Am J Transl Res 2024;16(6):2290-2300
www.ajtr.org /ISSN:1943-8141/AJTR0154339

Original Article

Gynostemma pentaphyllum Makino exerts cardioprotective effects by improving hemodynamic, biochemical and histopathological changes through activation of PI3K signalling in isoproterenol-induced myocardial infarction in rats

Diwyanshi Zinzuvadia, Jalpa Suthar, Alkeshkumar Patel, Umang Shah, Nilay Solanki, Hardik Koria

Ramanbhai Patel College of Pharmacy, Charotar University of Science and Technology, CHARUSAT Campus, Changa 388421, India

Received November 9, 2023; Accepted May 28, 2024; Epub June 15, 2024; Published June 30, 2024

Abstract: Objectives: To evaluate the cardioprotective effects of *Gynostemma pentaphyllum Makino* in isoproterenol-induced myocardial infarction in rats and to evaluate the role of phosphatidylinositol 3-kinases (PI3K) in cardioprotection. Methods: The protective effect of the hydroalcoholic leaf extract of *Gynostemma pentaphyllum* (LEGP) on the heart was investigated against isoproterenol (ISO)-induced MI in rats. Preliminary phytochemical screening was performed followed by molecular docking. For the in vivo studies Wistar albino rats (Male) were divided among different groups. Different parameters were evaluated such as heart weight index, Electrocardiogram (ECG) analysis, triphenyl tetrazolium chloride assay, cardiac enzyme markers, oxidative stress, antioxidant enzymes, PI3K levels, and histopathology of cardiac tissue. Results: Results showed that LEGP improved the electrocardiogram, reduced infarct size, and decreased the levels of cardiac enzyme markers and oxidative stress, while antioxidant enzymes and PI3K levels were increased. Conclusion: LEGP protected the heart against ISO-induced MI in rats by improving hemodynamic, biochemical and histological attributes. These protective effects were produced by the phytoconstituents of the LEGP through modulation of the PI3K signalling pathway.

Keywords: Myocardial infarction, isoproterenol, *Gynostemma pentaphyllum*, PI3K, cardioprotection, cardiac markers

Introduction

An acute myocardial infarction (AMI) is a death of cardiac myocytes that results from a sudden reduction in the amount of oxygenated blood that flows to the myocardium of the heart. This condition is often brought on when a thrombus blocks blood flow through an artery that is already affected by atheromatous plaque [1]. 49.2% of all cardiovascular diseses (CVD) deaths are due to myocardial infarction (MI) [2]. Cigarette smoking, diabetes, hypertension, obesity, and dyslipidemia are major risk factors for MI [3]. Symptoms of MI include substernal pain, breathing difficulty, sweating, nausea, vomiting, anxiety, and fatigue. Research has revealed that the degree of ST-segment elevation correction is an excellent indicator of both the immediate and delayed prognosis [4]. It is widely acknowledged as the most reliable indicator of tissue perfusion. During MI, a growing number of pathophysiologic modifications occur, which include thrombosis, calcium overload, oxidative stress, myocardial inflammation, and altered signaling pathways [5]. There is an overlap between the phases of fibrosis, angiogenesis, and inflammation in the damaged myocardium that results after MI [6].

Various signalling pathways play a role in the development of MI and its complications. Among them, the PI3K signalling pathway is crucial for the regulation of numerous responses such as cell cycle regulation, growth, and proliferation by regulation of downstream effectors [7]. Several traditional medicines exert cardio-

https://doi.org/10.62347/GHIX8452

protective effects by modulating activity the of PI3K signalling pathway [8]. Pre-treatment can reduce the damage caused by MI, which also decreases the probability of complications associated with MI. In recent years, several research studies have discovered multiple beneficial effects of *Gynostemma pentaphyllum* Makino [9, 10]. Gypenosides/Gynosaponins are saponins that are isolated from the leaves of *G. pentaphyllum* [11]. The nature of phytoconstituents present and their properties renders *Gynostemma pentaphyllum* (LEGP) a suitable candidate to evaluate its cardioprotective effect against ISO-induced MI in experimental rats [12].

Materials and methods

Chemicals and reagents

Isoproterenol and Wortmannin were procured from TCI Chemical Pvt Ltd. Hydroalcoholic leaf extract of *Gynostemma pentaphyllum* was obtained from Nutan Ayurveda. The biochemical kits of lactate dehydrogenase (LDH) and creatine kinase-myocardial band (CK-MB) were obtained from Loba Chemie Pvt Ltd, and PI3K enzyme-linked immunosorbent assay ELISA kit was obtained from Krishgen Biosystems.

Animals

66 Wistar albino rats (Male) weighing 200-250 grams (5-7 weeks) were procured from Sync Bio Research Private Limited. Polypropylene cages were used for the housing of animals. 3 animals were housed per cage and corn cob was used as the bedding material. Temperature (18-29°C) and humidity (30-70%) were maintained according to the Committee for Control and Supervision of Experiments on Animals (CCSEA) and Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. All the animals were fed with commercially available rat pellets along with drinking water. The animals were acclimatized to the laboratory environment for a minimum duration of 7 days before the commencement of the study. The entire experiment was performed as per the guidelines and regulations of the CCSEA, and Institutional Animal Ethics Committee approved the research protocol. The approval number of the protocol is RPCP/IAEC/2022-2023/R4.

Experimental design

In the study, Animals were divided in 7 groups.

(1) Control group: Normal saline was administered for 30 days.

(2) Isoproterenol (ISO) control group: ISO (100 mg/kg) was administered through subcutaneous (SC) route for last 2 days of the study.

(3) Carvedilol + ISO group: Carvedilol (1 mg/kg) was administered through oral route for 30 days and ISO (100 mg/kg) was administered through SC route for last 2 days of the study.

(4) Low dose of LEGP + ISO group: LEGP (125 mg/kg) was administered through oral route for 30 days, and ISO (100 mg/kg) was administered through SC route for last 2 days of the study.

(5) Medium dose of LEGP + ISO group: LEGP (250 mg/kg) was administered through oral route for 30 days, and ISO (100 mg/kg) was administered through SC route on the last 2 days of the study.

(6) High dose of LEGP + ISO group: LEGP (500 mg/kg) was administered through oral route for 30 days, and ISO (100 mg/kg) was administered through SC route on the last 2 days of the study.

(7) Wortmannin + ISO group: Wortmannin (100 µg/kg) was administered through intraperitoneal (IP) route for 30 days, and ISO (100 mg/kg) was given through SC route on the last 2 days of the study.

Preliminary phytochemical screening

The hydroalcoholic LEGP was evaluated for the presence of active phytoconstituents. The preliminary tests were performed to detect the presence of phytoconstituents like saponins [13], flavonoids [14], phenolic acids [15], alkaloids, proteins, and steroids [12] in the LEGP using standard procedures [16].

Molecular docking

Autodock vina v.1.2.0. was used to perform molecular docking to determine the binding efficiency of ligands present in the LEGP with

proteins [17]. The structures of proteins including Phosphoinositide-3 kinase α (PI3Kα), Phosphoinositide-3 kinase β (PI3Kβ) and Protein kinase B (Akt1) were identified from the database of the protein data bank (PDB). The PDB code of PI3Kα is 5sxA, PI3Kβ is 4G11 and Akt1 is 6HHG. The 3D structures of phytoconstituents including Gypenoside A (GYPA), Gypenoside 3 (GYP3), Gypenoside 17 (GYP17), Gallic acid, Quercetin and Rutin were collected from the database of PubChem. All the phytoconstituents were docked against all the three protein targets [18].

ECG analysis

Ketamine (75 mg/kg) and xylazine (10 mg/kg) were administered through intraperitoneal route to induce anaesthesia in the experimental rats. BIOPAC MP36 System [19] was used to record an electrocardiogram (ECG). Electrodes were attached to the right arm, left arm, and left leg of the rat to record the rat's ECG [20]. ST segment [21], QT interval [22], Q wave [23], QRS complex [22], and PR interval [22] were measured.

Heart weight index evaluation

From the animals of each group, hearts were removed and washed with phosphate buffer to remove excess blood. Then the hearts were dried with the help of filter paper and weighed. The following formula was used to get values for the heart weight index (HWI) [24]:

$$HWI\% = \frac{\text{Weight of heart}\,(g)}{\text{Weight of rat}\,(g)} * 100$$

Triphenyl tetrazolium chloride (TTC) assay

From each group hearts were isolated and frozen at -20°C for an hour. The hearts were sliced into 1 mm thick pieces, and incubated in 1% 2,3,5-triphenyl tetrazolium chloride (TTC) for 30 minutes. After incubation slices were placed in a 4% formaldehyde solution overnight. Normal myocardium stained red and the infarcted area stained pale grey/greyish [25]. The heart slices were analysed by Image J software. The percentage infarcted area was calculated by the following formula:

$$\%\text{Infarct area} = \frac{\text{Infarcted area}}{\text{Area at risk}} * 100$$

Detection of serum cardiac injury markers

Ketamine (75 mg/kg) and xylazine (10 mg/kg) were administered through the intraperitoneal route to induce anaesthesia in the experimental rats before blood was drawn from the retro-orbital plexus. The blood was centrifuged at 5000 rpm for 10 minutes at 4°C. The serum was stored at -20°C until additional analysis could be performed. Serum was analysed for the cardiac injury markers using commercially available kits. The measurements were performed as per the manufacturer's specifications.

Estimation of phosphoinositide-3-kinase (PI3K)

The estimation of PI3K from homogenate of hearts from each experimental groups was performed using enzyme linked immunosorbent assay (ELISA) kit obtained from Krishgen Biosystem.

Estimation of oxidative stress and antioxidant values

For euthanasia, high dose of barbiturates was administered in the rats and hearts were isolated and washed with phosphate buffer saline (PBS). Heart tissues were homogenised in 10 mM Tris-HCl buffer and centrifuged at 5000 rpm for 10 minutes at 4°C. Oxidative stress marker malondialdehyde (MDA) [26] and antioxidant parameters such as glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were analysed in the supernatant [27-29].

Histopathologic analysis

Following euthanasia, hearts from each experimental group were extracted and cleaned with normal saline in order to remove excess blood. For cell fixation, the cardiac tissue was immersed in 10% formalin. The samples were submitted to a pathology laboratory to determine alterations in cardiac tissue architecture.

Statistical analysis

All the data were evaluated using One-way ANOVA (One-way analysis of variance) followed by Tukey's multiple comparison test. The data were expressed as mean ± standard deviation (S.D.). A value of $P<0.05$ was considered significant.

Table 1. Docking score of the interaction between between various phytoconstituents of leaf extract of *Gynostemma pentaphyllum* (LEGP) and protein targets of Phosphoinositide-3 kinase (PI3K) signalling

Phytoconstituents	Docking Score (kcal/mol)		
	PI3Kα	PI3Kβ	AKT1
Gypenoside A (GYPA)	-10.8	-10.7	-9.5
Gypenoside 3 (GYP3)	-9.8	-7.0	-7.5
Gypenoside 17 (GYP17)	-10.6	-9.5	-9.2
Quercetin	-6.4	-5.3	-5.8
Rutin	-8.2	-8.7	-9.3
Gallic acid	-8.2	-8.7	-7.3

Phytoconstituent Gypenoside A (GYPA) has the highest docking score whereas, quercetin achieved the lowest docking score for all three proteins Phosphoinositide-3 kinase α (PI3Kα), Phosphoinositide-3 kinase β (PI3Kβ) and Protein kinase B (Akt1).

Results

Preliminary phytochemical screening of LEGP

The preliminary examination of the hydroalcoholic leaf extract of *Gynostemma pentaphyllum* was carried out to check the presence of various phytoconstituents present in the extract. Results showed that saponins, flavonoids, phenolic acids, proteins, and steroids were present, but alkaloids were absent.

Results of molecular docking

Molecular docking was performed to check the interaction between various phytoconstituents of LEGP and protein targets of PI3K signalling. The docking score of the phytoconstituents of *Gynostemma pentaphyllum* with the targets is shown in **Table 1**. Among the investigated phytoconstituents, GYPA had the highest docking score whereas, quercetin achieved the lowest docking score for all three proteins PI3Kα, PI3Kβ and Akt1.

Effect of LEGP on ECG parameters

ECG analysis was carried out to check the abnormalities in the heart induced by ISO. On administration of ISO the ST-segment elevation, QT interval widening and Q wave depression were observed in the ISO control group and Wortmannin + ISO group (PI3K inhibitor group) (**Figure 1**). Results of electrocardiographic changes (ECG) in the treatment groups showed correction in these measurements and there was significant correction in ST segment elevation, QT interval widening, and Q wave depression in the high dose LEGP group as compared to the ISO group ($P<0.05$) (**Figure 2**).

Effect of LEGP on heart weight index (HWI)

The induction of MI in the ISO control group led to significant animal weight loss and increased heart weight which resulted in increased HWI. While comparing the ISO control group to Wortmannin + ISO group, there was no significant difference for HWI (**Figure 3**). HWI was decreased in the LEGP treatment groups and there was a significant decrease in HWI in the high dose LEGP group ($P<0.05$).

Effect of LEGP on cardiac injury markers

CK-MB and LDH were measured in the blood serum to detect heart tissue damage. A rise in the concentrations of LDH and CK-MB was observed in the blood serum of the ISO control group and Wortmannin + ISO group (**Figure 4**). The level of these cardiac enzyme markers decreased significantly in the LEGP treated groups ($P<0.05$).

Effect of LEGP on PI3K pathway

PI3K level in the heart tissue homogenate was checked to assess its expression in the heart of various groups. The findings revealed that the level of PI3K was low in the ISO control group and Wortmannin + ISO group (**Figure 5**). PI3K level was increased significantly in the LEGP treated groups ($P<0.05$).

Effect of LEGP on infarct size

TTC assay was carried out to check the viability of the heart after MI induction by ISO in various groups. The area of the infarcted region in the hearts of the rats in the LEGP treated control group was less in size compared to ISO control group and Wortmannin + ISO group (**Figure 6**). The percentage of infarct size was 69.83% for ISO control group, 27.29% for carvedilol + ISO group, 57.92% for the low dose of LEGP + ISO group, 35.51% for the medium dose of LEGP + ISO group, 30.31% for the high dose of LEGP +

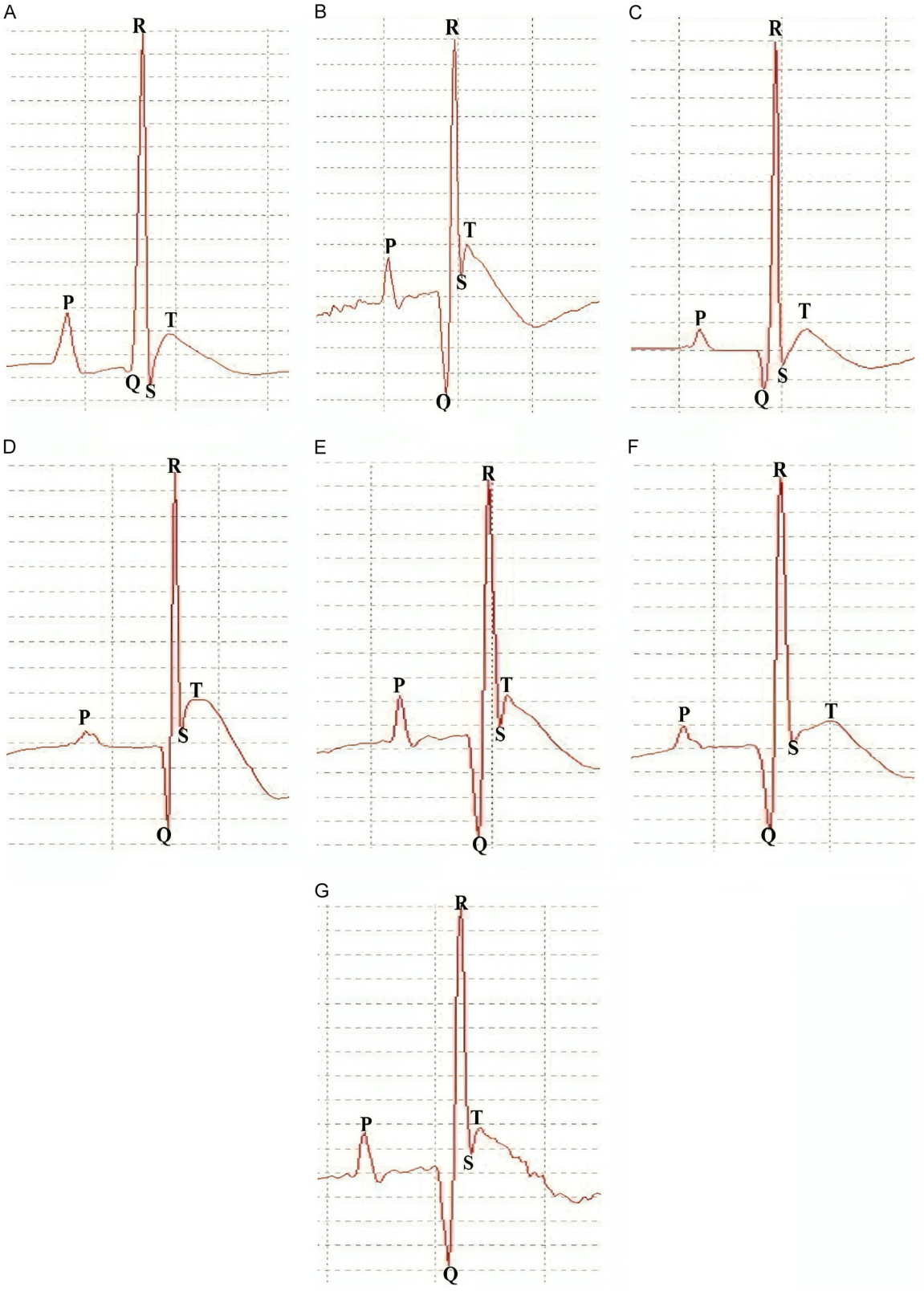

Figure 1. Representative images of electrocardiogram from different experimental groups. ST-segment, QT interval widening and Q wave depression were observed in the ioproterenol (ISO) control group. These features were corrected in the leaf extract of *Gynostemma pentaphyllum* (LEGP) treated groups. (A) Control group, (B) ISO control group, (C) Carvedilol + ISO group, (D) Low dose of LEGP + ISO group, (E) Medium dose of LEGP + ISO group, (F) High dose of LEGP + ISO group, and (G) Wortmannin + ISO group.

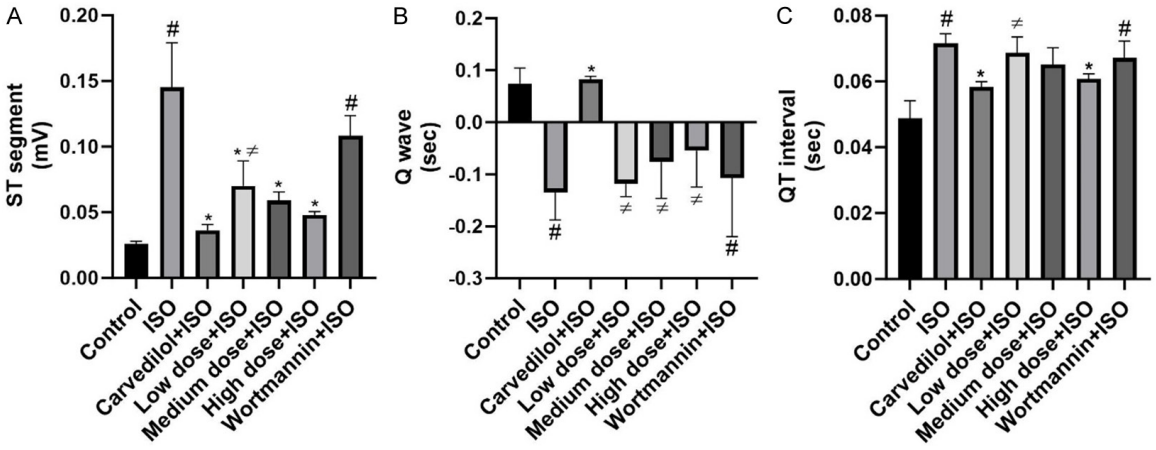

Figure 2. Effect of leaf extract of *Gynostemma pentaphyllum* (LEGP) on Electrocardiogram (ECG) changes. (A) ST segment, (B) Q wave, (C) QT interval. All values are stated as mean ± SD (n=6). The probability value is P<0.05, where # indicates statistical difference from the control group, * indicates statistical difference from the Isoproterenol-induced MI group, and ≠ indicates statistical difference from the Carvedilol + ISO control group, using one-way ANOVA, followed by Tukey's multiple comparison test.

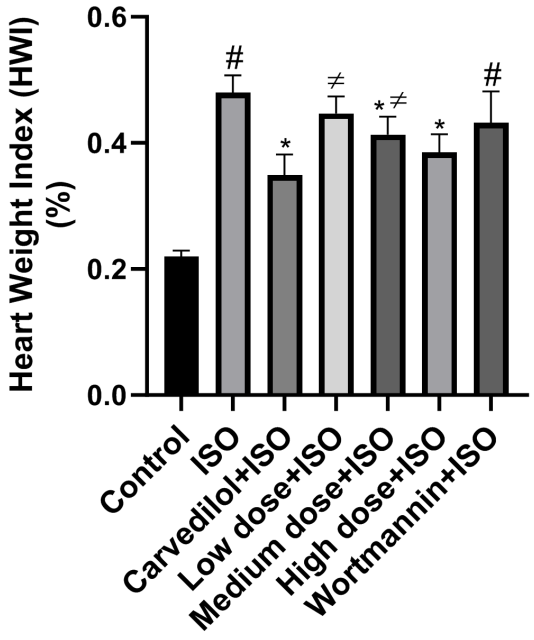

Figure 3. Effect of leaf extract of *Gynostemma pentaphyllum* (LEGP) on heart weight index (HWI). HWI was increased in the ISO control and Wortmannin group, whereas it was reduced in treated groups. All values are stated as mean ± SD (n=6). The probability value was P<0.05, where # indicates statistical difference from the control group, * indicates statistical difference from the Isoproterenol-induced MI group, and ≠ indicates statistical difference from the Carvedilol + ISO control group, using one-way ANOVA, followed by Tukey's multiple comparison test.

ISO group, and 60.45% for the Wortmannin + ISO group.

Effect of LEGP on oxidative stress and antioxidant parameters

MDA was analysed to check the extent of lipid peroxidation and subsequent oxidative stress. Increased oxidative stress was observed in the ISO control group and Wortmannin + ISO group. There was significant improvement in the antioxidant level and reduction in oxidative stress in the LEGP treated groups (P<0.05) (**Figure 7**).

Effect of LEGP on histopathology of the heart

Myocardial tissue architecture was normal in the control group (**Figure 8**). Dense and elongated myocardial fibres (double arrow), normal cardiomyocyte nuclei (single arrow) and erythrocytes in the blood vessels (arrowhead) were noticed in the control group. Neutrophil infiltration (rectangle), edema (circle), wavy heart muscle (double arrow), pyknotic nuclei (single arrow), and necrosis (star) were seen in the ISO control group. Denser myocardial fibres (double arrow), low neutrophilic infiltration (rectangle) and normal cardiomyocyte nuclei (single arrow) were observed and there was no evidence of necrosis or edema in the carvedilol + ISO group. Neutrophil infiltration (rectangle), pyknotic nuclei (single arrow), oedema, and necrosis (star), were more in the low dose of LEGP + ISO group compared to the medium dose of LEGP + ISO group. The least damage was observed in the higher dose of LEGP + ISO group. Necrosis and edema were not found in

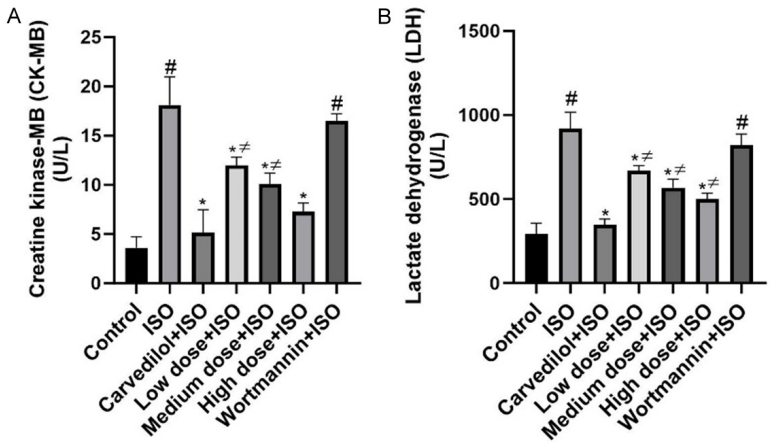

Figure 4. Effect of leaf extract of *Gynostemma pentaphyllum* (LEGP) on (A) Creatine kinase-MB (CK-MB) and (B) Lactate dehydrogenase (LDH) levels. Cardiac injury markers were increased in the ISO control and Wortmannin group, whereas they were reduced in treated groups. All values are stated as mean ± SD (n=6). The probability value is P<0.05, where # indicates statistical difference from the control group, * indicates statistical difference from the Isoproterenol-induced MI group, and ≠ indicates statistical difference from the Carvedilol + ISO control group, using one-way ANOVA, followed by Tukey's multiple comparison test.

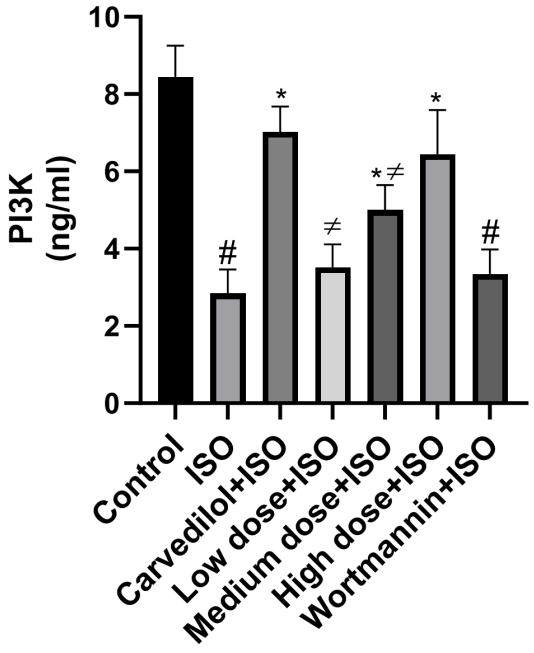

Figure 5. Effect of leaf extract of *Gynostemma pentaphyllum* (LEGP) on Phosphoinositide-3 kinase (PI3K) levels. PI3K level was increased in LEGP treated groups. PI3K was decreased in ISO control and Wortmannin + ISO groups. All values are stated as mean ± SD (n=6). The probability value is P<0.05, where # indicates statistical difference from the control group, * indicates statistical difference from the Isoproterenol-induced MI group, and ≠ indicates statistical difference from the Carvedilol + ISO control group, using one-way ANOVA, followed by Tukey's multiple comparison test.

the higher dose of LEGP + ISO group. The Wortmannin + ISO group's architectural changes were similar to those of the ISO control group. In the ISO control group and the Wortmannin + ISO group, damage was maximal, while in the treatment group LEGP was able to prevent neutrophilic infiltration, necrosis, and edema.

Discussion

This study aimed to evaluate the cardioprotective potential of LEGP in ISO-induced myocardial infarction and its possible mechanism. The preliminary phytochemical analysis revealed the presence of saponins, flavonoids, phenolic acids, proteins, and steroids. These phytoconstituents have been reported to possess various protective activities such as antioxidant activity, anti-inflammatory activity, and anti-apoptotic activity [30]. A molecular docking study was carried out which suggested that the phytoconstituents of LEGP may interact with various effectors of PI3K signalling to exert cardioprotection. MI was induced through administration of isoproterenol which resulted in hemodynamic, biochemical, and histological changes. ECG changes in the disease control group showed abnormal heart functioning due to myocardial damage. Same changes were improved in the LEGP treated groups as evident from the ECGs which suggests that LEGP may have prevented the damage of the cardiac myocytes. The induction of MI led to significant weight loss of animal and increased heart weight in the ISO control group, resulting in increased heart weight index. The results of the HWI in the treatment group suggests that LEGP may have prevented the hypertrophy and fibrotic changes in the myocardial tissues. Cardiac enzyme markers such as lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) are released in the blood stream from necrotic heart muscle in MI [31]. The low levels of the cardiac markers in the blood of the treatment groups indicates that necrosis was prevented by LEGP in the cardiac myocytes. Results of TTC assay suggests

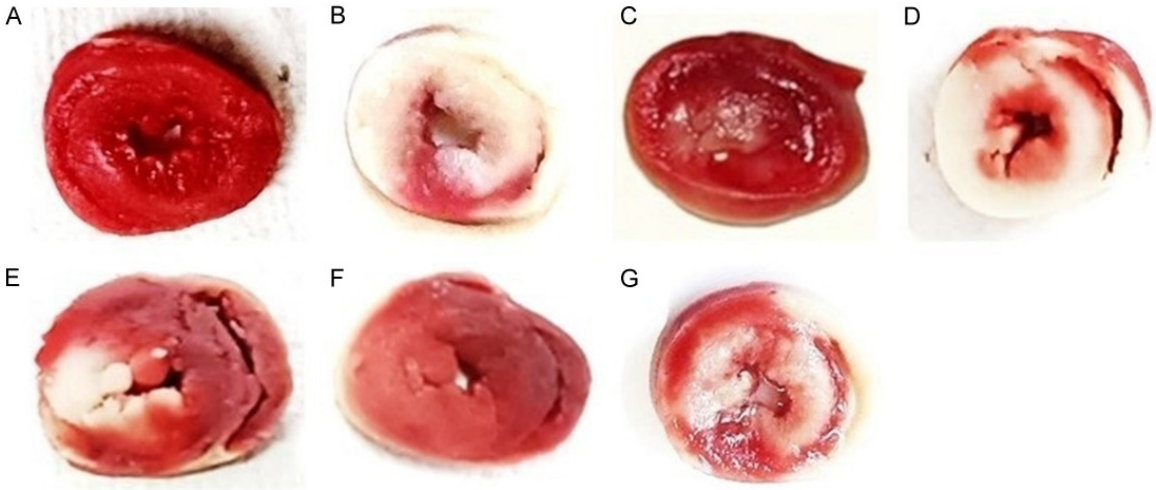

Figure 6. Representative triphenyl tetrazolium chloride assay (TTC) images of hearts isolated from different experimental groups. (A) Control group, (B) ISO control group, (C) Carvedilol + ISO group, (D) Low dose of LEGP + ISO group, (E) Medium dose of LEGP + ISO group, (F) High dose of LEGP + ISO group, and (G) Wortmannin + ISO group. The area of infarcted regions (white region) in the hearts of the rats in the LEGP treated control group was less compared to the ISO control group and Wortmannin + ISO group.

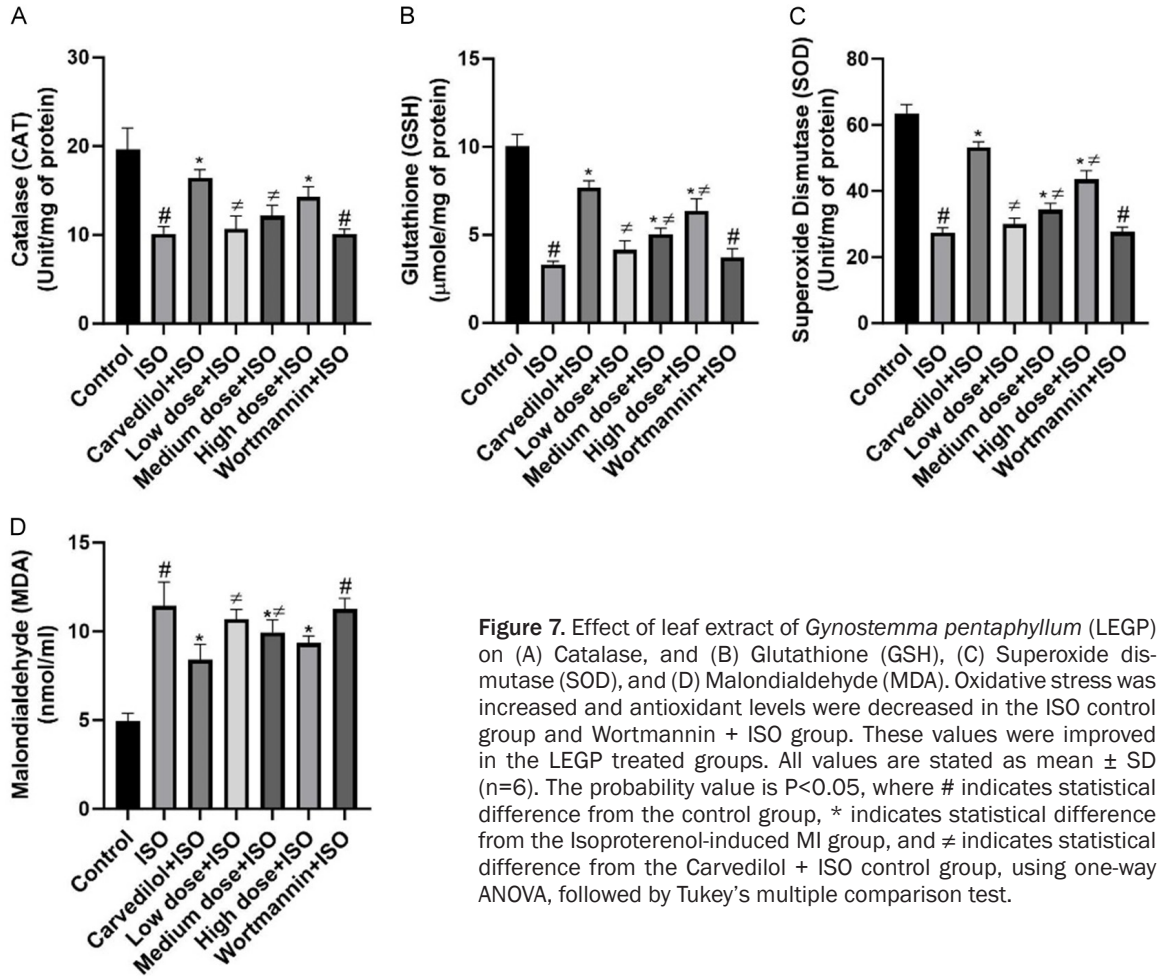

Figure 7. Effect of leaf extract of *Gynostemma pentaphyllum* (LEGP) on (A) Catalase, and (B) Glutathione (GSH), (C) Superoxide dismutase (SOD), and (D) Malondialdehyde (MDA). Oxidative stress was increased and antioxidant levels were decreased in the ISO control group and Wortmannin + ISO group. These values were improved in the LEGP treated groups. All values are stated as mean ± SD (n=6). The probability value is $P<0.05$, where # indicates statistical difference from the control group, * indicates statistical difference from the Isoproterenol-induced MI group, and ≠ indicates statistical difference from the Carvedilol + ISO control group, using one-way ANOVA, followed by Tukey's multiple comparison test.

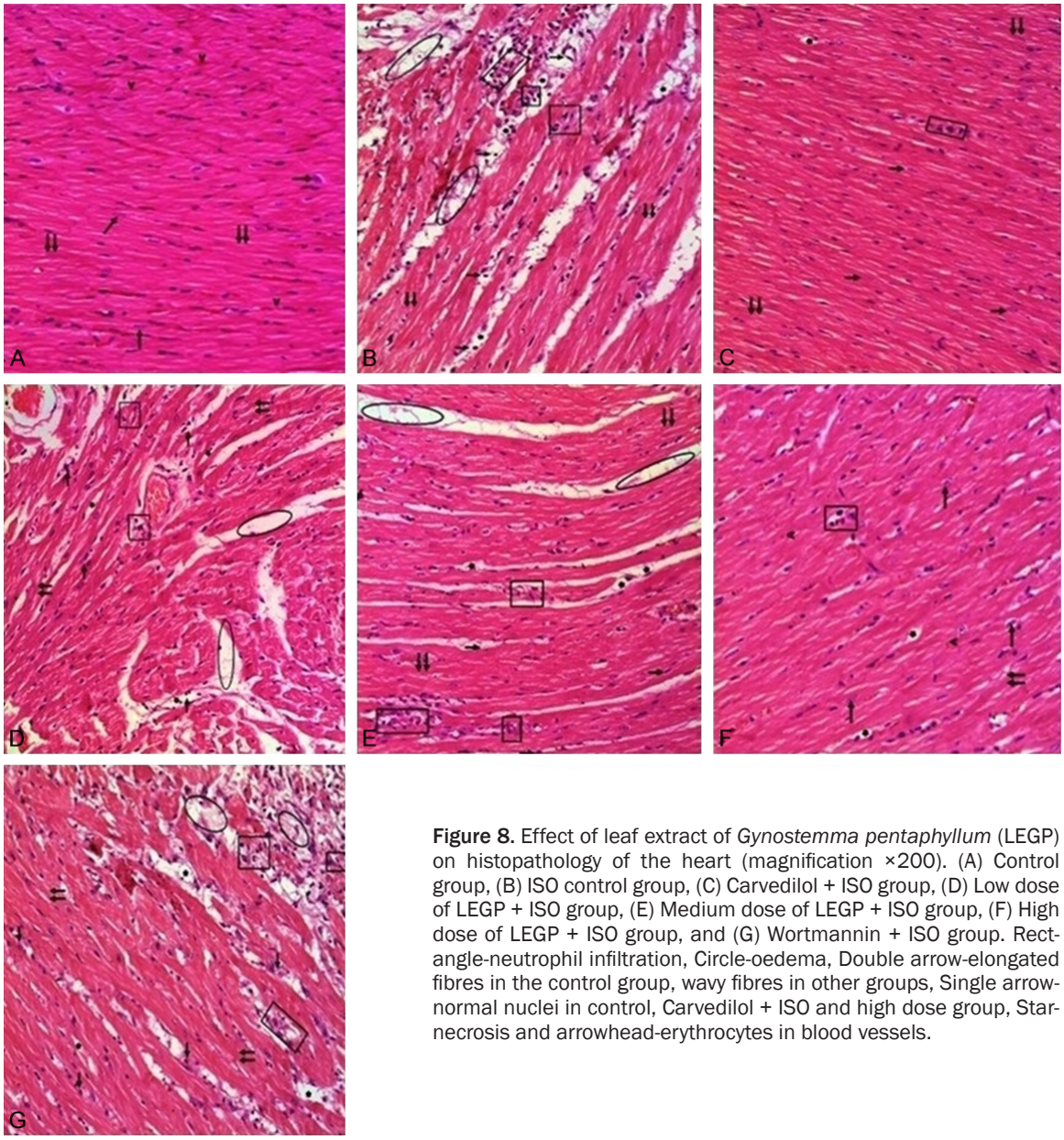

Figure 8. Effect of leaf extract of *Gynostemma pentaphyllum* (LEGP) on histopathology of the heart (magnification ×200). (A) Control group, (B) ISO control group, (C) Carvedilol + ISO group, (D) Low dose of LEGP + ISO group, (E) Medium dose of LEGP + ISO group, (F) High dose of LEGP + ISO group, and (G) Wortmannin + ISO group. Rectangle-neutrophil infiltration, Circle-oedema, Double arrow-elongated fibres in the control group, wavy fibres in other groups, Single arrow-normal nuclei in control, Carvedilol + ISO and high dose group, Star-necrosis and arrowhead-erythrocytes in blood vessels.

that LEGP has prevented the infarction possibly by inhibiting apoptosis and necrosis in the cardiac myocytes. Levels of oxidative stress marker and antioxidants such as CAT, SOD, and GSH in the treatment groups suggest that LEGP has reduced oxidative stress. Histopathology showed that LEGP reduced apoptosis, necrosis, and inflammation in the myocardium. All these protective changes were not observed when animals were treated with the Wortmannin (PI3K inhibitor) which suggests that phytoconstituents of LEGP exert cardioprotection through PI3K signalling as evident from an increased level of PI3K in LEGP treated groups and a good binding score of major phytoconstituents of LEGP with various proteins of PI3K signalling. Once the PI3K pathway is activated, many signalling proteins are activated such as Vascular endothelial growth factor (VEGF), Glycogen synthase kinase 3β (GSK-3β), Forkhead box subfamily O (FOXO), Mammalian target of rapamycin (mTOR), and Endothelial nitric oxide synthase (eNOS). These proteins may be involved in various protective effects produced by LEGP [32].

Conclusion

Our study revealed that the hydroalcoholic leaf extract of *Gynostemma pentaphyllum*

protected the heart against ISO-induced MI in rats. LEGP improved the hemodynamic, biochemical and histopathological parameters. These protective effects were produced by the phytoconstituents of the LEGP through the modulation of the PI3K signalling pathway.

Acknowledgements

We would like to thank Ramanbhai Patel College of Pharmacy, Charotar University of Science and Technology (CHARUSAT), CHARUSAT campus, Changa 388421, India for providing all the support and facilities to complete the study and authoring this article.

Disclosure of conflict of interest

None.

Abbreviations

Akt1, Protein kinase B; AMI, Acute myocardial infarction; ANNOVA, One way analysis of variance; BPM, Beats per minute; CAT, Catalase; CCSEA, Committee for the control and supervision of experiments on animals; CK-MB, Creatine kinase-MB; CVDs, Cardiovascular diseases; ECG, Electrocardiogram; ELISA, Enzyme-linked immunosorbent assay; eNOS, Endothelial nitric oxide synthase; FOXO, Forkhead box subfamily O; GSH, Glutathione; GSK-3β, Glycogen synthase kinase 3β; GYPA, Gypenoside A; GYP3, Gypenoside 3; GYP17, Gypenoside 17; HWI, Herat weight index; IAEC, Institutional Animal Ethic Committee; ISO, Isoproterenol; LDH, Lactate dehydrogenase; LEGP, Leaf extract of *Gynostemma pentaphyllum*; MDA, Malondialdehyde; MI, Myocardial infarction; mTOR, Mammalian target of rapamycin; PBS, Phosphate buffer saline; PI3K, Phosphoinositide-3 kinase; PI3Kα, Phosphoinositide-3 kinase α; PI3Kβ, Phosphoinositide-3 kinase β; PUFA, Polyunsaturated fatty acids; SD, Standard deviation; SOD, Superoxide dismutase; TTC, 2,3,5-triphenyl tetrazolium chloride; VEGF, Vascular endothelial growth factor.

Address correspondence to: Hardik Koria, Pharmacology Department, Ramanbhai Patel College of Pharmacy, Charotar University of Science and Technology, Changa 388421, India. Tel: +91-9731835384; ORCID: 0000-0001-7950-2975; E-mail: hardik.koria@gmail.com; hardikkoria.ph@charusat.ac.in

References

[1] Golforoush P, Yellon DM and Davidson SM. Mouse models of atherosclerosis and their suitability for the study of myocardial infarction. Basic Res Cardiol 2020; 115: 73.

[2] Roth GA, Mensah GA and Fuster V. The global burden of cardiovascular diseases and risks: a compass for global action. J Am Coll Cardiol 2020; 76: 2980-2981.

[3] Boateng S and Sanborn T. Acute myocardial infarction. Dis Mon 2013; 59: 83-96.

[4] Matetzky S, Novikov M, Gruberg L, Freimark D, Feinberg M, Elian D, Novikov I, Di Segni E, Agranat O, Har-Zahav Y, Rabinowitz B, Kaplinsky E and Hod H. The significance of persistent ST elevation versus early resolution of ST segment elevation after primary PTCA. J Am Coll Cardiol 1999; 34: 1932-1938.

[5] Garg M and Khanna D. Exploration of pharmacological interventions to prevent isoproterenol-induced myocardial infarction in experimental models. Ther Adv Cardiovasc Dis 2014; 8: 155-169.

[6] Wu X, Reboll MR, Korf-Klingebiel M and Wollert KC. Angiogenesis after acute myocardial infarction. Cardiovasc Res 2021; 117: 1257-1273.

[7] Ghafouri-Fard S, Khanbabapour Sasi A, Hussen BM, Shoorei H, Siddiq A, Taheri M and Ayatollahi SA. Interplay between PI3K/AKT pathway and heart disorders. Mol Biol Rep 2022; 49: 9767-9781.

[8] Upadhyay S and Dixit M. Role of polyphenols and other phytochemicals on molecular signaling. Oxid Med Cell Longev 2015; 2015: 504253.

[9] Shang X, Chao Y, Zhang Y, Lu C, Xu C and Niu W. Immunomodulatory and antioxidant effects of polysaccharides from Gynostemma pentaphyllum Makino in immunosuppressed mice. Molecules 2016; 21: 1085.

[10] Xie Z, Huang H, Zhao Y, Shi H, Wang S, Wang TT, Chen P and Yu L. Chemical composition and anti-proliferative and anti-inflammatory effects of the leaf and whole-plant samples of diploid and tetraploid Gynostemma pentaphyllum (Thunb.) Makino. Food Chem 2012; 132: 125-133.

[11] Takemoto T, Arichi S, Arihara S, Nakajima T, Okuhira M and Uchida Y. Gynosaponins, their use and a process for preparing the same. 1982.

[12] Razmovski-Naumovski V, Huang THW, Tran VH, Li GQ, Duke CC and Roufogalis BD. Chemistry and pharmacology of gynostemma pentaphyllum. Phytochem Rev 2005; 4: 197-219.

[13] Ling Y, Lei Z, Li X, Yan X, Wang S, Yang W, Nie Q, Zhang Q, Bao H, Yu J and Jin H. Characteriza-

tion and identification of the chemical constituents of Gynostemma pentaphyllum using high performance liquid chromatography-electrospray ionization-quadrupole time-of-flight tandem mass spectrometry (HPLC-ESI-QTOF-MS/MS). Anal Lett 2020; 53: 760-773.
[14] Xie Z, Zhao Y, Chen P, Jing P, Yue J and Yu LL. Chromatographic fingerprint analysis and rutin and quercetin compositions in the leaf and whole-plant samples of di- and tetraploid gynostemma pentaphyllum. J Agric Food Chem 2011; 59: 3042-3049.
[15] Šamec D, Valek-Žulj L, Martinez S, Grúz J, Piljac A and Piljac-Žegarac J. Phenolic acids significantly contribute to antioxidant potency of Gynostemma pentaphyllum aqueous and methanol extracts. Ind Crops Prod 2016; 84: 104-107.
[16] Khandelwal K. Practical pharmacognosy. Pragati Books Pvt. Ltd.; 2008.
[17] Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK and Olson AJ. Automated docking using a lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem 1639; 19: 16391662.
[18] Patel A, Patel A, Shah R, Zinzuvadia D and Mahant S. Discovering the potential of natural remedies in the post COVID-19 complications based on in silico techniques. Org Commun 2022; 15: 95.
[19] Marques V and Farah A. Chlorogenic acids and related compounds in medicinal plants and infusions. Food Chem 2009; 113: 1370-1376.
[20] Sembulingam K and Sembulingam P. Essentials of medical physiology. JP Medical Ltd; 2012.
[21] Sajid A, Ahmad T, Ikram M, Khan T, Shah AJ, Mahnashi MH, Alhasaniah AH, Al Awadh AA, Almazni IA and Alshahrani MM. Cardioprotective potential of aqueous extract of fumaria indica on isoproterenol-induced myocardial infarction in SD rats. Oxid Med Cell Longev 2022; 2022: 2112956.
[22] Mohamed ME, Abduldaium MS and Younis NS. Cardioprotective effect of linalool against isoproterenol-induced myocardial infarction. Life (Basel) 2021; 11: 120.
[23] Ahmad T, Khan T, Tabassum T, Alqahtani YS, Mahnashi MH, Alyami BA, Alqarni AO, Alasmary MY, Almedhesh SA and Shah AJ. Juglone from walnut produces cardioprotective effects against isoproterenol-induced myocardial injury in SD rats. Curr Issues Mol Biol 2022; 44: 3180-3193.
[24] Wu H, Dai Z, Liu X, Lin M, Gao Z, Tian F, Zhao X, Sun Y and Pu X. Pharmacodynamic evaluation of shenfu injection in rats with ischemic heart failure and its effect on small molecules using matrix-assisted laser desorption/ionization-mass spectrometry imaging. Front Pharmacol 2019; 10: 1424.
[25] Lie JT, Pairolero PC, Holley KE and Titus JL. Macroscopic enzyme-mapping verification of large, homogeneous, experimental myocardial infarcts of predictable size and location in dogs. J Thorac Cardiovasc Surg 1975; 69: 599-605.
[26] Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-8.
[27] Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82: 70-77.
[28] Aebi H. Catalase. In: Methods of enzymatic analysis. Elsevier; 1974. pp. 673-684.
[29] Marklund S and Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974; 47: 469-74.
[30] Su X, Zhou M, Li Y, Zhang J, An N, Yang F, Zhang G, Yuan C, Chen H, Wu H and Xing Y. Protective effects of natural products against myocardial ischemia/reperfusion: mitochondria-targeted therapeutics. Biomed Pharmacother 2022; 149: 112893.
[31] Aldous SJ. Cardiac biomarkers in acute myocardial infarction. Int J Cardiol 2013; 164: 282-294.
[32] Zhang Q, Wang L, Wang S, Cheng H, Xu L, Pei G, Wang Y, Fu C, Jiang Y, He C and Wei Q. Signaling pathways and targeted therapy for myocardial infarction. Signal Transduct Target Ther 2022; 7: 78.