, and Raja Ballabh Nighantu have described safed musli as "Vajikaran" or an aphrodisiac, which is a special kind of immunomodulator. A contentious medication, Safed Musli is made from a variety of plants and is used by Indian medical practitioners The pharmacological characteristics of Chlorophytum borivilianum L. also included a broad spectrum of activities such as antimicrobial, analgesic, anti-inflammatory, antipyretic, hepatoprotective, antioxidant, hypolipidemic, antistress, antiarthritic, antidiabetic, aphrodisiac, immunomodulatory, antiulcer, anticancer, anthelmintic, and larvicidal effects.(5)

The earlier studies showed that extract of Chlorophytum borivilianum l have cardioprotective activity, so further we are fractionating the particular chemical phytoconstituents and evaluate the cardioprotective activity, hence the Chlorophytum borivilianum l. Extract is selected for the in-silico and in-vivo studies.

Modern In-silico technology known as "**Network pharmacology**" applies on the principles of network science, bioinformatics, and pharmacology to determine the mechanism of disease. Using the potential of natural products as the main source of cumulative and synergistic impact, they hope to produce creative remedies.9 It offers a complete perspective by establishing an extensive network involving drugs, target genes, pathways, and disease. By examining common targets or paths via the network, it assists researchers in finding drugs for specific diseases and helps them find a way around issues associated with drug discovery. Hence, NP analysis not only aids in the optimisation of medication regimens or combination treatments, but it also helps to identify the adverse effects that drugs cause.(6)

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Molecular docking is a computer-aided tool used in molecular biology and drug discovery. Its purpose is to predict and investigate the interaction between target proteins-like receptors, carbohydrates, and nucleic acids and small molecules, called ligands. Understanding the structural and kinetic aspects of ligand-protein interactions is aided by this technique.(8)
The molecular docking approach can be utilised in model for the interaction between a protein & small molecule at the atomic level, which can allow us to characterize the behaviour of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes.(9) The aim of molecular docking is Predicting the binding mode, binding affinity & binding energy between a potential drug and its target macromolecule in terms of scoring function(10). Therefore, combining network pharmacology with molecular docking provides framework for researching the intricate relationships between natural medicines with the human body.

2. MATERIALS AND METHODS

Collection and authentication of plant materials
whole plant of chlorophytum borivilianum L were procured and authenticated in month of August 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/2023-24).

Preparation of Ethanolic extract:(11)
For extraction of plant material Soxhlet extraction method used in the study by using 70% ethanol as solvent. Roots of plant material washed and dried in shade, after that plant material is powdered coarsely & placed inside thimble and RBF (Round bottom flask) filled with Ethanol which is heated to reflux. The solvent vapor rises, condenses in the condenser, and drips onto the solid material in the Soxhlet extractor. The solvent dissolves the target compounds from the solid, and the solution is then drained back into the flask. This extraction process continues cyclically, until the solvent flowing from extraction chamber not leave any residue behind, allowing for efficient extraction of the desired compounds. Solvent from the extract was then removed using steam distillation to have gummy concentrate of extract which were used in the present study. Extraction method already done in this study and we are fractionating the plant material for further study.

Fractionation method: Fractionation done by separation funnel method, for fractionation we selected Petroleum ether, Ethyl acetate, Chloroform, aqueous Chlorophytum borivilianum (CB) fractions based on polarity. fractionation begins by dissolving crude extract with 250mL of CB fraction solvent added, shaking it & allowed for settle, subsequently aqueous layer is removed from bottom of funnel are transferred into clean container to obtain a fraction. This same cycle is repeated for the rest of the CB fraction, the residual portion left after fractionation is referred as aqueous fraction, since the extract was initially dissolved in water.

Chemicals used:
Doxorubicin used as negative control in the study in a cumulative dose & CB fraction such as a Petroleum. Ether, Ethyl acetate, Chloroform used for treatment and enzyme assay kits are used for the study.

Selection of animals:
For the study healthy adult animals are taken weighing 180-200g were used for the study. approved by INSTITUTIONAL ANIMAL ETHICAL COMMITTEE (IAEC). (Proposal no: Mph/NC0222010/KLECOPH/23). All the animals were individually housed in polyethylene cages & maintained fed with standard pellet diet and water ad-libitum.

Screening of active compounds of Chlorophytum borivilianum. L:
The active ingredients of Chlorophytum borivilianum. L were identified using databases such as IMPPAT, Traditional medicinal books, ChEBI.
Drug-likeness property of phytocompounds:
Data such as molecular weight, molecular formula, canonical SMILES were collected from the chemistry database PubChem. Drug-likeness model score (DLS), HBD (hydrogen bond donors), HBA (Hydrogen bond acceptor), MolLogP and BBB scores for each molecule were predicted using his MolSoft (https://molsoft.com/mprop/). DL values shows that the compound will be more useful for clinical use. So, the compounds with DLS>0 considered as candidate for further studies.
Pathway and Network analysis: Using STRING (https://string-db.org/), the target/protein(s) accountable for the pathogenesis of CVS that is regulated by the drugs was compiled. The Kyoto Encyclopaedia of Genes (KEGG) pathway was used to compile the data for the enriched pathways. Compound-Target-Pathway network was constructed and analysed using Cytoscape 3.6.1(https://cytoscape.org/). Using the command "Network Analyzer," the network was examined and handled as a direct network. The layout (attributed circle layout) was used for better visualization and the edge count represented the gene and compound's interaction.
Molecular docking: Chemical components and molecular targets with a high edge count, as reported in network pharmacology, were acquired for receptor-ligand molecular docking. The PubChem database was utilized to retrieve the 3D structure of each compound in SDF format. Protein molecular X-ray crystallographic structures were sourced from the RCSB PDB database (https://www.rcsb.org/) in .pdb format. AutoDock software was employed to prepare the protein by eliminating water molecules, removing heteroatoms, adding polar hydrogens, and applying Kollman charges to optimize the structure, which was then saved in PDBQt format. (https://doi.org/10.1007/978-1-59745-177-2_19).
The ligands were transformed into pdbqt format utilizing Pyrex 0.8v software. A grid box was generated for docking, and the docking outcomes were employed to pinpoint the most favorable binding poses and determine the binding affinity. The protein-ligand complex with the highest binding affinity was visualized using Discovery Studio Visualizer 2021. (12)
Phytochemical analysis: Preliminary phytochemical investigation carried out.

Acute oral toxicity
The dose selection of plant extract was selected based on previous literature. according to the Organization for Economic Cooperation and Development (OECD) revised up-and-down procedure for acute toxicity testing (OECD guideline 425), the extract had no adverse effects When administered to the animals at levels up to 2000 mg/kg(13). Further dose selection for fraction on the base of % yield of Chlorophytum borivilianum. L of fractions i.e. 200 mg/kg.
Study design:
After one week of acclimatization, the animals were divided into 7 groups of six animals each.
Group I – Received vehicle 5mL/kg for two weeks followed by saline i.p for two weeks. 2.
Group II - Animal will be treated with DOX (2.5 mg/kg body weight i.p) in 6 equal injections alternatively for 2 weeks to make a total cumulative dose of 15 mg/kg body weight 3.
Group III - Animal will be pre-treated with Vitamin E (100 mg/kg body weight p.o) followed by DOX administration as in group 2.
Group IV - Animal will be treated with Petroleum ether fractions (200 mg/kg body weight p.o) for 2 weeks followed by DOX administration as in group 2
Group V - Animal will be Ethyl acetate fractions (200 mg/kg body weight p.o) for 2 weeks followed by DOX administration as in group 2.

Group VI - Animal will be Chloroform fractions (200 mg/kg body weight p.o) for 2 weeks followed by DOX administration as in group 2.
Group VII - Animal will be Aqueous fractions (200 mg/kg body weight p.o) for 2 weeks followed by DOX administration as in group 2.

Parameters:

- Food & water intake was observed daily throughout the study.
- ECG was recorded in anaesthetized animals using combination of ketamine and xylazine i.p administration at a dose of 70 and 10 mg/kg respectively and recordings were taken using lead II method in BIOPAC MP36, Inc Santa Barbara, USA.(14)
- **Estimation of Specific Cardiac Biomarkers**: After a day of the last treatment, blood samples were obtained from orbital under light ether anaesthesia using heparinized microcapillaries for the estimation of following cardiac biomarkers.

➢ Creatinine kinase-MB (15)
➢ Lactate dehydrogenase (16)
➢ Cardiac Troponin I (cTn I)(17)

Using a semi-autoanalyzer, CK-MB And LDH were measured in accordant with the established methods provided with the respective kits.

In-vivo antioxidant activity: The animals were euthanized, and their hearts were promptly removed and divided into two halves. One half was utilized to measure various antioxidant enzymes, while the other half was designated for histopathological examination.

1. Glutathione peroxidase (GSH)(18)
2. Malondialdehyde (MDA) (19)
3. Superoxide dismutase (SOD) (20)
4. Catalase (CAT)(21)

Histopathology study: (22)

After taking samples of blood for the assessment of various markers, the animal was sacrificed, the heart was isolated, then cleaned with saline, and the weight of the heart was calculated by comparing its weight to that of the body. For histopathological studies., the separated hearts were kept in 10% neutral formalin solution for isolated heart.

Statistical analysis:

The experimental data were statistically analysed using one-way analysis of variance ANOVA followed by Bonferroni's Multiple Comparison Test. Results will be expressed as Mean ± SEM and difference were considered significant at $p<0.05$ using GraphPad Prism® software.

3. RESULTS

In-Silico Studies

Network Pharmacology: Compounds were identified in Chlorophytum borivilianum. L including Alkaloids, Flavonoids, carbohydrates, saponins, Phenols. Among 32 compounds, 6 compounds have showed Positive DLS in roots of Chlorophytum borivilianum that were used for the network analysis. In that Stigmast-5-en-3-ol has showed highest DLS i.e., 0.78. The targets were obtained from TTD and KEGG pathway which are related to Cardiovascular system

Molecular Docking:

Phytocompounds such as Chloromaloside-a, Stigmasterol, Stigmast-5-en-3-ol & target genes MAPK3, PRKCB, SRC reported that, these are having highest edge count in network pharmacology. Among them stigmast & stgmast-5-en-3-ol were docked. Stigmast-5-en-3-ol has highest binding affinity of -9 with Proto-oncogene tyrosine- protein kinase (SRC) target.

Plant Material:

Extraction method: % yield of ethanolic extract of coarse powdered Chlorophytum borivilianum. L obtained by Soxhlet extraction process using 70% ethanol as solvent was found to be 26.6% w/v.

Fractionation method: % yield of Chlorophytum borivilianum. L fractions based on their polarity.

Phytochemical investigation: Qualitative chemical analysis of Fractions of Chlorophytum borivilianum. L was finds out the Alkaloids, carbohydrates, Flavonoid's, Saponins, Phenols& polyphenol

Effect on Body weight of Chlorophytum borivilianum. L fractions

Based on general appearance of animals throughout the study period. Doxorubicin administered groups forms red exudates around eyes, pink tint & fur become scruffy and soft watery faecal matter. and these changes were not observed in vitamin E and Fractions treated groups.

Dox treated class, body weight declined when comparison with the control group (control vs. DOX, $p<0.001$). Standard Vitamin E treated group shown significant increase in body weight when compared with Dox (Vitamin, $p< 0.001$). & CB fractions treated groups has shown significant elevated body wt. when compared to standard group.

Effect on food & water intake of Chlorophytum borivilianum. L fractions:

In Negative control treated group food and water intake were significantly reduced as when compared with normal control (control vs DOX $p< 0.001$). Vitamin treated group & CB fractions treated group shown significant changes with food and water consumption when compared with Dox ($p< 0.001$)

Effect of Specific Serum Cardiac Biomarkers of Chlorophytum borivilianum. L fractions:

In comparison with Normal control group only Dox treated group animals has shown significant elevation in CK-MB, LDH enzymes (control vs DOX $p< 0.001$). Vitamin. E treated group & CB fraction treated group i.e. Ethyl acetate fraction group has shown significant decrease in cardiac biomarker when compared to doxorubicin treated group. (DOX vs Vit. E $p< 0.001$, CB ethyl acetate fraction $p< 0.01$).

Presence of Cardiac troponin I was identified using one step rapid test. Only Dox treated groups showed the presence of cTn I which is cardiac specific biomarker. Normal, Std+ dox and Fractions treated groups showed the absence of Cardiac troponin I when compared to Dox treated group

Effect of Electrocardiogram (ECG) of Chlorophytum borivilianum. L fractions:

In Dox administered group animals PR & QT intervals elevated, slowdowns heart rate and decrease in QRs complex has been observed in ECG (control vs DOX $p< 0.001$). Normal control, Vitamin. E treated showed normal pattern electrocardiogram observation and little less aqueous fraction of CB has shown normal ECG. Whereas CB Ethyl. Acetate fraction & Aqueous fraction treated group has shown protective effect against Dox induced alters in PR & QT interval (Vit.E vs CB fraction $p<0.001$) and QRS complex, heart rate.

Impact of In-vivo antioxidant activity on Chlorophytum borivilianum. L fractions:

Levels of GSH, SOD, and CAT were significantly lower in DOX-treated rats than those reported in the control group, while Standard group Vitamin. E and CB-fractions i.e. Ethyl acetate and aqueous significantly raised the standby levels GSH, SOD, CAT. GSH reductase, and SOD activity were considerably greater in the control group than in the DOX-treated group. Levels of MDA were higher in the DOX-treated group than those in the control group, however, CB fractions and Vitamin E significantly raised the MDA levels.

FIGURES:

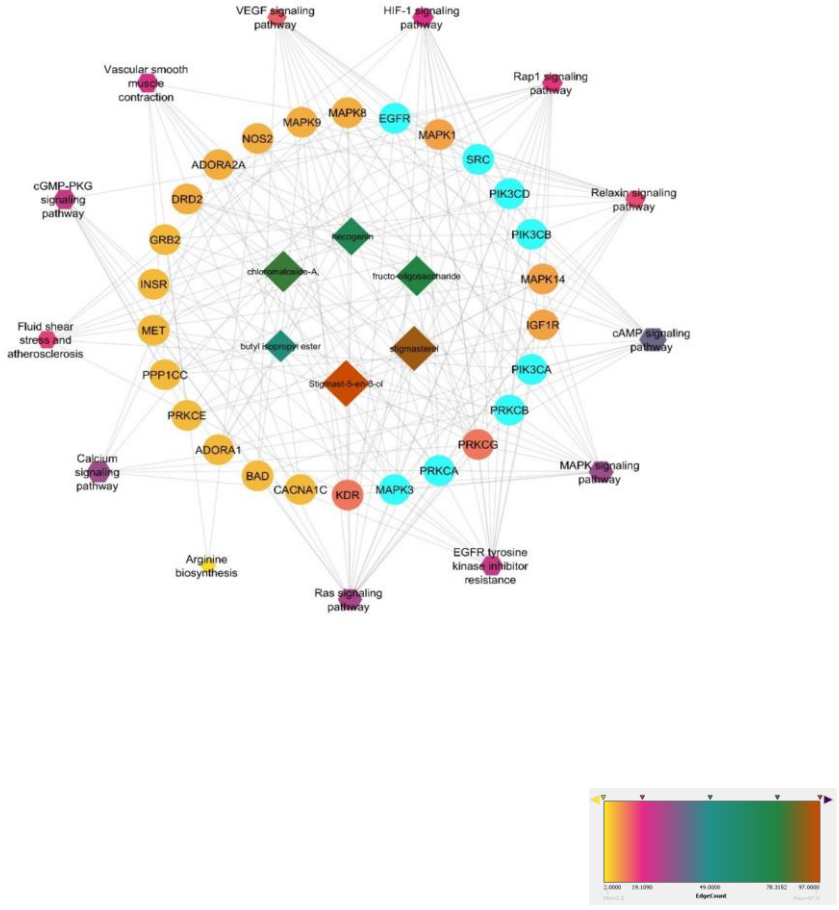
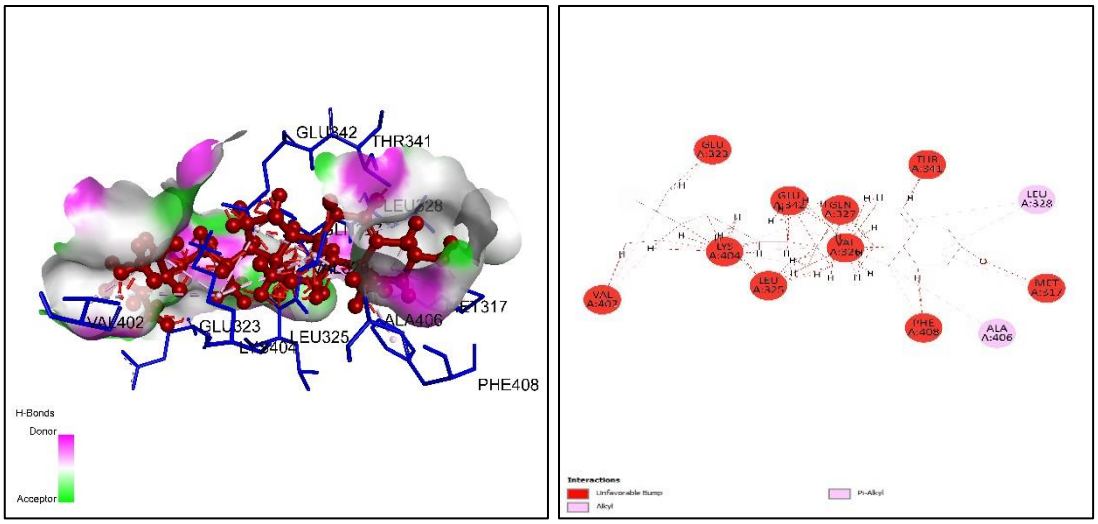


Figure 1: Network Analysis of Chlorophytum Borivilianum. L



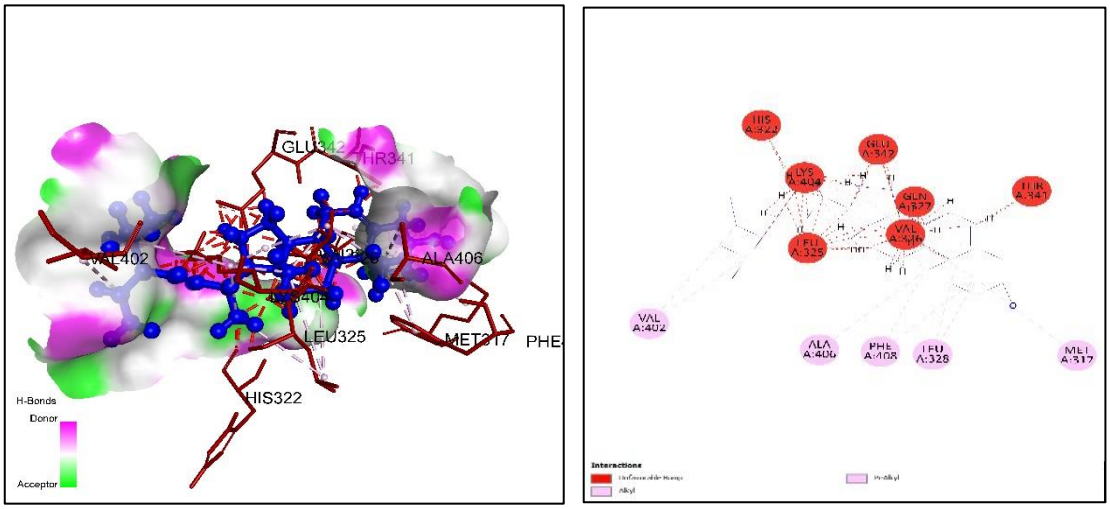


Figure 2: Molecular docking Interactions of Stigmasterol with SRC 3d & 2d model

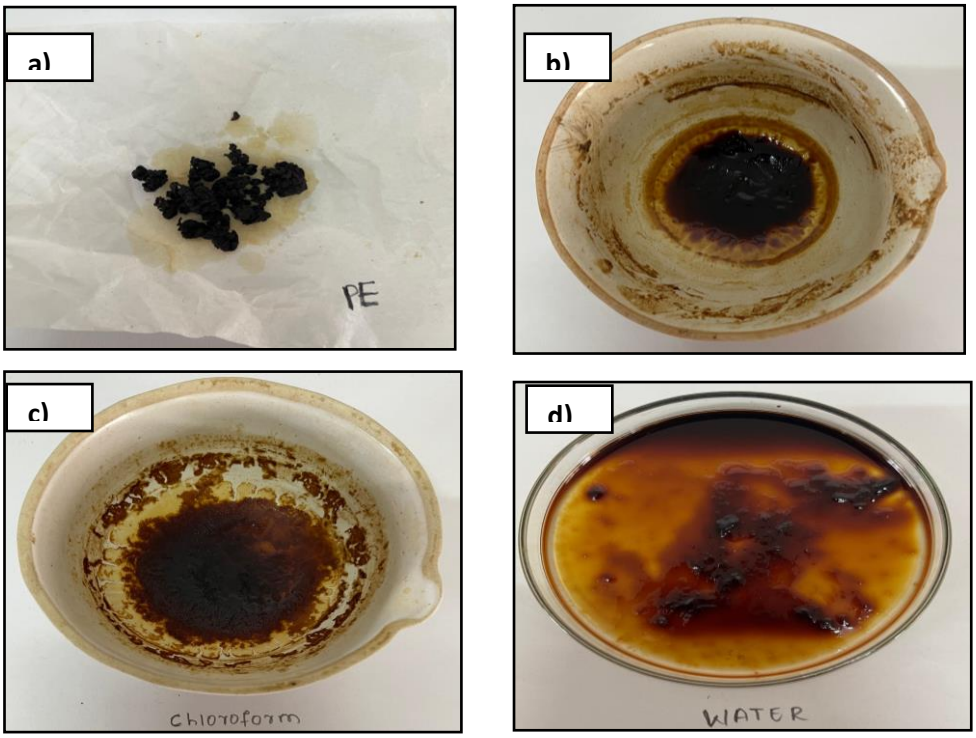


Figure 3: Molecular docking Interactions of Stigmast-5-en-3-ol with SRC 3d & 2d model

Figure 4: Chlorophytum borivilianum. L fractions a) Petroleum ether b) Ethyl acetate c) Chloroform d) Aqueous

Figure 5: Phytochemical Screening

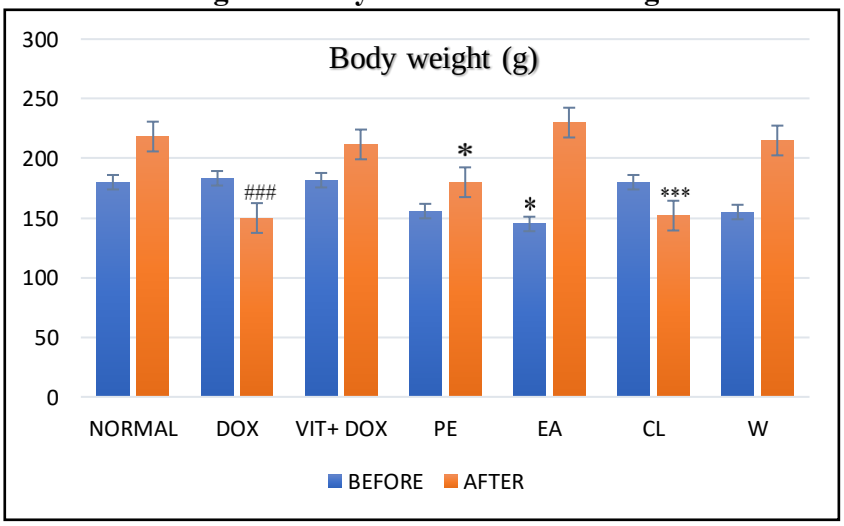


Figure 6: Effect on Body weight of Chlorophytum borivilianum. L fractions

The values are expressed in Mean ± SEM for Body weight using one-way ANOVA (Bonferroni's Multiple Comparison Test). ### p< 0.001 when compared to control, *** p< 0.001, ** p< 0.01, when compared to DOX.
PE – Petroleum Ether fractions of CB
EA – Ethyl acetate fractions of CB
CL – Chloroform fractions of CB
W- Aqueous fractions of CB

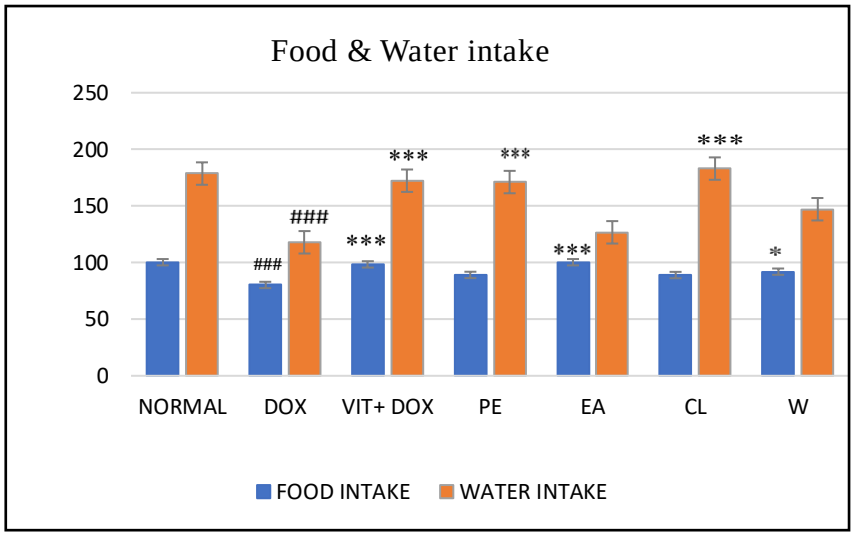
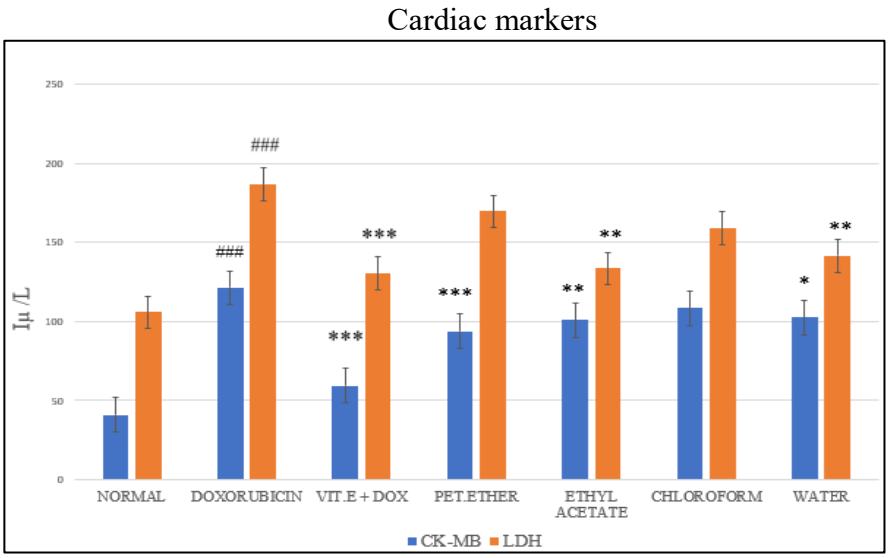


Figure 7: Effect on food & water intake of Chlorophytum borivilianum. L fractions



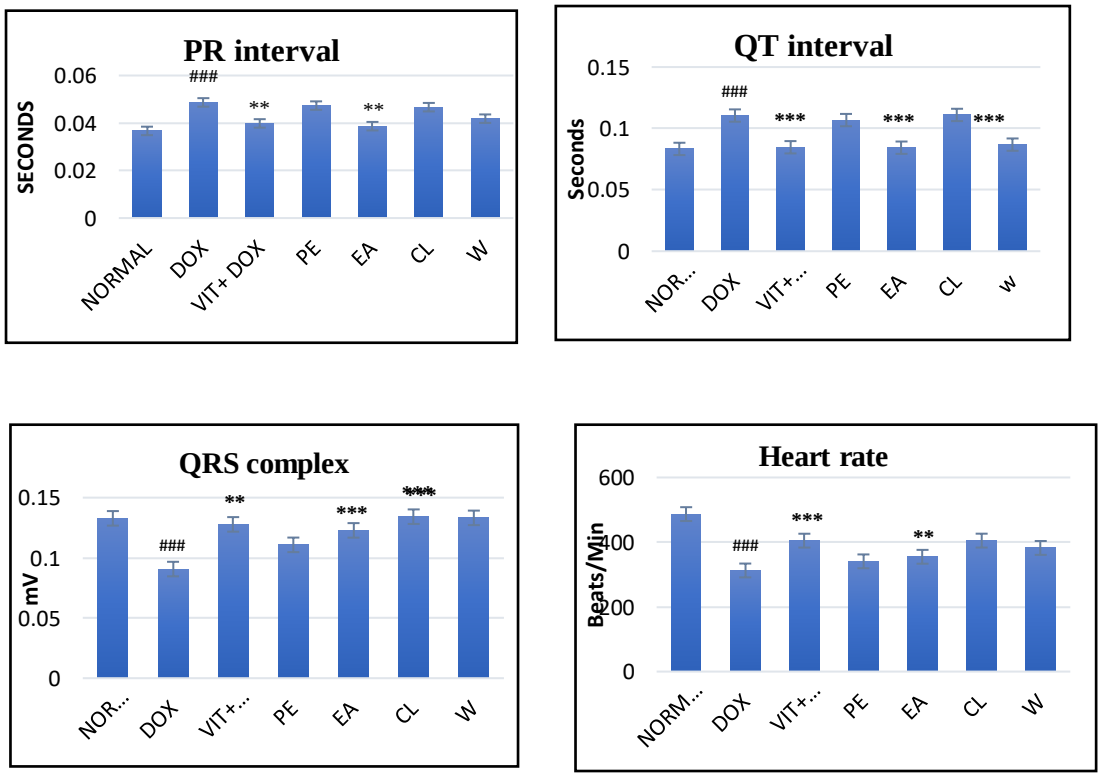
Values are Mean+ SEM; n=6 in each group, ### p< 0.001 when compared to control, *** p< 0.001, ** p< 0.01 when compared to DOX using one-way ANOVA (Bonferroni's Multiple Comparison Test).

Figure 8: Effect of Specific Serum Cardiac Biomarkers of Chlorophytum borivilianum. L fractions:

Values are Mean+ SEM; n=6 in each group, ### p< 0.001 when compared to control, *** p< 0.001, ** p< 0.01 when compared to DOX using one-way ANOVA (Bonferroni's Multiple Comparison Test).

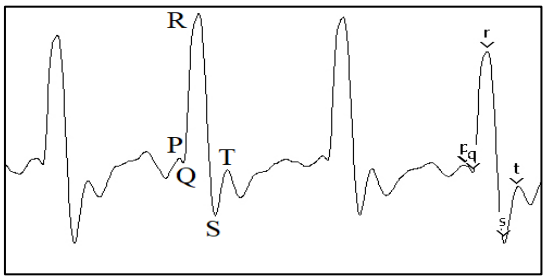
(A) (B) (C) (D) (E) (F) (G)

Figure 9: Results of Cardiac troponin results

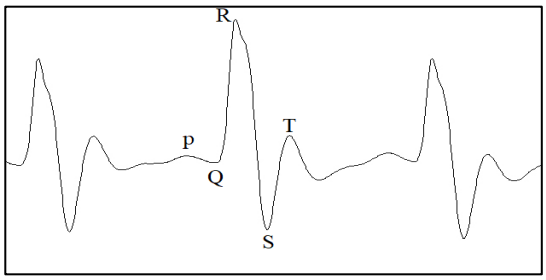


A) Normal control
B) Doxorubicin
C) Dox +Vitamin. E
D) Chlorophytum borivilianum (CB) pet. ether fraction
E) CB Ethyl aetate fraction
F) CB Chloroform fraction
G) CB aqueous fraction

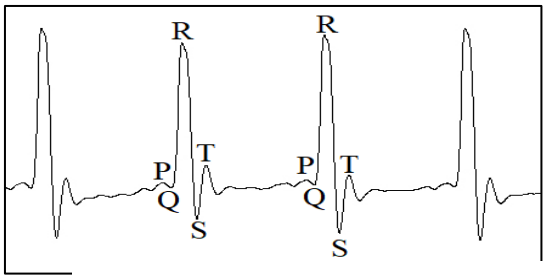
Figure 10: Effect of Electrocardiogram (ECG) of Chlorophytum borivilianum. L fraction



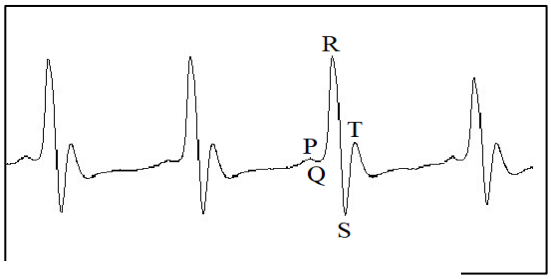
NORMAL



DOXORUBICIN



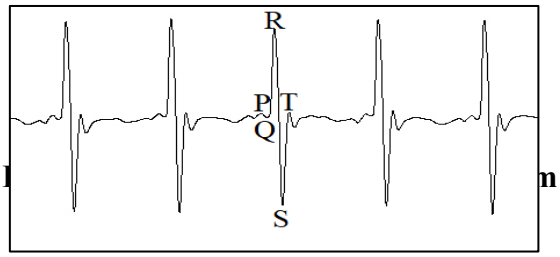
ETHYL ACETATE



CHLOROFORM

DOXORUBICIN +VIT.E

PETROLEUM.ETHER



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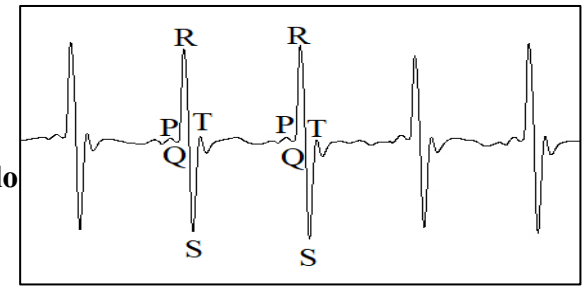
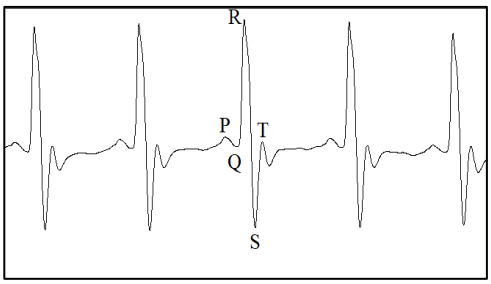




AQUEOUS

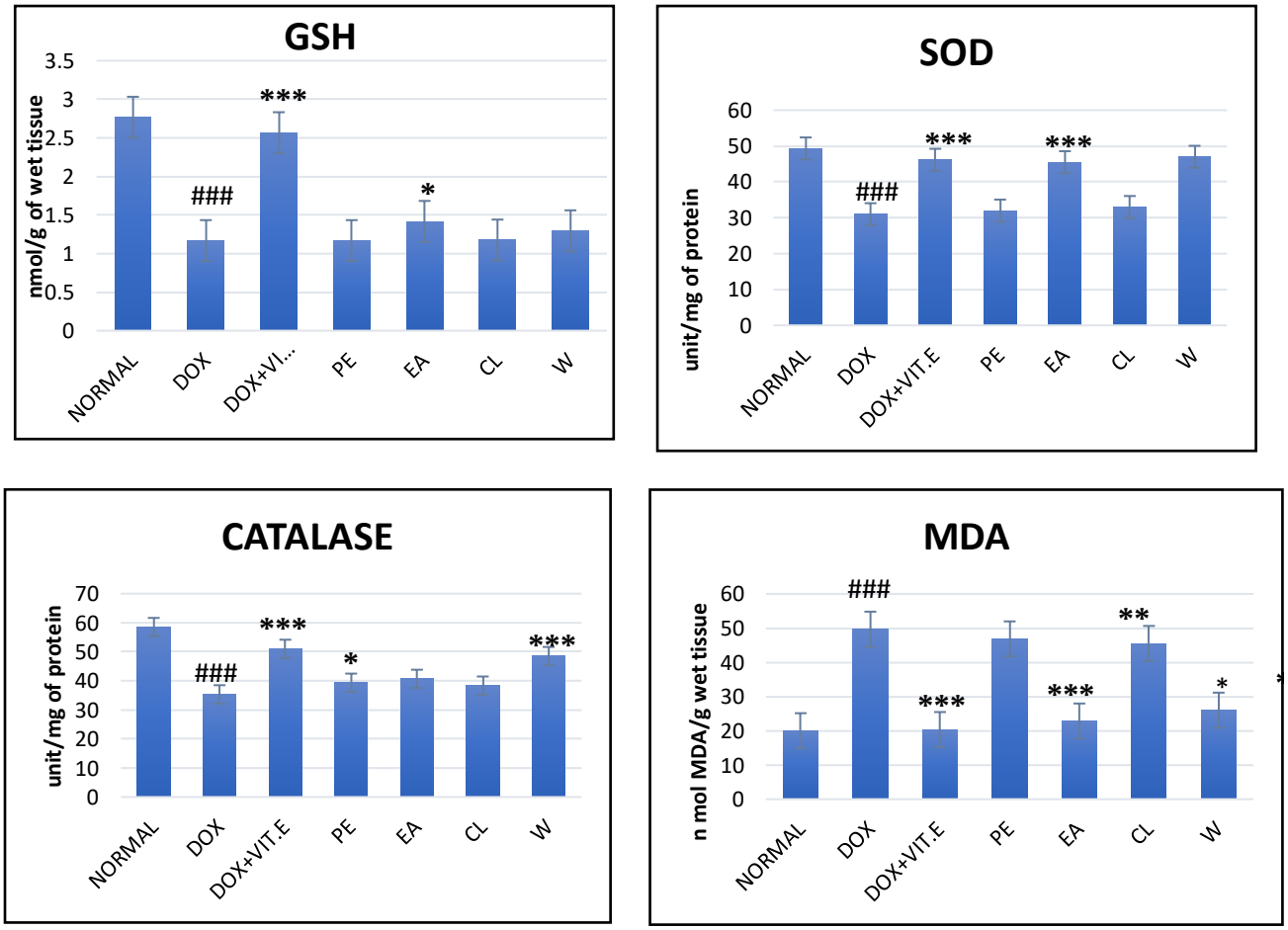
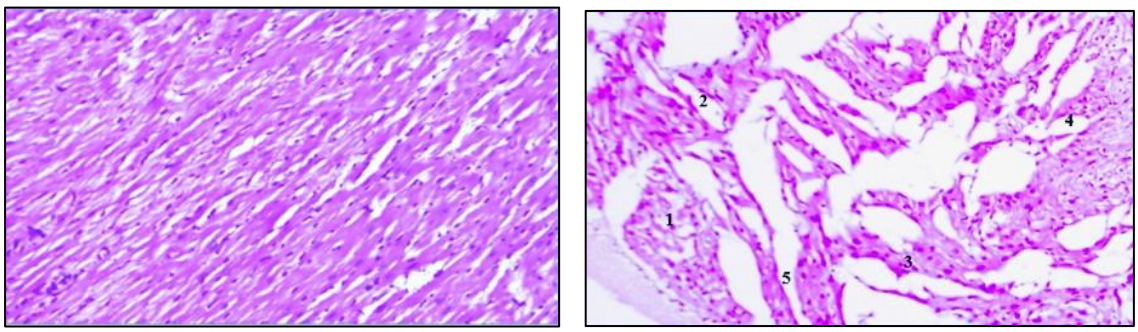
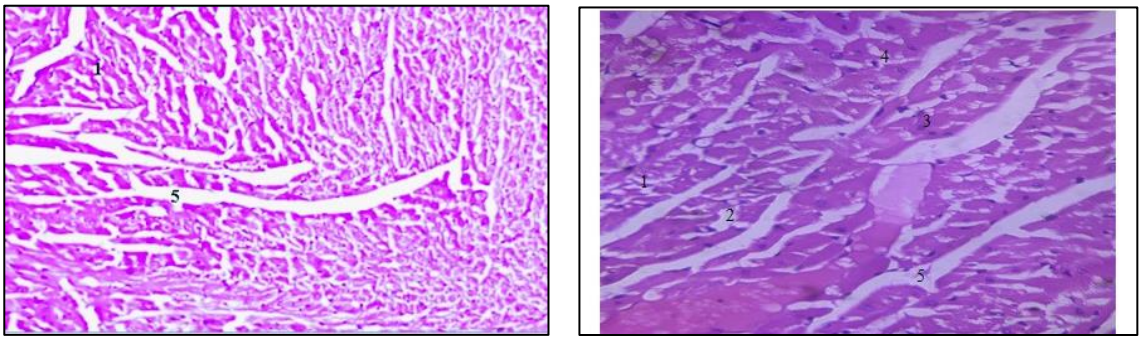


Figure 12: Impact of In-vivo antioxidant activity on Chlorophytum borivilianum. L fractions:

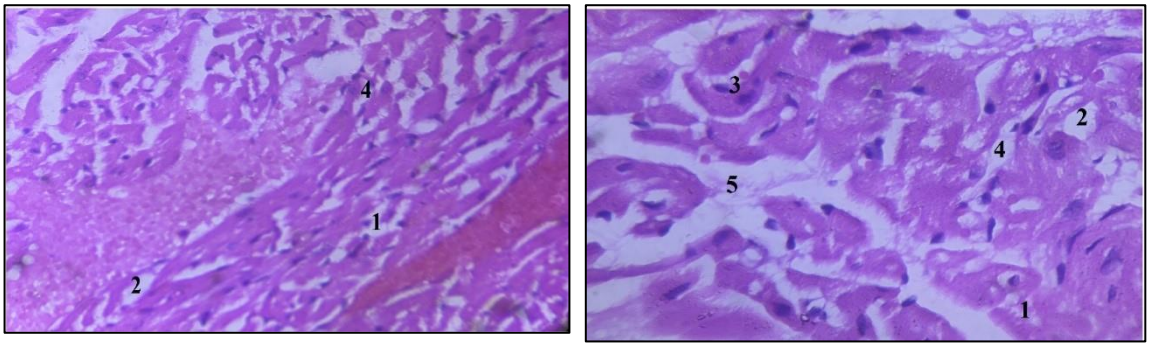
Values are Mean+ SEM; n=6 in each group, ### p< 0.001 when compared to control, *** p< 0.001, ** p< 0.01 when compared to DOX using one-way ANOVA (Bonferroni's Multiple Comparison Test).



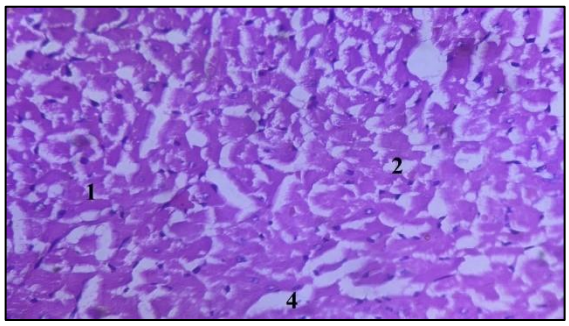
a) Normal b) Doxorubicin



c) Dox + Vit E d) PE fraction



e) EA fraction f) CL fraction



g) AQ fraction

1. Cardiomyocyte degeneration
2. intermuscular edema
3. Inflammatory cell infiltration
4. Vacuolization
5. Myofibrillar loss

Figure 13: Histopathology resultsTABLES:

Table 1: Results of positive Drug-likeness property and Target prediction

PHYTOCHEMICALS	PUBCHEM ID	MF	MW	HBA	HBD	LOGP	DLS
Butyl isopropyl ester	66984153	$C_{15}H_{26}O_4$	270.18	4	0	3.67	0.01
Chloromaloside-a,	151156	$C_{50}H_{80}O_{23}$	1048.5	23	12	0.34	0.36
Hecogenin	91453	$C_{27}H_{42}O_4$	430.31	4	1	3.72	0.04
Stigmasterol	5280794	$C_{29}H_{48}$ O	412.37	1	1	3.94	0.62
Fructo-oligosaccharide	135394093	$C_{48}H_{82}O_{41}$	1314.4	41	26	0.34	0.17
Stigmast-5-en-3-ol	22012	$C_{29}H_{50}O$	414.39	1	1	3.94	0.78

Table 2: Results Binding affinity of targets.

Binding Affinity		
Target	**Stigmasterol**	**Stigmast-5-en-3-ol**
MAPK3	-8	-8.5
PRKCB	-8.4	--
SRC	-8.6	-9

Fractions	% yield of fractions of CB (w/v)
Pet. ether	2.3
Ethyl acetate	5.7
Chloroform	3.2
Aqueous	28.3

Table 3: % yield of Chlorophytum borivilianum. L fractions

Table 4: Results of Phytochemical investigation

Phytoconstituents test	Results
Alkaloids	+
Carbohydrates	+
Flavonoids	+
Steroids	-
Saponins	+
Amino acids	-
Quinone	-
Phenols	+

'+' - Present, '-'- Absent

Table 5: Effect of Chlorophytum borivilianum. L fractions on Histopathological study.

	Cardiomyocyte degeneration	Intermuscular edema	Inflammatory cell infiltration	Vacuolization	Myofibrillar loss
Normal	-	-	-	-	-
Doxorubicin	++	+	+	++	++
Dox+vit.E	+	-	-	-	+
Pet.ether	++	++	+++	+	+
Ethyl acetate	+	+	-	+	-
Chloroform	++	+++	+	+	+
Water	+	+	-	+	-

+ = Present - = Absent

+++ = Severe, ++ = Moderate, + = Mild, - = Nil

4. DISCUSSION:

The aim of the current study was to investigate the impact of Chlorophytum borivilianum L. on the treatment of cardiovascular diseases using network pharmacology combined with docking studies.

Numerous internal and external research projects are being carried out to address complicated conditions such as cardiovascular diseases (CVSs). Network pharmacology is a well-known method for drug discovery that is one such strategy. Numerous attempts have been made to clarify the molecular processes of holistic treatments in the treatment of complicated disorders by use of network pharmacology(23).

We constructed a network depicting the interactions between phytoconstituents, their targets, and possible pathways. The findings of network pharmacology shows that Butyl isopropyl ester, Chloromaloside-a, Hecogenin, Stigmasterol, Fructo-oligosaccharide, Stigmast-5-en-3-ol potential phytoconstituents that may interact with multiple protein molecules involved in the pathogenesis of cardiovascular diseases. Among them Stigmasterol and stigmast-5-en-3-ol plays an important role in transferring signals in cardioprotective activity in network.

By modifying 5 important pathways, Chlorophytum borivilianum L. has a vital role in the management of cardiovascular disorders, based on KEGG pathway analysis, MAP signalling pathway, EGFR tyrosine kinase inhibitor resistance, RAS signalling pathway, Calcium signalling pathway, RAP1 signalling pathway.

The protein MAPK3 is mitogen-activated protein kinase 3 in which protein encoded by MAP kinase family also known as extracellular signal-regulated kinases (ERKs), act in a This kinase is a component of a signalling cascade that, in response to various extracellular stimuli, controls a number of cellular processes, such as proliferation, differentiation, and cell cycle progression. It travels to the nucleus after being activated by upstream kinases, where it phosphorylates nuclear targets. Different protein isoforms are also produced by polymorphisms in the spliced transcript(24).

PRKCB: A class of serine- and threonine-specific protein kinases known as protein kinase C (PKC) is triggered by calcium and the second messenger diacylglycerol. Members of the PKC family are essential to many cellular signalling pathways and phosphorylate a wide range of protein targets. Furthermore, PKC proteins serve as the main receptors for the class of tumour promoters known as phorbol esters. Every member of the PKC family has a distinct expression profile and is thought to carry out certain biological tasks. This particular protein kinase is involved in the activation of B cells, the induction of apoptosis, the proliferation of endothelial cells, and the absorption of sugars by the digestive tract. Studies conducted on mice suggest that this kinase is linked to fear-induced conflict behaviour after stress and may potentially affect neural processes. Moreover, transcript variants with alternative splicing that encode distinct isoforms have been discovered. (25)

SRC: This gene has similar resemblance to the Rous sarcoma virus's v-src gene. It could be involved in controlling cell growth and embryonic development as a proto-oncogene. Tyrosine-protein kinase, the protein that this gene encodes, can have its activity reduced by phosphorylation by c-SRC kinase. This gene's mutations may have a role in the colon cancer's malignant development. For this gene, two transcript variants that encode the same protein have been found(26).

The aim of this study was to assess the cardioprotective effects of plant fractions from Chlorophytum borivilianum (CB) on doxorubicin-induced cardiotoxicity in albino rats. Cardiotoxicity was induced by administering 2.5 mg/kg of doxorubicin via intraperitoneal injection on alternate days for two weeks(27). Characteristic signs of cardiotoxicity include decreased body weight, reduced food and water intake, altered ECG patterns, elevated cardiac markers (LDH, CK-MB), increased serum markers, elevated antioxidant enzyme levels (GSH, SOD, CAT), reduced MDA levels, and histopathological changes in the heart. Antioxidants, such as vitamins, have been reported to reduce or prevent doxorubicin-induced cardiotoxicity.

Cardiotoxicity induced by Doxorubicin:

Doxorubicin is a highly cytotoxic antibiotic widely used in various chemotherapy regimens for treating haematological and other solid tumours. Its antineoplastic mechanism involves DNA

intercalation, which hinders replication and protein synthesis, and inhibition of topoisomerase-II, which prevents the relegation of double-strand breaks. Despite its effectiveness as an anticancer drug, doxorubicin is associated with dose-dependent cardiotoxicity, leading to acute or early/late-onset chronic progressive cardiomyopathy, a common side effect that limits its therapeutic use(28). Doxorubicin accumulates in cardiac tissue over time in a dose-dependent manner, ultimately causing cardiac dysfunction(29). Previous studies have shown that chronic administration of doxorubicin generates free radicals in heart tissue.

Doxorubicin produces reactive oxygen species (ROS) through a number of different processes(30)(31). By reducing doxorubicin by one electron, mitochondrial reductase may generate free radicals known as anthracycline semiquinone. These unstable radicals easily deplete molecular oxygen to produce reactive oxygen species (ROS), including hydrogen peroxide and superoxide anions, when aerobic conditions are met(32). Increased production of free radicals in cardiomyocytes causes oxidative stress, which in turn causes a host of negative consequences, such as disruptions to the mitochondrial energy balance, build-up of p53, activation of the p38 and JNK pathways, and eventually, cell death(33).

- **Preparation and effect of CB fractions:**

Preparation of fractions of CB from plant extract was done by hot extraction method in which coarse powder of plant has shown yield 26.6 % using 70 percent w/v ethanol as solvent. phytochemical investigation revealed the presence of Alkaloids, Carbohydrates, saponins, phenols, Flavonoids.

Effect of fractions of Chlorophytum borivilianum. L on food & water, body weight: reported that rats which treated with DOX has reduced food and water intake within few days(34) . due to anorexia (lack of appetite) that was adverse event produced by DOX. Which stimulates autophagy for the cell to cellular recycle build during times of energy status(35) & inducing of autophagy requires optimal production of ROS and damage in DNA responds to starvation(36). Previous investigations have shown that fasting causes glutathione to be depleted, which leads to oxidative stress. Fasting modifies molecular pathways in cells that are essential to both cell life and death(37). Dehydration and water restriction cause the RAAS to become activated and stress hormones like cortisol to be released(38)(39). Reduced food and water consumption in doxorubicin-treated rats led to a steady reduction in body weight, which is in line with earlier findings and is probably caused by anorexia, a side effect of doxorubicin. Rats that had received a fraction of CB and vitamin E.

Effect of ECG patterns on Chlorophytum borivilianum root:

An essential factor in properly diagnosing cardiac damage is abnormalities on the electrocardiogram (ECG). Furthermore, in line with other research, ECG alterations indicate the degree of myocardial damage caused by doxorubicin. With increasing doxorubicin dosages, notable ECG abnormalities include QT lengthening, a reduction in the QRS complex, a drop in the amplitude of the R wave, T wave flattening, an extension of the ST gap, and a decrease in heart rate(40). Although the precise processes causing these transient ECG alterations are unknown, they could be connected to the reversible myocardial edema caused by doxorubicin(41)(42).Treatment with CB and Vitamin E reduced QT interval prolongation, suggesting a decrease in oxidative stress. The increase in R wave amplitude indicated reduced right and left ventricular hypertrophy, while the improved heart rate demonstrated a protective effect against doxorubicin-induced ECG alterations. This treatment also eliminated acute fatal complications and protected against cell membrane damage, likely due to its protective or membrane-stabilizing effects on the myocardium.

Specific cardiac markers: Doxorubicin damages the myocardium, it releases cardiac indicators like lactate dehydrogenase, creatine kinase-MB, and troponins into the circulation. It also releases biochemical markers like transaminases (aspartate and alanine) and alkaline

phosphatase. These markers function as cardiac tissue injury diagnostic indications(43). Measured often in clinical practice to diagnosis cardiac necrosis and toxicity(42), serum LDH, CK-MB, and cTnI are thought to be specific cardiac indicators for diagnosing cardiac toxicity. Due to a shortage of oxygen or glucose, these enzyme levels are markedly increased in rats given doxorubicin, which causes due to myocyte damage, the cardiac membrane becomes more brittle and bursts, allowing enzymes to escape.

In-vivo antioxidant:

In the present study, doxorubicin-treated rats exhibited a significant increase in malondialdehyde (MDA) levels, indicating heightened lipid peroxidation, and a decrease in GSH, SOD, and CAT levels, indicating an increase in free radicals, consistent with earlier reports. However, treatment with CB and Vitamin E resulted in decreased MDA levels, suggesting a reduction in lipid peroxidation, and increased GSH, SOD, and CAT levels, demonstrating the free radical scavenging properties of the extracts and their protective effect against oxidative damage to myocytes.

Histopathology of heart:

Rats administered with CB fraction & Vit.E in this study showed decreased inflammatory cells, decreased myocardial fibre loss, decreased vacuolated cell presence, and decreased edema. These findings imply that the extracts shielded the heart against doxorubicin-induced myocardial damage. Furthermore, there was an increase in heart weight as well, as shown by the ratio of heart weight to body weight. This rise may have been brought on by enlarged, hypertrophic, and dilated ventricles in addition to mitochondrial swelling. But in rats given CB, all of these alterations were noticeably reduced.

Preparation and evaluation of fractions

Preparation of fractions of CB contains Alkaloids, flavonoids, saponins, phenols which might responsible for DOX induced cardiotoxicity further fractionation done by separation funnel method according to elevated polarity values: Petroleum ether, Ethyl acetate, Chloroform, aqueous from Chlorophytum borivilianum extract. Previous studies have indicated that polyphenols have a direct impact on various molecular signal transduction pathways, including those related to inflammation cascade, oxidative stress, and metabolic disorders, which play crucial roles in the development of numerous non-communicable diseases(43). Polyphenols, such as flavonoids, are known to participate in the elimination or inactivation of reactive oxygen species (ROS) at the cellular level. The bioactive ethyl acetate fraction's phytochemical analysis revealed that CB contained polyphenols. Plants contain Saponins & polyphenols, which serve a variety of defensive purposes. It has been suggested that saponins scavenge harmful free radicals that are produced by the body in excess and Recent research indicates that polyphenols can also indirectly combat oxidative stress by promoting the production of natural antioxidant enzymes. Hence in current study these polyphenols and saponins may be responsible for cardioprotective effect in doxorubicin induced cardiac toxicity and this may be due to anti-oxidant and free radical scavenging capacity. Further study is required to isolate and characterize the ethyl acetate.

5. CONCLUSION:

Recent research indicates that Chlorophytum borivilianum. L can prevent and treat cardiovascular diseases (CVSs) through a complex regulatory network involving multiple connections, targets, and signalling pathways. Utilizing Network pharmacology and molecular docking, our study aims to provide new insights and theoretical base for exploring the active compounds in Chlorophytum borivilianum. L Root and their potential mechanisms in CVS

prevention and treatment. The outcomes from both in vitro and in vivo experiments corroborated the findings from molecular docking studies.

Based on the results of general appearance, specific cardiac markers, ECG studies, biochemical markers, anti-oxidant activity and histopathologic studies, it may be concluded that, the Fractions of Chlorophytum borivilianum L. Root exhibited cardioprotective activity in doxorubicin induced cardiotoxicity. Ethyl acetate Chlorophytum borivilianum L fractions of Stigmasterol & Stigmast-5-en-3-ol present in the roots might be responsible for cardioprotective activity.

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CONFLICT OF INTEREST

Authors declare no conflict of interests.

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