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In Vivo and *In Silico* Assessment of the Cardioprotective Effect of *Thymus linearis* Extract against Ischemic Myocardial Injury

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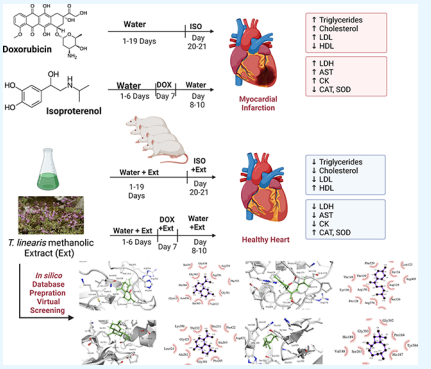
ABSTRACT: Myocardial infarction is irreversible cardiac tissue necrosis due to the blockage of one of the arteries. It leads to an insufficient supply of oxygen and nutrients, creating muscular damage in the affected regions. In the present study, aqueous methanolic extract of *Thymus linearis* was prepared to evaluate its activity against ischemic stress due to free radical production. GC−MS analysis was performed to evaluate the phytochemicals present in the plant extract. A chemical database of 30 compounds was virtually screened against NF-κB, COX2, and MCL, where γ-cadinene, β-bisabolene, and β-caryophyllene were found to be the best interacting ligands. To systematically assess cardioprotective activity against ischemia, isoproterenol and doxorubicin were used to induce cardiotoxicity in rats. The prepared extract of *T. linearis* (100 mg/kg) was given daily to animals for 21 days before injecting isoproterenol (85 mg/kg of animal weight) subcutaneously in two doses on the 20th and 21st days. In the case of doxorubicin, cardiotoxicity was induced in rats by a single injection (15 mg/kg) on the seventh day, and the extract was given to animals for 10 consecutive days. Animals' blood samples were used to monitor cardiac, liver, and other marker enzymes, including LDH, CPK, and AST. Superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were also assayed in blood plasma to determine the degree of oxidative stress. H&E staining was performed to evaluate cardioprotection by plant extract, showing significant preventive effects in reducing cardiac injury induced by isoproterenol and doxorubicin.



INTRODUCTION

Cardiovascular disorders (CVDs) are the leading cause of morbidity and mortality in the developing world. However, hypertension has been one of the key risk factors for many CVDs, such as atherosclerosis, coronary artery disease (CAD), heart failure, myocardial ischemia (MI), stroke, and renal insufficiency.[1] According to WHO, nearly one-third of the population worldwide undergo hypertension, and this occurrence has increased faster due to lifestyle changes. MI causes irreversible cardiac tissue necrosis ruptured with an atherosclerotic plaque in the coronary arteries. The prevalence of hypertension is one in every four middle-aged adults in Pakistan.[2]

In CAD, the coronary arteries, pumping blood to the heart, become narrowed or blocked by atheroma rupture and/or blood clot formation. In the case of the complete blockage of the artery in the heart, there has been a lack of perfusion or blood supply with insufficient oxygen (hypoxia) and nutrients, causing ischemia leading to MI, which causes muscular damage in the affected regions of the heart.[3] Creatine kinase-MB and troponin biomarkers are up-regulated and used in the prognosis and diagnosis of MI. Cardiac troponin and some other enzymes are released through heart muscles/myocytes and correlate directly with infarct size.[4,5]

Treatment of MI has considered restoring the perfusion (i.e., reperfusion) and recovering the damaged heart muscle supply by using pharmacological or mechanical methods like coronary artery bypass graft and/or percutaneous coronary intervention. After reperfusion, chemotactic factors have been improved, such as monocyte chemoattractant protein-1, complement component-5a, interleukin-1, intercellular adhesion molecule-1, and growth factor-β. These factors progress to the infiltration of monocytes and neutrophils into the vulnerable myocardium, provoking an inflammatory response that initiates the repair process, fibrosis, and remodeling of myocardial tissues.[6] Many drugs like β-blockers, glyceryl trinitrate and possibly ACE inhibitors, and fibrinolytic and antihyperlipidemic agents have been used for treatment, but

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Table 1. Antioxidant Activities of the Aqueous Methanolic Extract of *T. linearis* through Different Antioxidant Assays[a]

	TPC	TFC	DPPH (% inhibition)	reducing power
T. linearis	78.02 ± 7.30	45.40 ± 4.20	55.03 ± 4.50	24.89 ± 3.60

[a]Values indicate mean ± standard error of mean (n = 3) measurements. TFC (mg catechin equivalent/g dry extract); TPC (mg GAE/g dry extract); DPPH = 2,2-diphenyl-1-picrylhydrazyl; reducing power = absorbance @ 0.5 ppm.

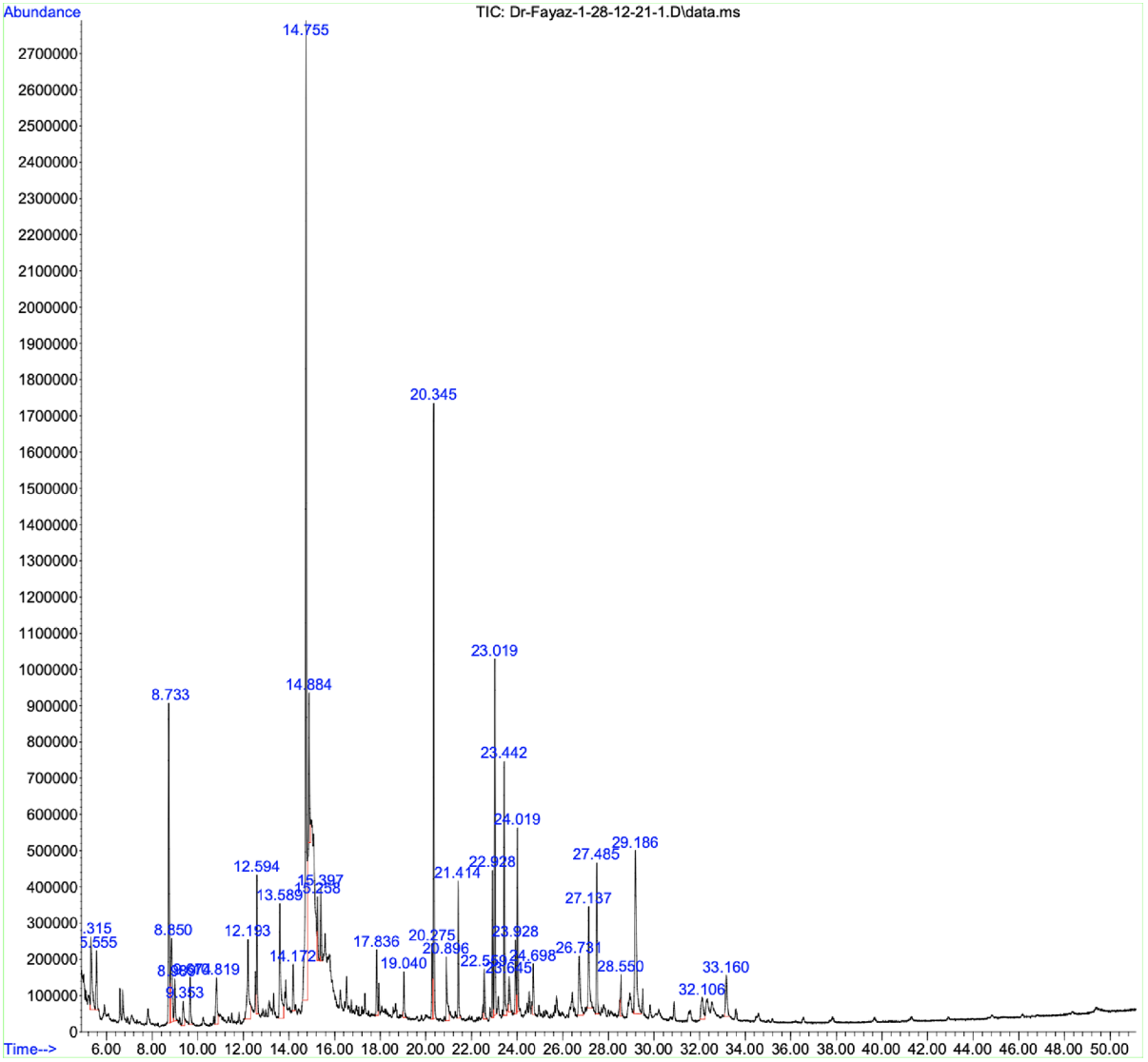

Figure 1. GC−MS spectrum of the aqueous methanolic extract of *T. linearis*.

they provoke many adverse effects like kidney failure, liver damage, GIT problems, etc. Recently, there has been growing interest in alternative therapies, especially from plant sources, due to their apparent lower side effects, affordability, and ease of accessibility.[7,8]

Thymus linearis is one of the most important genera of the Lamiaceae family, with common name Tumuro; aerial parts were used and comprised more than 350 species. It has been traditionally used for its carminative, antiseptic, analgesic, gout, antibacterial, antifungal, and antioxidant properties.[9,10] Active constituents of *T. linearis* include thymol, thymyl acetate, carvacrol, β-caryophyllene, cardiac glycosides, and saponins. Here, we have evaluated the cardioprotective effects of *T. linearis* extract in isoproterenol- and doxorubicin-induced cardiac injuries and have discussed the possible role of phytoconstituents by *in silico* studies.

RESULTS

Phytochemical Investigations. *T. linearis* with potent active phytoconstituents has a possible role in reducing the CVD burden. These constituents include considerable amounts of alkaloids, cardiac glycosides, and flavonoids.[11,12] The plant extract contained high amounts of flavonoids and phenols compared to tannins and alkaloids, while anthraquinone was absent (Table 1 and Tables S1 and S2).

In the case of total phenolic contents (TPCs) and total flavonoid contents (TFCs), the contents were found to be 78.02 ± 7.30 GAE mg/g DW (dry weight) and 45.40 ± 4.20 CE (catechin equivalents) mg/g DW, respectively (Table 3).

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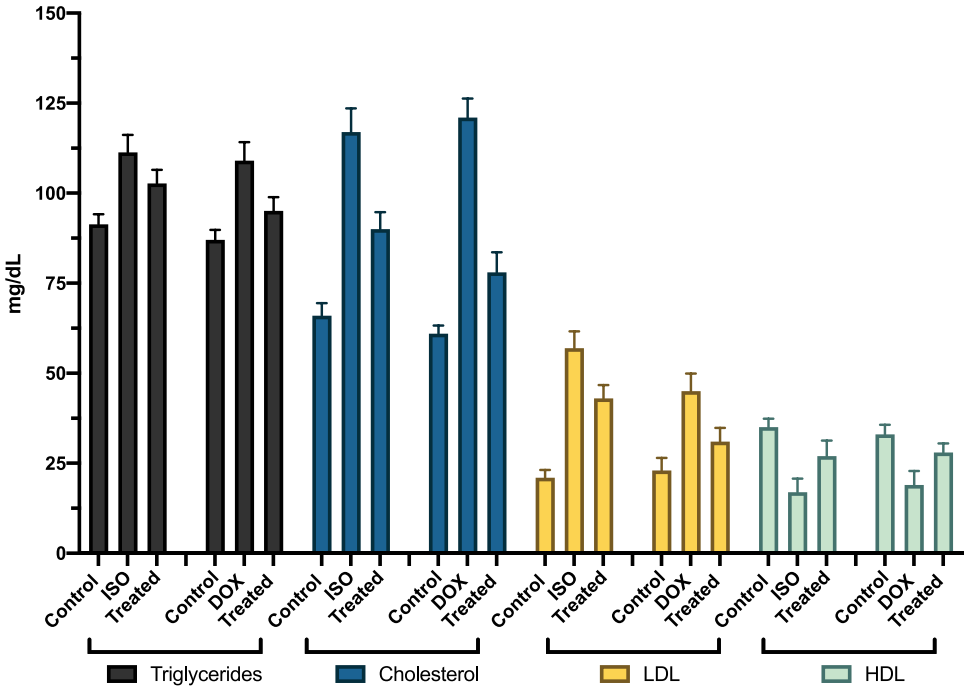

Figure 2. Effects of aqueous methanolic extract on ISO/DOX-induced heart injury in rats with respect to serum lipid level (mg/dL) changes. Data are represented as means ± SEM of five rats. $^{*}p < 0.05$ vs control, while $^{\#}p < 0.05$ vs isoproterenol group.

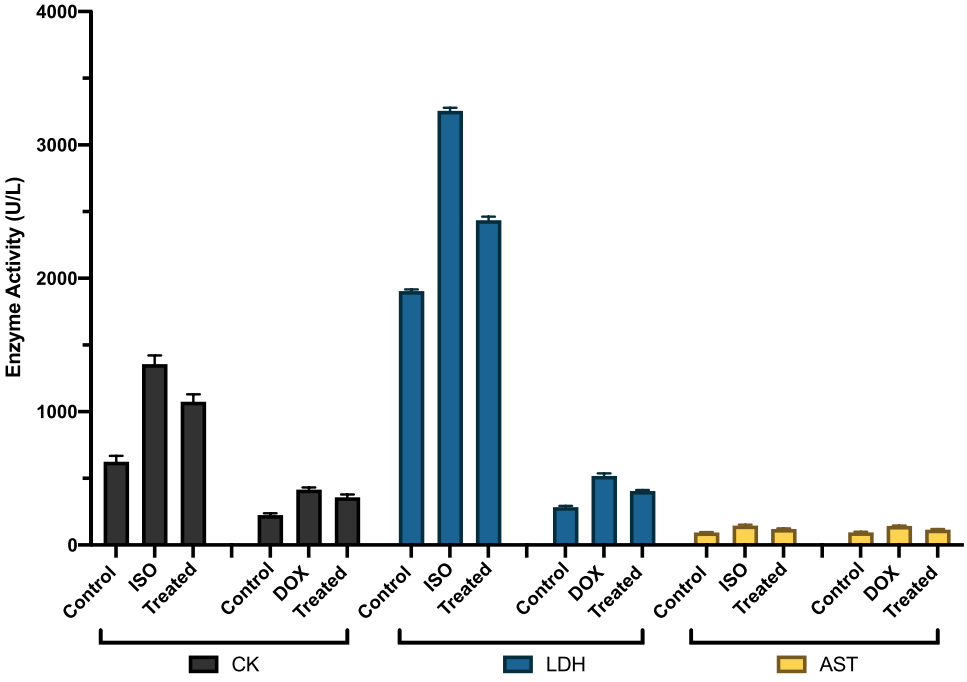

Figure 3. Effects of administration of aqueous methanolic extract *T. linearis* on creatine phosphokinase, lactic dehydrogenase, and aspartate aminotransferase in ISO/DOX-induced heart injury in SD rats. Values are represented as the means ± SEM ($n = 5$); plant extract of *T. linearis* was administered orally to rats with a dose of 100 mg/kg/day for 21 successive days, while isoproterenol (85 mg/kg, s.c.) was injected on days 20 and 21. $^{*}p < 0.05$ vs comparison with control animals, while $^{\#}p < 0.05$ vs isoproterenol group.

The *T. linearis* extract has shown considerable scavenge superoxide and DPPH (1,1-diphenyl-2-picrylhydrazyl) inhibition activities and improved ferric reducing antioxidant power (Table 3).[26,27] Furthermore, the analysis of polyphenols by HPLC showed the presence of gallic acid (0.151 mg/g), caffeic acid (0.124 mg/g), and benzoic acid (10.81 mg/g) (Figure S1). GC−MS showed the presence of 82 different compounds based on identified peaks in the GC−MS spectra (Figure 1). These compounds include flavonoids, phenolics, and some alkaloids (Table S1).

***In Vivo* Studies.** *Effect of T. linearis on the Lipid Profile, Cardiac Enzymes, and Antioxidants.* Ischemic muscle rapidly generates the radical species derived from reactive oxygen species (ROS) when the levels of cellular antioxidant enzymes decline.[13] The current assessment was intended to evaluate the potential preventive/protective effects of the extract of *T.*

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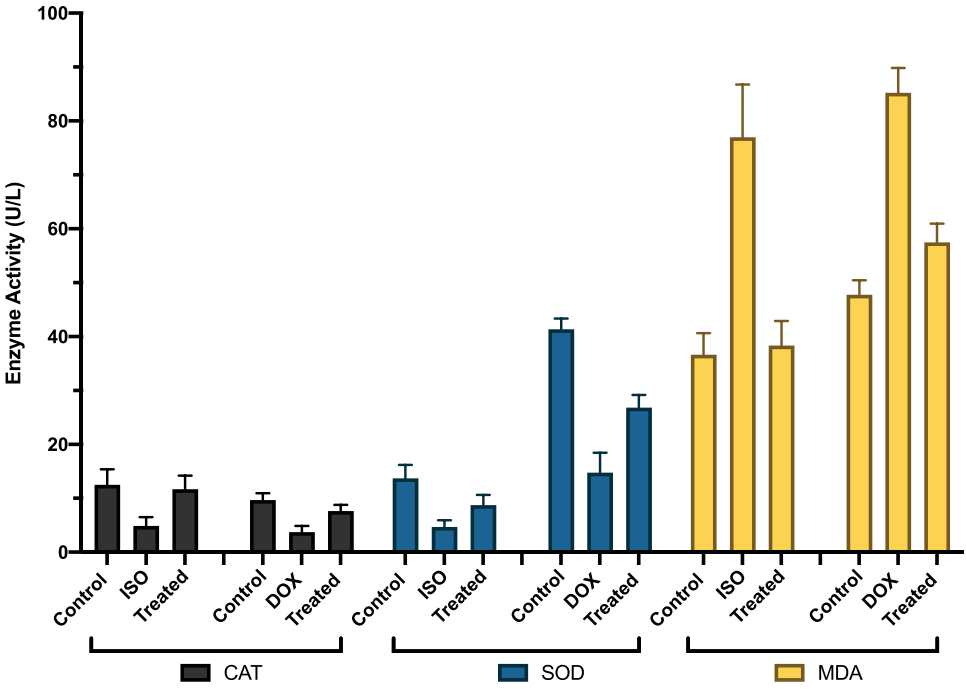

Figure 4. Effects of aqueous methanolic extract on CAT, SOD, and MDA in ISO/DOX-induced heart injury in rats. *T. linearis* (100 mg/kg/day for 21 successive days) was given orally to rats, while isoproterenol (85 mg/kg; subcutaneous) was injected on 2 (20th and 21st) days. Data are represented as the means ± SEM of five animals. CAT = catalase, SOD = superoxide dismutase, MDA = malondialdehyde. Values are expressed as means ± SEM (n = 5). *T. linearis* was given orally to rats for 10 successive days, having a dose of 100 mg/kg/day, while doxorubicin (15 mg/kg; intraperitoneally) was injected on the 7th day of the protocol. $^{*}p < 0.05$ vs control, while $^{\#}p < 0.05$ vs doxorubicin group.

linearis (crude extract) against isoproterenol-induced cardiotoxicity in rats, as shown in Figures 2−4. In both ISO/DOX treatment groups, the triglyceride levels were slightly high, but cholesterol and LDL levels were significantly higher (Figure 2). In comparison, the *T. linearis*-treated group showed normal cholesterol and LDL levels that agree with the antihyperlipidemic effects of *T. linearis*. Furthermore, HDL levels were slightly increased in the *T. linearis*-treated group.

The increased levels of CK, LHD, and AST depict the ISO/DOX-induced myocardial ischemia. Isoproterenol with a dose of 85 mg/kg subcutaneously given in two doses on the 20th and 21st days and doxorubicin in a dose of 15 mg/kg intraperitoneally given on 7th day injections to different rat groups significantly elevated serum CPK, LDH, and MDA levels, which are secreted from damaged heart muscles, which are sensitive markers or indicators of heart injury (Figure 3).

The antioxidant levels were also measured in all treatment groups. Catalase (CAT) and superoxide dismutase (SOD) levels were considerably lowered in ISO/DOX-induced heart injury in rats, while MDA levels were increased (Figure 4). The higher MDA levels show overall oxidative damage to cell membranes and may indicate damage to heart muscles.

Histopathological Investigations. Histopathological investigations were done according to average grading, which indicates the myocardial texture in the current study, as shown in (Figure 5). Isoproterenol and/or doxorubicin administration to different groups of animals caused significant changes in the myocardium like edema, neutrophilic infiltration, and necrosis in the diseased group as compared to the normal control group. Furthermore, animals treated with the extract of *T. linearis* showed reversed effects or improved myocardium tissues compared to isoproterenol and/or doxorubicin control groups. The plant extract-treated groups have shown almost similar parameters in relation to the control group.

Acute Toxicity. Albino mice of both sexes were used for the acute toxicity study and were randomly divided into five groups having n = 5. Normal saline (10 mg/kg) was administered (i.p.) in group 1, which was considered a control. The remaining groups, 2 to 4, were administered various doses of plant extract orally in increasing/ascending order like 1000, 2000, and 3000 mg/kg i.p. body weight. The animals were kept for 24 h to check their mortality. Further, the highest dose, which did not kill the animal, and the lowest dose, which killed one animal, were noticed. The geometric means of the two highest and the lowest one were used to calculate LD_{50}, while our plant extract has shown the median lethal dose (LD_{50}) at the dose of 3000 mg/kg in albino mice. Meanwhile, the extract did not show symptoms like alteration in body weights or organ weight in animals in the subacute toxicity study.

***In Silico* Activities.** A database of 30 phytochemical structures from *T. linearis* was prepared (Table S3) and optimized before molecular docking. NF-κB, COX2, and MCL were optimized and prepared as receptor molecules. Virtual screening was performed to obtain ligand−protein interactions, and binding energies were calculated along with dissociation constants to determine the ligand−protein affinity.

In the case of NF-κB, γ-cadinene (**4**) was found to be the best ligand with a binding energy of −7.86 kcal/mol and a dissociation contact of 1.7 μM (Table 2). The binding site residues consisted of Phe353, Arg354, Phe355, Arg356, Val358, Gly361, Pro362, Ser363, His364, Gly365, Gly366, Val412, Gly413, Lys414, Asn436, Leu437, Gly438, Ile439, and Leu440 (Figure 6, left panel). The ligands' interactions with the amino acid residues are maintained by hydrophobic interactions (Figure 6, left panel). β-Bisabolene (**6**), thymol

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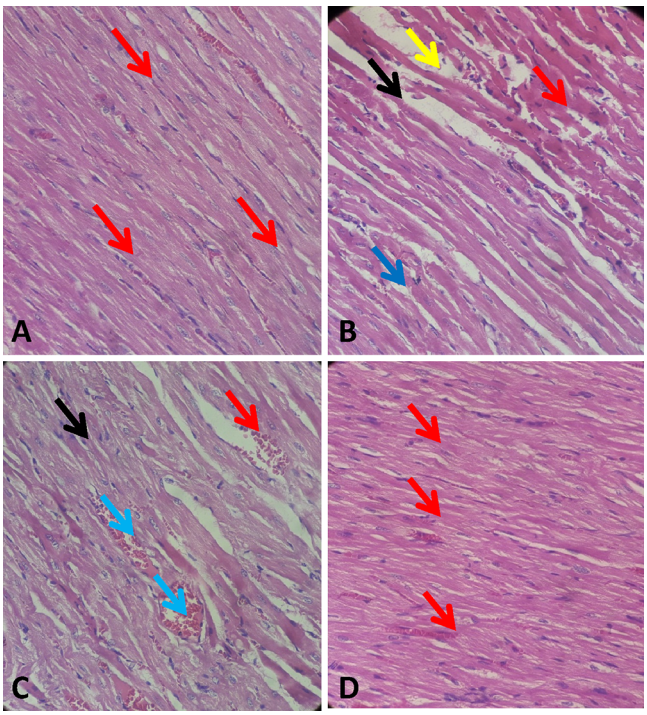

Figure 5. Photomicrograph of a rat's heart: H&E staining, 40×. (A) Normal morphology revealing normal cardiac architecture with the uniform myofibrillar arrangement (red arrows) without any abnormal histopathological changes. (B) Isoproterenol-induced mildly distorted cardiac architecture (blue arrow), mild edema (black arrow), mild degenerative changes (red arrows), and mild lymphocytic infiltration (yellow arrow). (C) Doxorubicin-induced mildly distorted cardiac architecture with mild edema (red arrow), dilated blood vessels (blue arrows), and mild lymphocytic infiltration (black arrow). (D) Normal morphology revealing normal cardiac architecture with the uniform myofibrillar arrangement (red arrows) without any abnormal histopathological changes.

acetate (**20**), and α-bisabolene (**5**) are found to be the next best binding ligands with binding energies of −7.42, −7.30, and −7.11 kcal/mol, respectively (Table 2). The binding energies and dissociation constant for the remaining compounds are given in Table S3.

β-Bisabolene (**6**) showed the string binding to COX2 with a binding energy of −8.19 kcal/mol and a dissociation contact of 0.98 μM (Table 3). The binding site consisted of Leu123, Ile124, Asp125, Ser126, Pro127, Pro128, Thr129, Tyr130, Thr149, Arg150, Ala151, Leu152, Gln374, Asn375, Arg376, Ala378, Arg469, Phe470, Phe529, and Lys532 residues. The ligands' interactions are maintained by the hydrophobic interactions (Figure 6, right panel). β-Caryophyllene (**27**), α-bisabolene (**5**), and α-hummulene (**3**) are found to be the next best binding ligands with binding energies of −7.93, −7.71, and −7.63 kcal/mol, respectively (Table 3). The binding energies and dissociation constants for the remaining compounds are given in Table S4.

For MCL, the best binding is shown by γ-cadinene (**4**), which binds with an energy of −8.12 kcal/mol and a dissociation contact of 1.12 μM (Table 4). The binding site residues consisted of Val230, Pro231, Thr232, His235, Ala262, Ala263, Leu264, Pro265, Asp268, Lys390, Ser391, Gly392, Gly393, Asp421, Pro422, Asp423, Leu424, Gly425, and Glu426 (Figure 7). Caryophyllene (**27**), germacrene D (**12**), and β-bisabolene (**6**) were found to be the next three ligands with binding energies of −7.25, −7.18, and −6.92 kcal/mol, respectively (Table 4).

DISCUSSION

Reactive oxygen species (ROS) or free radicals damage the tissues and biomolecules, progressing to various diseases, especially degenerative diseases (cardiovascular disorders, diabetes, endothelial dysfunction, etc.).[14] Multiple synthetic drugs exhibit substantial protection against oxidative stress/damage, but they have many adverse effects like cardiovascular diseases, hepatotoxicity, nephrotoxicity, and gastrointestinal tract problems.[15,16] Alternative options for safer use are the consumption of traditional medicine, natural antioxidants from food supplements, and herbal and polyherbal formulations.[17] Doxorubicin has been used extensively and effectively as a broad-spectrum anticancer drug, although its medical use has been limited due to its severe dose-dependent cardiotoxicity in humans.[18] Experimental and clinical analysis suggested that increased oxidative stress and/or ROS play a crucial role in consequent cardiomyopathy and cardiac failure related to the doxorubicin treatment model. Because oxidative stress and/or ROS are a keystone in doxorubicin-induced cardiotoxicity in humans or animals, it was sensible to understand the levels of oxidants and/or antioxidants in rats of this study.[19] Many phytochemicals have been reported to protect against DOX-induced cardiotoxicity.[20]

Isoproterenol or isoprenaline is a β-adrenergic agonist, a synthetic catecholamine derivative of noradrenaline. The administration of isoproterenol (85 mg/kg s.c.) causes oxidative stress mostly through the stimulation of β-1 adrenergic receptor found in the heart muscles,[21] which are accountable for the acute chronotropic and positive inotropic effects on cardiac tissues, leading to an imbalance between energy intake by the blood flow and increased oxygen demand progressing to myocardial ischemia.

T. linearis shows various biological activities, including antimicrobial, antifungal, hepatoprotective, antihypertensive, and cardioprotective activities.[8,22,23] In a previous study, 25 mg/kg isoproterenol was used to induce toxicity, and *T. linearis*

Table 2. Ligands' Interactions with NF-κB

ligand	name	bind. energy [kcal/mol]	dissoc. constant[μM]	contacting receptor residues
4	γ-cadinene	−7.86	1.73	Phe353, Arg354, Phe355, Arg356, Val358, Gly361, Pro362, Ser363, His364, Gly365, Gly366, Val412, Gly413, Lys414, Asn436, Leu437, Gly438, Ile439, Leu440
6	β-bisabolene	−7.42	3.64	Phe353, Arg356, Tyr357, Val358, Cys359, Glu360, Gly361, Pro362, Ser363, His364, Gly365, Gly366, Val412, Gly413, Lys414, Asn436, Leu437, Gly438, Ile439, Leu440
20	thymol acetate	−7.3	4.49	Phe353, Arg354, Phe355, Arg356, Gly361, Pro362, Ser363, His364, Gly365, Gly366, Val412, Gly413, Asn436, Leu437, Gly438, Ile439, Leu440
5	α-bisabolene	−7.11	6.15	Phe353, Arg356, Tyr357, Val358, Cys359, Glu360, Gly361, Pro362, Ser363, His364, Gly365, Gly366, Val412, Gly413, Asn436, Leu437, Gly438, Ile439, Leu440, His441

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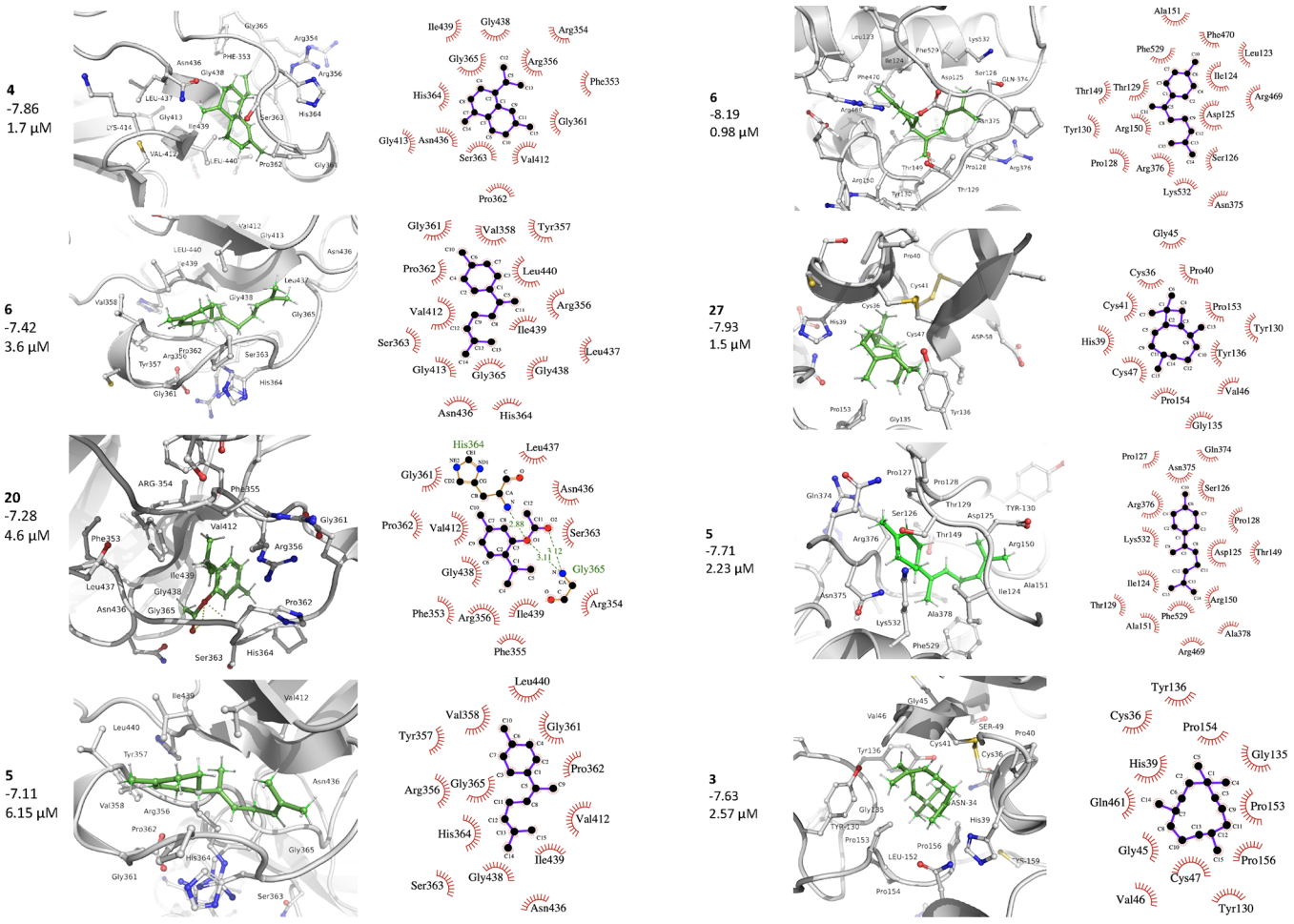

Figure 6. In the case of NF-κB (left panel), γ-cadinene (**4**), β-bisabolene (**6**), thymol acetate (**20**), and α-bisabolene (**5**) show the best binding energies, while COX2 (right panel) shows the best binding to β-bisabolene (**6**), β-caryophyllene (**27**), α-bisabolene (**5**), and α-hummulene (**3**).

Table 3. Ligands' Interactions with COX2

lig.	name	bind. energy [kcal/mol]	dissoc. constant [μM]	contacting receptor residues
6	β-bisabolene	−8.19	0.98	Leu123, Ile124, Asp125, Ser126, Pro127, Pro128, Thr129, Tyr130, Thr149, Arg150, Ala151, Leu152, Gln374, Asn375, Arg376, Ala378, Arg469, Phe470, Phe529, Lys532
27	β-caryophyllene	−7.93	1.53	Cys36, His39, Pro40, Cys41, Gly45, Val46, Cys47, Met48, Tyr130, Gly135, Tyr136, Leu152, Pro153, Pro154, Pro156, Gln461
5	α-bisabolene	−7.71	2.23	Leu123, Ile124, Asp125, Ser126, Pro127, Pro128, Thr129, Tyr130, Thr149, Arg150, Ala151, Gln374, Asn375, Arg376, Ile377, Ala378, Arg469, Phe529, Lys532
3	α-hummulene	−7.63	2.57	Asn34, Cys36, His39, Pro40, Cys41, Gly45, Val46, Cys47, Tyr130, Gly135, Tyr136, Leu152, Pro153, Pro154, Val155, Pro156, Gln461

Table 4. Ligands' Interactions with MCL

lig.	name	bind. energy [kcal/mol]	dissoc. constant [μM]	contacting receptor residues
4	γ-cadinene	−8.12	1.12	Val230, Pro231, Thr232, His235, Ala262, Ala263, Leu264, Pro265, Asp268, Lys390, Ser391, Gly392, Gly393, Asp421, Pro422, Asp423, Leu424, Gly425, Glu426
27	β-caryophyllene	−7.25	4.84	Val141, Leu183, Pro184, His187, Val188, His189, Gly190, Val230, Ser261, Arg283, Met286, Thr287, Met291, Lys390, Gly392, Gly393, Tyr394, Lys395
12	germacrene D	−7.18	5.48	Thr143, Leu183, Pro184, His187, Val188, His189, Gly190, Val230, Ser261, Arg283, Gly285, Met286, Thr287, Met291, Lys390, Gly393, Tyr394, Lys395
6	β-bisabolene	−6.92	8.49	Thr143, Ser144, Leu183, Pro184, His187, Val188, His189, Gly190, Leu191, Val230, Ser261, Thr287, Lys390, Gly393, Tyr394, Lys395

aqueous methanolic (30:70) root extract was used to observe and obtain cardioprotective effects.[24] This study uses the aerial parts of *T. linearis* for extract preparation and to evaluate the protection against isoproterenol- and/or doxorubicin-induced

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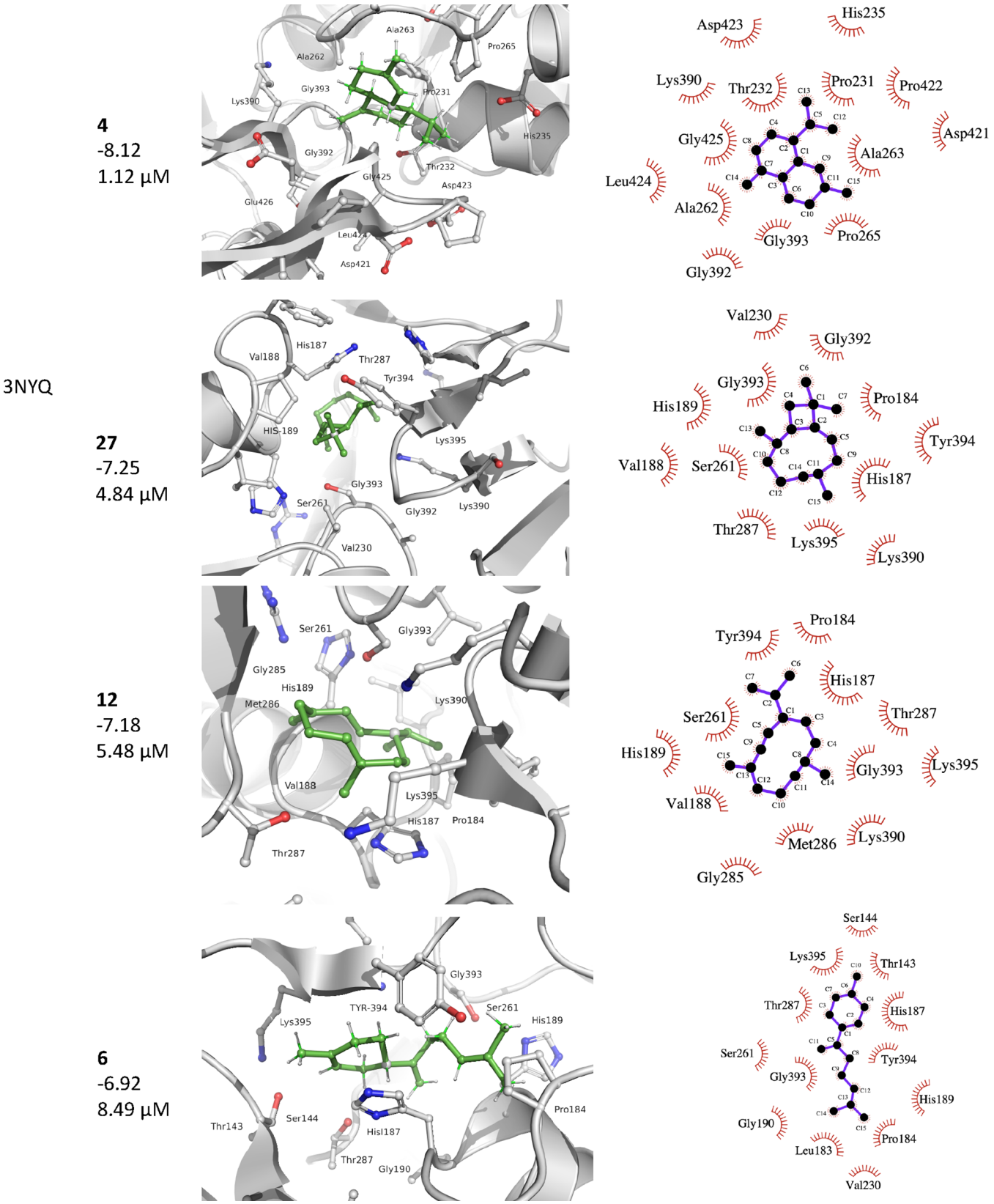

Figure 7. In the case of MCL, γ-cadinene (**4**), caryophyllene (**27**), germacrene D (**12**), and β-bisabolene (**6**) showed the best binding energies.

heart injury. Our research data clearly shows overt oxidative stress induced in animals, which is in line with a previous report by scientists.[25] The current assessment was to investigate the potential protective or preventive effect of the aqueous methanolic extract of *T. linearis* against doxorubicin-induced myocardial injury in the rat model.

Due to cardiac damage, CAT and SOD levels were reduced, while MDA and oxidized glutathione were extensively elevated

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in doxorubicin-induced animals compared to control animals. Pretreatment of SD rats with *T. linearis* significantly protected against the oxidative stress measured in isoproterenol and doxorubicin animal groups. In the ISO/DOX treatment groups, SOD and CAT were extensively reduced, while MDA was considerably elevated compared to control rats. Furthermore, data suggested that our results are in line with earlier reports by different researchers.[26,27] The *T. linearis* extract treatment normalized the antioxidant enzyme activities, which may be elucidated based on their exhaustion in combating observed oxidative stresses in ISO/DOX-induced heart injury.

Our study results have also shown that plant extract treatment decreased the depletion of the antioxidant enzymes. This explains and confirms the previously observed GSH depletion and MDA and other enzyme accumulation in cardiac and other tissues of the body. This can be explained based on the active ingredients of plants with a high content of numerous antioxidants such as alkaloids, cardiac glycosides, flavonoids, and phenolics.[28,29]

T. linearis (carvacrol) is a big source of active compounds that inhibit oxidative stress/progression in the heart and other body tissues.[30] Cardiac enzymes also increased in ISO/DOX-induced heart injury,[18,31,32] while elevated lipid profile levels, including cholesterol, triglycerides, and LDL, were observed. HDL levels were decreased compared with the control group. The treatment with *T. linearis* extract significantly lowered the cardiac enzyme levels, such as LDH, CK, and AST, showing the reversal of enzyme levels for the protection of the cardiac muscles. Histological examination shows signs of reduced transverse striations, inflammation, and degeneration in both ISO/DOX-induced heart injuries, while after the treatment of *T. linearis* extract, considerable recovery of the heart muscle was observed with a well-preserved cardiac cell structure.

In the case of *in silico* studies with NF-κB, COX2, and MCL, the virtual screen shows that phytoconstituents including cadinene, caryophyllene, thymol acetate, bisabolene, and germacrene show considerable inhibition of the selected proteins. Fruit oils of cinnamon are rich in caryophyllene and cadinene; these are reported for the management of cardiovascular diseases.[33] Caryophyllene and cadinene also show antimicrobial properties.[34] Germacrene is a major component of many oils from *Phlomis* species showing antioxidant and antiatherogenic properties.[35] NF-κB and COX2 play important roles in inflammation pathways, while MCL is malonyl-CoA, a fatty acid synthesis precursor. Previously, an *in silico* screening of some flavonols showed that morin potentially inhibited malonyl coenzyme A and may act as a cardioprotective agent.[36] A formulation (COMB) consisting of syringic acid (SA) and resveratrol (RV) was evaluated for cardioprotective activities; it lowered the lipid markers and showed potential binding with NF-κB in virtual screening.[37] Here, it seems that caryophyllene and cadinene in the *T. linearis* extract might inhibit NF-κB and COX2, reduce inflammatory responses, lower oxidative stress, and protect cells from stress-induced damage. Meanwhile, MCL inhibition shows reduced fatty acid biosynthesis and lower lipid profile markers. *In silico* studies correspond well with our *in vivo* and *in vitro* results in this study.

CONCLUSIONS

From the current study, it is evident that *T. linearis* possesses cardioprotective effects. The results obtained indicate its potential in protecting the rats from isoproterenol- and doxorubicin-induced myocardial infarction. The plant extract alleviated the biochemical parameters and prevented oxidative stress and other enzyme induction by isoproterenol and/or doxorubicin models. Therefore, the therapeutic success of *Thymus* in the folkloric claim of traditional remedies may be due to their antioxidant, antilipoperoxidation, and antihyperlipidemic activities.

MATERIALS AND METHODS

Reagents and Chemicals. The drugs and chemicals used in this study include isoproterenol and doxorubicin (Sigma Aldrich), methanol, and ethanol (Merck). Kits used for biochemical parameters were purchased from Randox Laboratories, UK. All the chemicals used were of analytical grades.

Plant Extract Preparation. *T. linearis* Benth. (aerial parts) was collected from part of Gilgit Baltistan, Pakistan. The medicinal plant was identified and authenticated, and a voucher specimen (AK-6031) was deposited in the Herbarium, University of Sargodha, Sargodha, Pakistan. The aerial parts were shade-dried and ground to powder, followed by extraction and maceration in aqueous methanol (30:70) for 3 days with occasional shaking. The plant extract was concentrated by using a rotary evaporator. The solidified extract was stored in a cool place in amber-colored bottles, and the percentage yield was calculated.

Phytochemical Analysis. Different qualitative and quantitative tests were conducted for an aqueous-methanolic extract for the presence of different phytoconstituents, including alkaloids, tannins, reducing sugars, saponins, flavonoids, phenolic contents, and cardiac glycosides using standard protocols.[38]

GC−MS Analysis. The plant extract was subjected to GC−MS analysis using a gas chromatography instrument (GC 7890A) coupled with MS (5977B, with single quadrupole) (Agilent, USA). Separation was performed using a 0.25 μm DB-5MS fused-silica capillary column (0.25 mm in diameter) (J&W Scientific, Folsom, CA). The mass spectrum was scanned (at a rate of 1.5 scans/s) from m/z 35 to 650, peaks were identified using the MassHunter library, and the peak area (%) was used for quantification. The MS source temperature was 230 °C, while the Quadrupole MS temperature was 150 °C. Helium gas was used as a carrier with a flow rate of 1 mL/min. For sample preparation, water contents were removed from the sample in a drying oven, and the semisolid sample was dissolved in methanol before injecting into the GC−MS instrument.

Total Flavonoid and Total Phenolic Assays. The total flavonoid contents (TFCs) and total phenolic contents (TPCs) of the *T. linearis* extract were determined using modified standard protocols.[39,40] The selective phenolic compounds were also analyzed by high-performance liquid chromatography (HPLC, Agilent, USA) using the phenolic standards, including chlorogenic acid, caffeic acid, gallic acid, synapic acid, and benzoic acid. The filtered aqueous-methanolic extract (20 μL, 0.22 μm filtered) was injected and subjected to fractionation at a speed of 0.6 mL/min using a mobile phase (dH_2O/formic acid (99:1, v/v) (solvent A) and acetonitrile/formic acid (99:1, v/v) (solvent B)).

Determination of Antioxidant Activities. *DPPH Scavenging and Reducing Power Assays.* Free radical scavenging activity was carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay with slight modifications.[41] Reducing power

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Table 5. Groups in Isoproterenol-Induced Cardiotoxicity

		treatment	
		days 1−19	days 20 and 21
group 1	control group	distilled water daily (1 mL/kg, orally)	distilled water daily (1 mL/kg, orally)
group 2	drug control (ISO)	distilled water daily (1 mL/kg, orally)	ISO (85 mg/kg, subcutaneous)[44]
group 3	treated group	plant extract (100 mg/kg) orally + water	plant extract (100 mg/kg) orally + ISO (85 mg/kg, subcutaneous) + water

Table 6. Groups in Doxorubicin-Induced Cardiotoxicity

		treatment		
		days 1−6	day 7	days 7−10
group 1	control group	distilled water daily (1 mL/kg, orally)	distilled water daily (1 mL/kg, orally)	distilled water daily (1 mL/kg, orally)
group 2	drug control (DOX)	distilled water daily (1 mL/kg, orally)	distilled water daily (1 mL/kg, orally) + DOX (15 mg/kg, i.p.)	distilled water daily (1 mL/kg, orally)
group 3	treated group	plant extract (100 mg/kg) orally + water (1 mL/kg, orally)	plant extract (100 mg/kg) orally + water (1 mL/kg, orally) + DOX (15 mg/kg, i.p.)	plant extract (100 mg/kg) orally + water (1 mL/kg, orally)

assays were performed using potassium ferricyanide and $FeCl_3$ method.[42]

***In Vivo* Studies.** In the current study, Sprague Dawley rats (weighing 200−250 g) and albino mice (25−30 g) of either sex were obtained and acclimatized in the Animal House, University of Sargodha, Sargodha. The animals were fed on the excess of commercial rat chow as guided by the National Institute of Research Council.[43] Ethical approval was given by the Biosafety and Ethical Review Committee, the University of Sargodha, with a reference number SU/ORIC/58.

Isoproterenol (ISO)-Induced Cardiotoxicity. After acclimatization for 1 week, rats were randomly divided into three groups; each group consisted of five rats and was treated as given in Table 5.

At the end of the experiment, the rats were euthanized within 24 h after the last isoproterenol injection. Blood samples were collected by cardiac punctures (after anesthesia induced by diethyl ether) in two test tubes for serum and plasma collection.

Doxorubicin (DOX)-Induced Cardiotoxicity. The Sprague Dawley rats were divided randomly into three groups (five rats per group), and a treatment set of 10 days[45] was selected, as shown in Table 6. After 24 h of the last treatment (day 11), rats were euthanized. The sera and plasma were collected for biochemical assays, as said above.

Biochemical Assays. Different kits were used for biochemical assays of cardiac markers, liver function enzymes, and lipid profiles according to the manufacturer's instructions. The sera collected from animals' blood were used for the estimation of lactate dehydrogenase (LDH), creatine phosphokinase (CPK), aspartate aminotransferase (AST), and lipid profile. Endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation such as malondialdehyde (MDA) levels were estimated from blood plasma.

Toxicological Studies. The acute toxicity test was carried out as stated by the OECD guidelines.[46] The albino mice of both sexes were randomly divided into two groups (n = 5). Group 1 served as a control and received normal saline (10 mL/kg). At the same time, group 2 was administered different doses of the aqueous-methanolic extract of *T. linearis* in an increasing concentration, i.e., 1000, 2000, and 3000 mg/kg i.p. The mortality rate was observed for 24 h, and mice were kept under observation for 24 h for behavioral changes (restlessness, dullness, and agitation) with signs of toxicity and mortality.[47] LD_{50} was determined at different extract concentrations.[48] The number of animals dying during the first 12 h and then in the next 24 h was observed.

Histopathological Examination of Heart Tissues. The animals were euthanized with anesthesia (diethyl ether). The animal hearts were then removed after thorax surgery and fixed in 10% buffered formalin. Heart tissues were embedded in paraffin with 5 μm sections and stained with H&E (hematoxylin and eosin) for histology evaluation. Myocardial fibrosis and necrosis were evaluated in each section of the heart tissue using a microscope attached to a digital camera.[44]

***In Silico* Studies.** A database of the *T. linearis* phytoconstituents was prepared by downloading their structures from PubChem (https://pubchem.ncbi.nlm.nih.gov/, accessed on 25 August 2021) and ChemSpider (https://chemspider.com, accessed on 25 August 2021) (Table S1). The structures were optimized by an MM1 force field in YASARA (ver. 20.7.4).[49] The protein structures for NF-κB (PDB ID: 3GUT), cyclooxygenase-2 (COX2) (PDB ID: 5IKR), and malonyl-CoA ligase (MCL) (PDB ID: 3NYQ) were downloaded from Protein 3NYQ Bank (PDB) (https://www.rcsb.org/). The protein structures were prepared for docking by removing the bound ligand and deleting extra chains. The YASARA Structure (ver. 20.7.4) software was used for virtual screening using a modified AutoDock-Lamarckian Genetic Algorithm and a standard parameter described elsewhere.[29,50,51] Protein−ligand interactions were studied to rank the best binding ligands with respect to binding energy and dissociation constants. The figures were prepared using PyMol and LigPlot.

Statistical Analysis. The results were obtained using statistical analysis of means ± SEM (n = 5). Statistical analysis was performed using one-way ANOVA (analysis of variance) followed by Tukey's multiple comparison tests using GraphPad Prism (ver. 8.0). $p < 0.05$ was considered statistically significant.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c04544.

Figure S1: chromatogram phenolic contents of the *T. linearis* extract with reference standards using HPLC; Table S1: GC−MS analysis of the plant extract; Table S2: phytochemical test of the selected plant *T. linearis*;

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Table S3: phytochemical screening against GUT3; Table S4: phytochemical screening against COX2 (PDF)

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Notes

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REFERENCES

(1) Roth, G. A.; Johnson, C.; Abajobir, A.; Abd-Allah, F.; Abera, S. F.; Abyu, G.; Ahmed, M.; Aksut, B.; Alam, T.; Alam, K.; Alla, F.; Alvis-Guzman, N.; Amrock, S.; Ansari, H.; Ärnlöv, J.; Asayesh, H.; Atey, T. M.; Avila-Burgos, L.; Awasthi, A.; Banerjee, A.; Barac, A.; Bärnighausen, T.; Barregard, L.; Bedi, N.; Belay Ketema, E.; Bennett, D.; Berhe, G.; Bhutta, Z.; Bitew, S.; Carapetis, J.; Carrero, J. J.; Malta, D. C.; Castañeda-Orjuela, C. A.; Castillo-Rivas, J.; Catalá-López, F.; Choi, J. Y.; Christensen, H.; Cirillo, M.; Cooper, L., Jr.; Criqui, M.; Cundiff, D.; Damasceno, A.; Dandona, L.; Dandona, R.; Davletov, K.; Dharmaratne, S.; Dorairaj, P.; Dubey, M.; Ehrenkranz, R.; el Sayed Zaki, M.; Faraon, E. J. A.; Esteghamati, A.; Farid, T.; Farvid, M.; Feigin, V.; Ding, E. L.; Fowkes, G.; Gebrehiwot, T.; Gillum, R.; Gold, A.; Gona, P.; Gupta, R.; Habtewold, T. D.; Hafezi-Nejad, N.; Hailu, T.; Hailu, G. B.; Hankey, G.; Hassen, H. Y.; Abate, K. H.; Havmoeller, R.; Hay, S. I.; Horino, M.; Hotez, P. J.; Jacobsen, K.; James, S.; Javanbakht, M.; Jeemon, P.; John, D.; Jonas, J.; Kalkonde, Y.; Karimkhani, C.; Kasaeian, A.; Khader, Y.; Khan, A.; Khang, Y. H.; Khera, S.; Khoja, A. T.; Khubchandani, J.; Kim, D.; Kolte, D.; Kosen, S.; Krohn, K. J.; Kumar, G. A.; Kwan, G. F.; Lal, D. K.; Larsson, A.; Linn, S.; Lopez, A.; Lotufo, P. A.; el Razek, H. M. A.; Malekzadeh, R.; Mazidi, M.; Meier, T.; Meles, K. G.; Mensah, G.; Meretoja, A.; Mezgebe, H.; Miller, T.; Mirrakhimov, E.; Mohammed, S.; Moran, A. E.; Musa, K. I.; Narula, J.; Neal, B.; Ngalesoni, F.; Nguyen, G.; Obermeyer, C. M.; Owolabi, M.; Patton, G.; Pedro, J.; Qato, D.; Qorbani, M.; Rahimi, K.; Rai, R. K.; Rawaf, S.; Ribeiro, A.; Safiri, S.; Salomon, J. A.; Santos, I.; Santric Milicevic, M.; Sartorius, B.; Schutte, A.; Sepanlou, S.; Shaikh, M. A.; Shin, M. J.; Shishehbor, M.; Shore, H.; Silva, D. A. S.; Sobngwi, E.; Stranges, S.; Swaminathan, S.; Tabarés-Seisdedos, R.; Tadele Atnafu, N.; Tesfay, F.; Thakur, J. S.; Thrift, A.; Topor-Madry, R.; Truelsen, T.; Tyrovolas, S.; Ukwaja, K. N.; Uthman, O.; Vasankari, T.; Vlassov, V.; Vollset, S. E.; Wakayo, T.; Watkins, D.; Weintraub, R.; Werdecker, A.; Westerman, R.; Wiysonge, C. S.; Wolfe, C.; Workicho, A.; Xu, G.; Yano, Y.; Yip, P.; Yonemoto, N.; Younis, M.; Yu, C.; Vos, T.; Naghavi, M.; Murray, C. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. *J. Am. Coll. Cardiol.* **2017**, *70*, 1−25.

(2) Liaquat, A.; Javed, Q. Current trends of cardiovascular risk determinants in Pakistan. *Cureus* **2018**, *10*, No. e3409.

(3) Saleh, M.; Ambrose, J. A. Understanding myocardial infarction. *F1000Res* **2018**, *7*, DOI: 10.12688/f1000research.15096.1.

(4) Mythili, S.; Malathi, N. Diagnostic markers of acute myocardial infarction. *Biomed. Rep.* **2015**, *3*, 743−748.

(5) Zhang, X.; Liu, L.; Chen, W. C.; Wang, F.; Cheng, Y. R.; Liu, Y. M.; Lai, Y. F.; Zhang, R. J.; Qiao, Y. N.; Yuan, Y. Y. Gestational Leucylation Suppresses Embryonic T-Box Transcription Factor 5 Signal and Causes Congenital Heart Disease. *Adv. Sci.* **2022**, 2201034.

(6) Birdsall, H. H.; Green, D. M.; Trial, J.; Youker, K. A.; Burns, A. R.; MacKay, C. R.; LaRosa, G. J.; Hawkins, H. K.; Smith, C. W.; Michael, L. H.; Entman, M. L.; Rossen, R. D. Complement C5a, TGF-β1, and MCP-1, in sequence, induce migration of monocytes into ischemic canine myocardium within the first one to five hours after reperfusion. *Circulation* **1997**, *95*, 684−692.

(7) Tibaut, M.; Mekis, D.; Petrovic, D. Pathophysiology of myocardial infarction and acute management strategies. *Cardiovasc. Hematol. Agents Med. Chem.* **2016**, *14*, 150−159.

(8) Qadir, M. I.; Parveen, A.; Abbas, K.; Ali, M. Analgesic, anti-inflammatory and anti-pyretic activities of *Thymus linearis*. *Pak. J. Pharm. Sci* **2016**, *29*, 591.

(9) Hussain, A. I.; Anwar, F.; Chatha, S. A. S.; Latif, S.; Sherazi, S. T. H.; Ahmad, A.; Worthington, J.; Sarker, S. D. Chemical composition and bioactivity studies of the essential oils from two Thymus species from the Pakistani flora. *LWT - Food Sci. Technol.* **2013**, *50*, 185−192.

(10) Taverne, Y. J. H. J.; Bogers, A. J. J. C.; Duncker, D. J.; Merkus, D. Reactive oxygen species and the cardiovascular system. *Oxid. Med. Cell. Longevity* **2013**, *2013*, 1.

(11) Naz, A.; Saeed, M.; Hussain, M. M.; Ishaq, M. S. *In vitro* phytochemical and antimicrobial screening of *Thymus linearis*. *Bangladesh J. Pharmacol.* **2015**, *10*, 21−26.

(12) Shirazi, M. *In Vivo* Biological Investigation of methanolic extract of *Thymus linearis* whole plant. *Am. J. Ethnomed.* **2018**, *5*, 2.

(13) Shahzad, S.; Mateen, S.; Naeem, S. S.; Akhtar, K.; Rizvi, W.; Moin, S. Syringic acid protects from isoproterenol induced cardiotoxicity in rats. *Eur. J. Pharmacol.* **2019**, *849*, 135−145.
(14) Batool, A. I.; Naveed, N. H.; Aslam, M.; da Silva, J.; Rehman, M. F. U. Coal dust-induced systematic hypoxia and redox imbalance among coal mine workers. *ACS Omega* **2020**, *5*, 28204−28211.
(15) Liu, Z.; Su, W.; Ao, J.; Wang, M.; Jiang, Q.; He, J.; Gao, H.; Lei, S.; Nie, J.; Yan, X.; Guo, X.; Zhou, P.; Hu, H.; Ji, M. Instant diagnosis of gastroscopic biopsy via deep-learned single-shot femtosecond stimulated Raman histology. *Nat. Commun.* **2022**, *13*, 1−12.
(16) Akram, M. S.; Pery, N.; Butler, L.; Shafiq, M. I.; Batool, N.; Rehman, M. F. U.; Grahame-Dunn, L. G.; Yetisen, A. K. Challenges for biosimilars: focus on rheumatoid arthritis. *Crit. Rev. Biotechnol.* **2021**, *41*, 121−153.
(17) Tang, R. Z.; Li, Z. Z.; Hu, D.; Kanwal, F.; Yuan, C. B.; Mustaqeem, M.; Batool, A. I.; Rehman, M. F. U. Sanjie Yiliu Formula Inhibits Colorectal Cancer Growth by Suppression of Proliferation and Induction of Apoptosis. *ACS Omega* **2021**, *6*, 7761−7770.
(18) Iqbal, M.; Dubey, K.; Anwer, T.; Ashish, A.; Pillai, K. K. Protective effects of telmisartan against acute doxorubicin-induced cardiotoxicity in rats. *Pharmacol. Res.* **2008**, *60*, 382.
(19) Takemura, G.; Fujiwara, H. Doxorubicin-induced cardiomyopathy: from the cardiotoxic mechanisms to management. *Prog. Cardiovasc Dis.* **2007**, *49*, 330−352.
(20) Ojha, S.; Al Taee, H.; Goyal, S.; Mahajan, U. B.; Patil, C. R.; Arya, D. S.; Rajesh, M. Cardioprotective Potentials of Plant-Derived Small Molecules against Doxorubicin Associated Cardiotoxicity. *Oxid. Med. Cell. Longevity* **2016**, *2016*, 5724973.
(21) Zhou, R.; Xu, Q.; Zheng, P.; Yan, L.; Zheng, J.; Dai, G. Cardioprotective effect of fluvastatin on isoproterenol-induced myocardial infarction in rat. *Eur. J. Pharmacol.* **2008**, *586*, 244−250.
(22) Nawaz, M.; Ahmad, T.; Mushtaq, M. N.; Batool, A. Hepatoprotective activity of *Thymus linearis* against paracetamol-and carbon tetrachloride-induced hepatotoxicity in albino mice. *Bangladesh J. Pharmacol* **2014**, *9*, 230−234.
(23) Kumar, A.; Kamal, A.; Singh, S.; Padalia, R. C.; Tandon, S.; Chauhan, A.; Saikia, D.; Verma, R. S. Chemical composition, antimicrobial activity, kinetics and mechanism of action of Himalayan-thyme (*Thymus linearis* Benth). *J. Essent. Oil Res.* **2020**, *32*, 59−68.
(24) Arshad, L.; Irfan, H.; Noor, N.; Alotaibi, N.; Alharbi, K.; Bukhari, A. P. D. S. N. A.; Bukhari, S. N. A.; Althobaiti, Y.; Ullah, A.; Khan, S. Cardioprotective potential of *Thymus linearis* Benth. *Pak. J. Pharm. Sci* **2022**, 281−285.
(25) Ibrahim, M. A.; Ashour, O. M.; Ibrahim, Y. F.; El-Bitar, H. I.; Gomaa, W.; Abdel-Rahim, S. R. Angiotensin-converting enzyme inhibition and angiotensin AT1-receptor antagonism equally improve doxorubicin-induced cardiotoxicity and nephrotoxicity. *Pharmacol. Res.* **2009**, *60*, 373−381.
(26) Ojha, S.; Golechha, M.; Kumari, S.; Arya, D. S. Protective effect of *Emblica officinalis* (amla) on isoproterenol-induced cardiotoxicity in rats. *Toxicol. Ind. Health* **2012**, *28*, 399−411.
(27) Razavi, B. M.; Karimi, G. Protective effect of silymarin against chemical-induced cardiotoxicity. *Iran. J. Basic Med. Sci.* **2016**, *19*, 916.
(28) Matouk, A. I.; Taye, A.; Heeba, G. H.; El-Moselhy, M. A. Quercetin augments the protective effect of losartan against chronic doxorubicin cardiotoxicity in rats. *Environ. Toxicol. Pharmacol.* **2013**, *36*, 443−450.
(29) Rehman, M. F.; Batool, A. I.; Qadir, R.; Aslam, M. Hesperidin and naringenin. In *A centum of valuable plant bioactives*, Elsevier, 2021; pp. 403−444, DOI: 10.1016/B978-0-12-822923-1.00027-3.
(30) Upaganlawar, A.; Balaraman, R. Protective effects of *Lagenaria siceraria* (Molina) fruit juice in isoproterenol induced myocardial infarction. *Int. J. Pharmacol.* **2010**, *6*, 645−651.
(31) Ahmed, H. H.; Mannaa, F.; Elmegeed, G. A.; Doss, S. H. Cardioprotective activity of melatonin and its novel synthesized derivatives on doxorubicin-induced cardiotoxicity. *Bioorg. Med. Chem.* **2005**, *13*, 1847−1857.
(32) Dianita, R.; Jantan, I.; Amran, A.; Jalil, J. Protective effects of *Labisia pumila* var. alata on biochemical and histopathological alterations of cardiac muscle cells in isoproterenol-induced myocardial infarction rats. *Molecules* **2015**, *20*, 4746−4763.
(33) Shang, C.; Lin, H.; Fang, X.; Wang, Y.; Jiang, Z.; Qu, Y.; Xiang, M.; Shen, Z.; Xin, L.; Lu, Y.; Gao, J.; Cui, X. Beneficial effects of cinnamon and its extracts in the management of cardiovascular diseases and diabetes. *Food Funct.* **2021**, 12194.
(34) Barbosa, D. C. D. S.; Holanda, V. N.; Ghosh, A.; Maia, R. T.; da Silva, W. V.; Lima, V. L. D. M.; Da Silva, M. V.; dos Santos Correia, M. T.; de Figueiredo, R. C. B. Q. Leishmanicidal and cytotoxic activity of essential oil from the fruit peel of *Myrciaria floribunda* (H. West ex Willd.) O. Berg: Molecular docking and molecular dynamics simulations of its major constituent onto Leishmania enzyme targets. *J. Biomol. Struct. Dyn.* **2021**, 1−16.
(35) Hemmati Hassan Gavyar, P.; Amiri, H. Chemical composition of essential oil and antioxidant activity of leaves and stems of *Phlomis lurestanica*. *Int. J. Food Prop.* **2018**, *21*, 1414−1422.
(36) Laskar, M. A.; Choudhury, M. D.; Chetia, P. *In silico* screening of cardioprotective activity of some flavonols. *Int. J. Pharm. Pharm. Sci.* **2014**, *6*, 528−531.
(37) Shaik, A. H.; Al Omar, S. Y.; Mohammad, A.; Kodidhela, L. D. Combined cardio-protective ability of syringic acid and resveratrol against isoproterenol induced cardio-toxicity in rats via attenuating NF-kB and TNF-α pathways. *Sci. Rep.* **2020**, *10*, 3426.
(38) Kalyana Sundaram, I.; Sarangi, D. D.; Sundararajan, V.; George, S.; Sheik Mohideen, S. Poly herbal formulation with anti-elastase and anti-oxidant properties for skin anti-aging. *BMC Complementary Altern. Med.* **2018**, *18*, 33.
(39) Ribarova, F.; Atanassova, M.; Marinova, D.; Ribarova, F.; Atanassova, M. Total phenolics and flavonoids in Bulgarian fruits and vegetables. *JU Chem. Metal.* **2005**, *40*, 255−260.
(40) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic* **1965**, *16*, 144−158.
(41) Yen, G.-C.; Chen, H.-Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.* **1995**, *43*, 27−32.
(42) Xiao, F.; Xu, T.; Lu, B.; Liu, R. Guidelines for antioxidant assays for food components. *Food Front.* **2020**, *1*, 60−69.
(43) Council, N. NRC. *Guide for the care and use of laboratory animals*; The National Academies Press: Washington, DC 2011.
(44) Fathiazad, F.; Matlobi, A.; Khorrami, A.; Hamedeyazdan, S.; Soraya, H.; Hammami, M.; Maleki-Dizaji, N.; Garjani, A. Phytochemical screening and evaluation of cardioprotective activity of ethanolic extract of *Ocimum basilicum* L. (basil) against isoproterenol induced myocardial infarction in rats. *DARU J. Pharm. Sci* **2012**, *20*, 1−10.
(45) Fadillioglu, E.; Oztas, E.; Erdogan, H.; Yagmurca, M.; Sogut, S.; Ucar, M.; Irmak, M. K. Protective effects of caffeic acid phenethyl ester on doxorubicin-induced cardiotoxicity in rats. *J. Appl. Toxicol.* **2004**, *24*, 47−52.
(46) Jujun, P.; Pootakham, K.; Pongpaibul, Y.; Duangrat, C.; Tharavichitkul, P. Acute and repeated dose 28-day oral toxicity study of *Garcinia mangostana* Linn. rind extract. *CMU J. Natl. Sci* **2008**, *7*, 199−208.
(47) Awodele, O.; Oreagba, I. A.; Odoma, S.; da Silva, J. A. T.; Osunkalu, V. O. Toxicological evaluation of the aqueous leaf extract of *Moringa oleifera* Lam. (Moringaceae). *J. Ethnopharmacol.* **2012**, *139*, 330−336.
(48) Miller, L. C.; Tainter, M. L. Estimation of the ED50 and its error by means of logarithmic-probit graph paper. *Proc. Soc. Exp. Biol. Med.* **1944**, *57*, 261−264.
(49) Krieger, E.; Vriend, G. YASARA View—Molecular graphics for all devices—From smartphones to workstations. *Bioinformatics* **2014**, *30*, 2981−2982.
(50) Rehman, M. F.; Akhter, S.; Batool, A. I.; Selamoglu, Z.; Sevindik, M.; Eman, R.; Mustaqeem, M.; Akram, M. S.; Kanwal, F.;

https://doi.org/10.1021/acsomega.2c04544
ACS Omega 2022, 7, 43635−43646

Lu, C.; Aslam, M. Effectiveness of Natural Antioxidants against SARS-CoV-2? Insights from the In-Silico World. *Antibiotics* **2021**, *10*, 1011.
(51) Fernandez-Poza, S.; Padros, A.; Thompson, R.; Butler, L.; Islam, M.; Mosely, J.; Scrivens, J. H.; Rehman, M. F.; Akram, M. S. Tailor-made recombinant prokaryotic lectins for characterisation of glycoproteins. *Anal. Chim. Acta* **2021**, *1155*, No. 338352.

https://doi.org/10.1021/acsomega.2c04544
ACS Omega 2022, 7, 43635−43646