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Original Article

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Modulation of *Inula racemosa* Hook Extract on Cardioprotection by Ischemic Preconditioning in Hyperlipidaemic Rats

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Objectives: Hyperlipidemia (HL) is a major cause of ischemic heart diseases. The size-limiting effect of ischemic preconditioning (IPC), a cardioprotective phenomenon, is reduced in HL, possibly because of the opening of the mitochondrial permeability transition pore (MPTP). The objective of this study is to see what effect pretreatment with *Inula racemosa* Hook root extract (IrA) had on IPC-mediated cardioprotection on HL Wistar rat hearts. An isolated rat heart was mounted on the Langendorff heart array, and then ischemia reperfusion (I/R) and IPC cycles were performed. Atractyloside (Atr) is an MPTP opener.
Methods: The animals were divided into ten groups, each consisting of six rats (n = 6), to investigate the modulation of *I. racemosa* Hook extract on cardioprotection by IPC in HL hearts: Sham control, I/R Control, IPC control, I/R + HL, I/R + IrA + HL, IPC + HL, IPC + NS + HL, IPC + IrA+ HL, IPC + Atr + oxidative stress, mitochondrial function, integrity, and hemodynamic parameters are evaluated for each group.
Results: The present experimental data show that pretreatment with IrA reduced the LDH, CK-MB, size of myocardial infarction, content of cardiac collagen, and ventricular fibrillation in all groups of HL rat hearts. This pretreatment also reduced the oxidative stress and mitochondrial dysfunction. Inhibition of MPTP opening by Atr diminished the effect of IrA on IPC-mediated cardioprotection in HL rats.
Conclusion: The study findings indicate that pretreatment with IrA e restores IPC-mediated cardioprotection in HL rats by inhibiting the MPTP opening.

Keywords: ischemic preconditioning, hyperlipidaemia, mitochondria, oxidative stress

INTRODUCTION

Ischemic heart disease (IHD), more specifically myocardial infarction, requires reperfusion of the ischemic heart. It is a leading cause of death and disability in both men and women worldwide [1]. The IHD leads to a cascade of adverse events, resulting in ischemia-reperfusion injury (I/R) injury. Endothelial dysfunction, oxidative stress, and apoptosis–necrosis–inhibition have been linked to I/R-induced damage. An endogenous technique for protecting the ischemic heart is ischemia preconditioning (IPC) [2], which can mitigate these effects [3, 4]. However, during the IPC, the myocardium undergoes a brief but potentially fatal bout of sublethal ischemia and reperfusion [5]. Mitochondrial ATP-sensitive potassium channels (KATP) form when the PI3K/Akt pathway is activated and the mitochondrial permeability transition pore (MPTP) opening is blocked [6-8]. However, the cardioprotective effect of the IPC diminishes under some pathological conditions such as aging [9], high blood pressure [10, 11], diabetes mellitus [12, 13], and heart failure [14, 15]. Hyperlipidemia (HL) is a major risk factor for IHD [16] and reduces the cardioprotective effects of the IPC. However, the underlying mechanism for this reduction is not yet clear.

There has recently been an uptick in interest on medicinal

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plants among scientists, which have long been hailed for their healing properties and as a potential resource for the development of novel treatments for heart diseases and other chronic illnesses [17]. *Inula racemosa* Hook. f. sp. root extract (IrA), also known as *Pushkarmool*, can help treat many cardiorespiratory and cardiovascular conditions. According to some indigenous and traditional medical systems, *Inula* species can be utilized in the treatment of anginal pain [18]. Previous studies have shown that various herbal formulations containing *I. racemose* can protect against myocardial necrosis, which has led to an increase on *Inula* species as a cardioprotective agent [19].

IPC-mediated inhibition of MPTP opening reduces under HL conditions [20]. However, it is not yet known whether IPC conducted with *I. racemosa* Hook root extract (IrA) has any cardioprotective effects in the treatment of patients with HL. This study investigates whether IrA could enhance the cardioprotective effects of IPC in HL rats.

MATERIALS AND METHODS

Wistar rats weighing 170-190 g were used in this investigation.

1. Drugs and chemicals

All of the chemicals and reagents used in this work were of analytical grade and freshly prepared before use.

2. Analysis of phytochemicals in plant materials

After air drying, the roots of *I. racemosa* Hook. f. sp. (IrA), a plant found in Kashmir and Himachal Pradesh [8], were broken into small pieces and processed into a fine powder (500 g). The cold maceration extraction was performed at 25℃ using a 50:50 mixture of methanol and water. Next, the hydroalcoholic extract was filtered and the solvent was evaporated under reduced pressure, producing a viscous mass.

3. Experimental hyperlipidemia

A 6-week high-fat diet (corn starch 44.74 g, sugar 10 g, butter 20 g, casein 14 g, fiber 5 g, mineral mixture 3.5 g, cholesterol 1 g, vitamin mixture 1 g, citric acid 0.25 g, choline 0.25 g, and terbutylhydroquinone 0.0008 g) was found to cause experimental HL [20]. Commercially available assays were used to measure the serum cholesterol and triglyceride levels to determine the HL (Span Diagnostics [P] Ltd, Surat, India).

4. Preparation of the rat heart in isolation

Sodium pentobarbital (60 mg/kg) was injected intramuscularly into the Wistar rats [21] to put them to sleep, followed by intraperitoneal administration of heparin (500 IU/L) as an anticoagulant (Gland Pharma Ltd., Hyderabad, India). The heart was promptly cut out and put in place [22]. The heart was kept at 37℃ using warm water. Kerbs–Henseleit (KH) buffer (NaCl 118 mM; $MgSO_4 \cdot 7H_2O$ 1.2 mM, KCl 4.7 mM, $CaCl_2$ 2.5 mM; KH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM; and $C_6H_{12}O_6$ 11 mm) was used for retrograde perfusion at pH 7.4 with 95% O_2 and 5% CO_2.

5. IPC induction and experimentation

Wistar rats of both sexes (n = 6) were placed in 10 separate groups. After a 7-day acclimatization period, the animals were divided at random. Fig. 1 shows a schematic of the test group organization. Isolated rat hearts were kept stable by perfusing with the KH buffer solution for 190 min without global ischemia.

After 10 min of stabilization, the isolated rat hearts were subjected to 30 min of global ischemia, followed by 120 min of reperfusion. They were then subjected to four cycles of preconditioning. In each cycle, the hearts were isolated for 5 min, followed by 5 min of reperfusion with the KH buffer and 10 min of stabilization. Next, global ischemia was conducted for 30 min, followed by 120 min of reperfusion for the rat hearts. The hearts of HL rats were stabilized for 10 min before they were subjected to global ischemia, after which they were re-oxygenated for 120 min (I/R in HL rats). A hydroalcoholic extract of IrA (100 mg/day, diluted in 0.9% normal saline once daily for 30 days) was administered to the hearts of HL rats after a 5-week diet that led to HL. The isolated rat hearts were kept stable for 10 min before being put into global ischemia for 30 min. They were then allowed to recover for another 120 min. After stabilization for 10 min, the HL heart was subjected to four cycles of preconditioning consisting of 5 min of occlusion, followed by 5 min of reperfusion with the KH buffer after 10 min of stabilization. The rat heart was then subjected to global ischemia for 30 min and reperfusion for 120 min. Another 30-min

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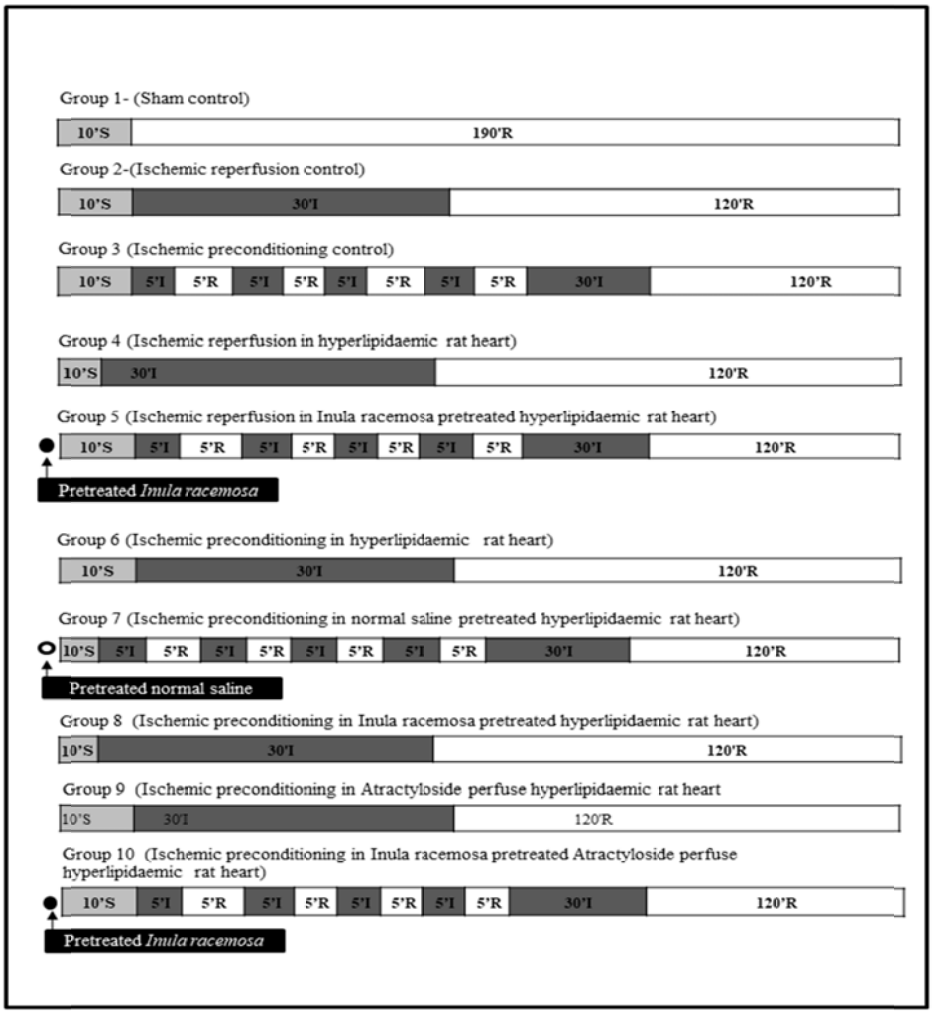

Figure 1. The graphical representation of the experimental methodology for distinct groups of animals.

period of global ischemia was followed by a 120-min period of reperfusion. Atr-perfused HL rat heart was subjected to four cycles of preconditioning, which included 5 min of occlusion, followed by 5 min of reperfusion with KH buffer after 10 min of stabilization. The heart was then subjected to global ischemia for 30 min, followed by 120 min of reperfusion. Next, Atr (20 M) reperfusion was conducted in the final episode of IPC. The heart isolated from HL rats was treated with a hydroalcoholic extract of IrA and then subjected to four cycles of preconditioning: 5 min of occlusion, followed by 5 min of reperfusion with the KH buffer after stabilization for 10 min (10). Next, IPC was conducted in the rat heart treated with IrA and Atr was administered intravenously. A 30-min period of global ischemia was followed by a 120-min period of reperfusion.

6. Determining the size of myocardial infarction

The isolated heart was kept at –85℃ for 25-30 min after the end of the cycle. The frozen heart was sliced from the top to bottom and the cross sections were examined. Each slice was 2-3 mm thick. The live cardiac tissue was dyed brick red using 1% triphenyl–tetrazolium chloride (TTC), while the infarct area was left untouched. ImageJ was used to obtain an idea about the infarct size as a percentage of the overall surface area of the heart [23].

7. Assessment of cell damage to the myocardium

A commercially available kit (Coral Clinical System Pvt. Ltd., India) was used to estimate the amounts of lactate dehydrogenase (LDH) and creatine kinase-myoglobin binding (CK-MB) responsible for coronary drainage to quantify the extent of

cell injury [24].

8. Hematological factor

A Langendorff assembler was installed in the heart. The left ventricular end-diastolic pressure (LVEDP) was equalized between 5 and 10 mm Hg using an inflated L–V balloon filled with Alatex. The heart rate (HR), LVEDP, and L–V pressures were recorded using the acquisition system software (PowerLab). A corresponding parameter was recorded in each set of experiments. The flow rate of the coronary arteries was recorded and used for subsequent tests. The HR and the occurrence of reperfusion-mediated ventricular fibrillation (VF) were noted in each set of tests at basal, 0, 30, and 120 min immediately following the induction of global ischemia in each experiment [25].

9. Release of troponin-T

A commercially available troponin-T test kit (Coral Clinical System Pvt. Ltd., India) [26] was used to assess the troponin-T release during cardiac preparation.

10. Assessment of myocardial tissue oxidative stress

Heart tissue samples were softened at 4℃ and homogenized for 5 min using a homogenizer at 6,000 rpm in 10% w/v ice-cold 0.1 M phosphate buffer (pH 7.4). First, 2 mL of the supernatant was collected after the homogenate was centrifuged for 10 min at 3,000 rpm at 4℃. The leftover tissue homogenate was used to separate the proteins. The supernatant was collected after centrifugation at 4℃ for 10 min at 4,000 rpm with an equal volume of 5% trichloroacetic acid (TCA) [27].

11. Evaluation of the activity of superoxide dismutase

Spectrophotometric analysis at 560 nm was used to measure the SOD activity of the heart. A 96-well plate configuration was used for each of the blank, standard (autoxidation), and tissue samples. The blank received 300 L of the tris buffer. For the other samples, 10 L of the tissue homogenate was added, followed by the addition of 280 L of the tris buffer. Finally, 10 L of pyrogallol was administered via a multichannel pipette to each well [28].

12. Catalase (CAT) activity measurement

Hydrogen peroxide accumulated in the myocardium in the presence of CAT. A 3-mL cuvette containing 1.95 mL of 50 mM phosphate buffer was used to collect the sample (pH 7.0). Finally, the absorbance changes were monitored for 15 s every 30 s at 240 nm by adding 1 mL of hydrogen peroxide to the solution and monitoring the changes for 30 min. [26]

13. Tests for glutathione (GSH) oxidation activity

Glutathione peroxidase activity was estimated using the GPX-catalyzed oxidation of glutathione by cumene hydroperoxide. The absorbance was measured at 412 nm after 10 min of incubation [26].

14. Isolation of heart-tissue mitochondria

After the heart was perfused in the control group, the mitochondria were promptly separated using the usual technique. After reperfusion, all remaining groups had their cardiac mitochondria extracted immediately and used as lipidemic models. The Lowry method was used to estimate the mitochondrial protein content [29].

15. Evaluation of mitochondrial performance

Spectroscopy was conducted at 595 nm to measure the amount of formazan produced. The amount of formazan produced to decrease (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used to quantify the mitochondrial function, which was expressed in milligram-per-minute-of-formazan production per milligram of protein.

16. Cardiac collagen and histopathological investigation

Formaldehyde was used to fix the cardiac collagen in 10% neutral buffered formalin. A microtome was used to cut an approximately 5-nm-thick transverse mid-ventricular segment from these tissues after they had been fixed. The size of myocytes involved in necrosis was determined. The myocytes were then stained with picrosirius red F3BA fibrosis (collagen content).

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17. Statistics

GraphPad Prism 7 was used for the statistical analysis. The mean standard deviation (SD) was used for each parameter. ANOVA and Bonferroni post-hoc tests were performed to analyze the coronary flow rate, hemodynamic, HR, LDH, and CK-MB enzyme activity. All additional statistical data analyses made use of one-way ANOVA and the Student–Newman–Keuls post-hoc test. p-values of < 0.05 were deemed statistically significant.

RESULTS

IrA potentiates the IPC-induced increase in the coronary flow of HL rat hearts. Fig. 2a shows the effect of IrA on the coronary flow in the HL rat heart, which mediates the IPC. Post-hoc tests did not show any significant differences in the coronary flow of the different groups at baseline. In contrast to the I/R control and IPC + HL groups, IrA enhanced the IPC-mediated increase in the coronary flow of HL rats.

The cardiac function of the HL rats was increased when the IPC-induced increase in the HR was inhibited. Fig. 2b shows the effect of IrA on IPC-mediated alterations in the HR in HL rats. The post-hoc test did not show any significant difference in the basal HR across groups in any of the studies. When ischemia commences in the rat heart, the HR declines in the I/R control, IPC control, and IPC + HL groups, whereas IrA stimulates the IPC-mediated increase in the HR of HL rat hearts. This effect was observed during the entire duration of the experiment.

Fig. 3 shows how IrA affects the IPC-mediated increase in the LVDP, $+dP/dt_{max}$, and $-dP/dt_{max}$ in the HL rat hearts. According to the post-hoc analysis of the data, no statistically significant differences were observed in the LVDP, $+dP/dt_{max}$, and $-dP/dt_{max}$ at baseline in this group of rats. However, a decrease in the LVDP, $+dP/dt_{max}$, and $-dP/dt_{max}$ were observed in the IPC-mediated HL-induced rat hearts, although IrA increased them in the control and IPC + HL rat hearts. This effect was observed during the entire experiment.

IrA facilitates the IPC-induced reduction in LDH levels

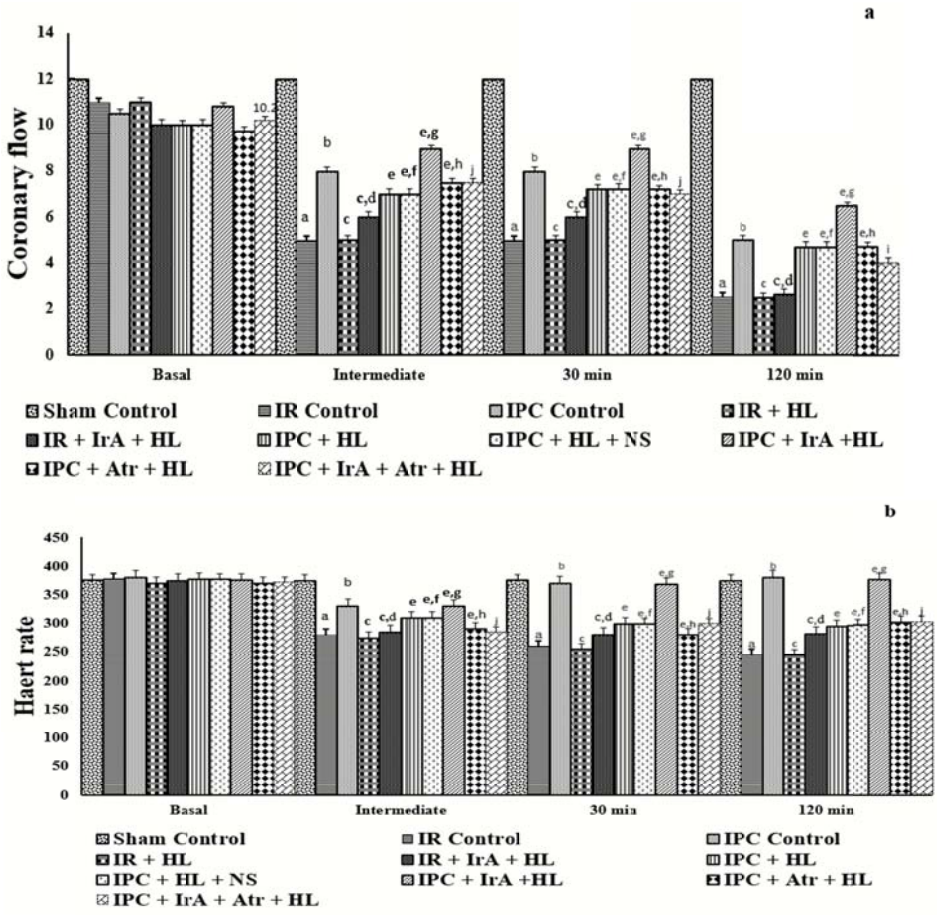

Figure 2. IPC-mediated alterations in coronary flow and heart rate in HL rats were recovered by *Inula racemosa*. Unless otherwise noted, all data are presented as mean standard deviation (n = 6). There was a significant difference between the control and I/R groups (a, b), the I/R control group (c), and the I/R + HL control group (d). $^{e}p < 0.05$ in comparison to the IPC control. $^{e,f}p < 0.05$ compared to IPC control and IPC + HL, respectively. The difference between IPC control and IPC + HL for $^{e,g}p$ was 0.05. There was a statistically significant difference between the IPC control and IPC + HL, and between the IPC control and IPC + IrA + HL ($^{e,h}p < 0.05$). (one way ANOVA followed by Bonferroni post hoc test).

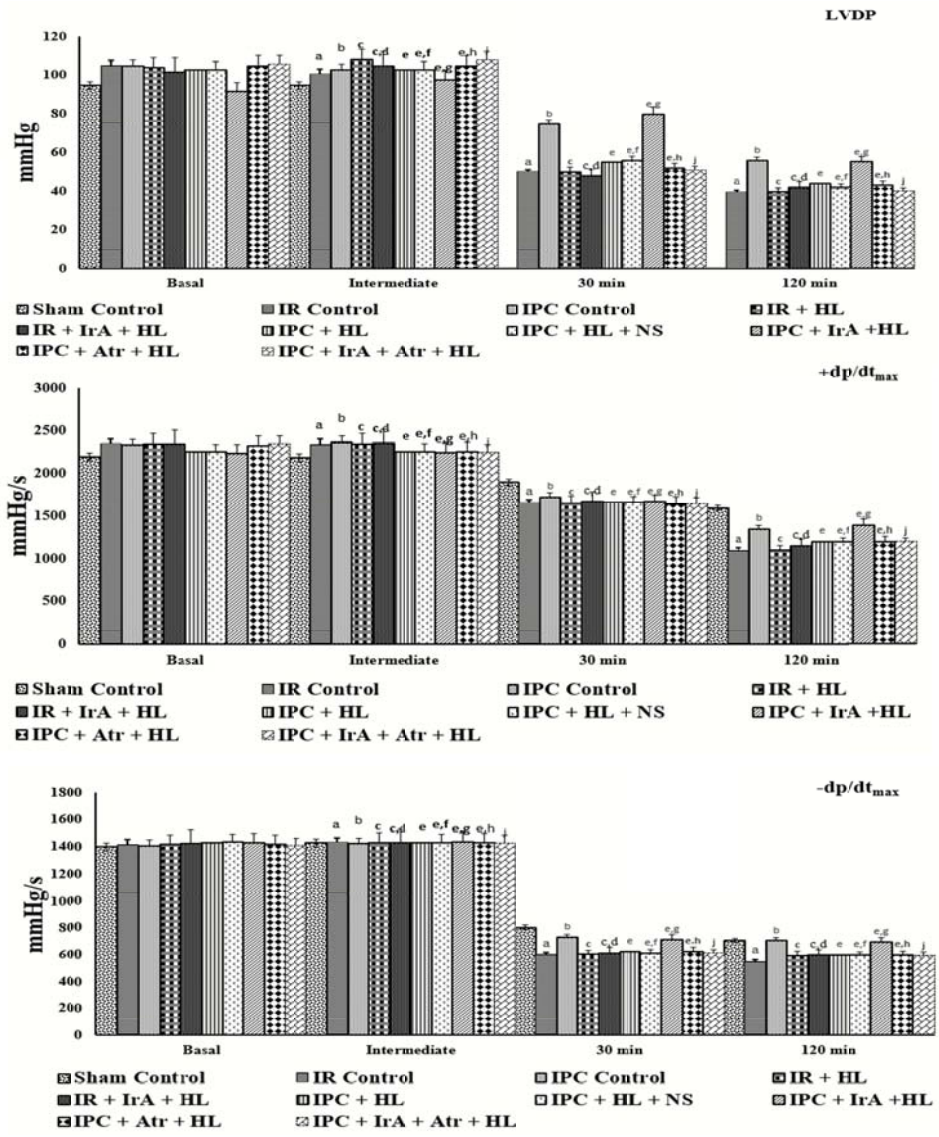

Figure 3. Figure shows how *Inula racemosa* restored in IPC mediated changes in LVDP, $+dp/dt_{max}$, and $-dp/dt_{max}$ in the hearts of rats with HL. Data are presented as mean standard deviation (n = 6). $^{a}p < 0.05$ compared with sham control, $^{b}p < 0.05$ compared with I/R control, $^{c}p < 0.05$ compared with IR control, $^{c,d}p < 0.05$ compared with I/R control and I/R + HL, $^{e}p < 0.05$ compared with IPC control, $^{e,f}p < 0.05$ compared with IPC control and IPC + HL, $^{e,g}p < 0.05$ compared with IPC control and IPC + HL, $^{e,h}p < 0.05$ compared with IPC control and IPC + HL, $^{j}p < 0.05$ compared with IPC control and IPC + IrA + HL (one way ANOVA followed by Bon-ferroni post hoc test).

in the HL rat heart. Fig. 4a shows the LDH activity in HL rat hearts owing to IPC-mediated alterations. A post-hoc analysis showed that there was no significant difference in the basal LDH levels in this group of rats. In contrast to the I/R control and IPC + HL rat hearts, the LDH level in the coronary effluent reduced in HL rat hearts owing to an IPC-mediated drop, while IrA decreased the LDH level. This effect was observed for the remaining experiment.

The CK-MB levels of HL rats reduce owing to the addition of IrA. IPC-mediated alterations, including in the CK-MB activity, are seen in Fig. 4b. The CK-MB levels of the rats of this group were not significantly different from those of the other groups for all of the studies, according to a post-hoc analysis. IPC-mediated reductions decreased the CK-MB levels in the coronary effluent of HL rats, while IrA decreased them in the I/R control and IPC + HL rats. This effect was observed for the entire duration of the experiment. In addition, Atr and IrA decreased the IPC-mediated reduction of CK-MB in the hearts of HL rats.

The infarct size and the VF of HL rat hearts were affected by IrA owing to IPC-mediated alterations (Fig. 5). According to a post-hoc analysis, the IPC considerably reduced the extent of myocardial infarctions and the VF generated by HL, which was then compared to those of the I/R control and IPC + rats. In HL rat hearts, Atr + IrA reduced the infarct size and VF less effectively than did IPC alone.

The IPC-induced decrease of the infarct size and VF of the HL rat heart can be facilitated by IrA. Fig. 6 how IrA causes

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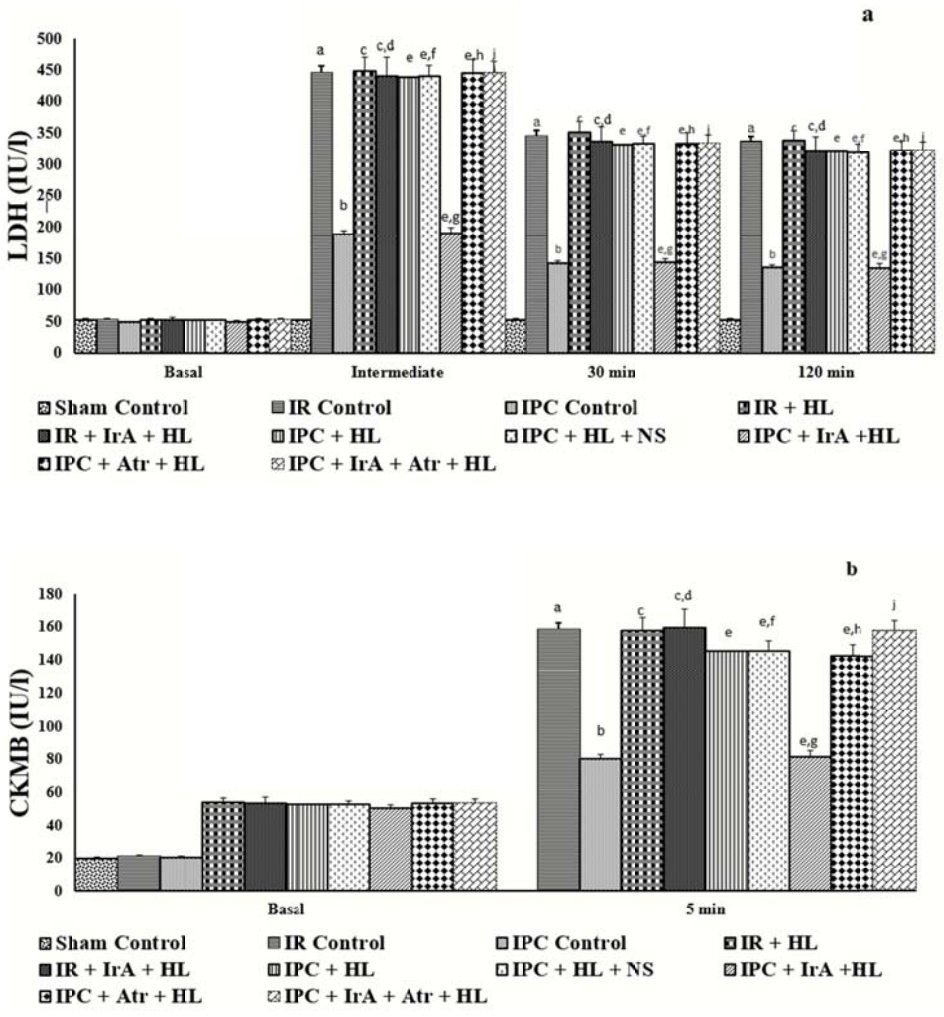

Figure 4. In HL rat hearts, *Inula racemosa* reversed IPC-mediated changes in LDH and CKMB. Unless otherwise noted, all data are presented as mean standard deviation (n = 6). I/R control was found to be more effective in terms of ^{a}p than sham, while I/R control was found to be more effective in terms of ^{c}p and ^{d}p than I/R control and I/R + HL. IPC control was found to be more effective in terms of anecdotal evidence, while IPC control and IPC + HL were found to be more effective in terms of objective evidence. When compared to IPC control and IPC + HL, $^{e,h,j}p < 0.05$ were found to be significantly lower than the IPC control and IPC + IrA + HL (one way ANOVA followed by Bonferroni post hoc test).

IPC-mediated alterations in the mitochondrial function, as measured by the formazan level and the fluorescence intensity of tetramethyl rhodamine methyl ester (TMRM) (integrity). When compared to a sham control, the post-hoc test found that the mitochondrial function was significantly impaired in HL rat hearts. In contrast to the control and IPC + HL groups, IrA and Atr reduced the IPC-mediated increase in the mitochondrial activity of HL rat hearts.

IPC-induced reduction in the oxidative stress of the HL rat hearts can be facilitated by the addition of IrA. Table 1 shows IPC-mediated alterations in the GSH, CAT, and SOD activities of HL rat hearts after the IrA treatment. Compared to the sham control, the GSH, CAT, and SOD (antioxidant) activities were not significantly reduced in the HL heart. IPC reduced the elevated activity of HL rat hearts. Compared to the I/R control and IPC + HL groups, IrA increases the IPC-mediated reduction of antioxidant levels in HL rat hearts. Interestingly, in HL rat hearts, Atr and IrA inhibited an increase in the oxidative stress indicators mediated by IPC.

Attenuation in the levels of troponin-T in HL rat hearts is facilitated by IrA in the presence of IPC. Fig. 7 demonstrates the role of IrA on IPC and troponin-T levels in the hearts of HL rats. While IPC significantly reduced the troponin-T rises relative to that of sham control hearts, IrA significantly reduced the rise in troponin-T levels in IPC + HL hearts. Hence, a combination of Atr and IrA reduces troponin-T levels in HL rat hearts.

Myocardial histological alterations of the HL rat hearts are attenuated by IrA, which is produced by IPC. Histological alterations in several rat cardiac groups are shown in various photomicrographs (Fig. 8).

The endocardium, epicardium, and papillary muscles and the heart's vascular system were found to be normal in the sham control. The microscopic injury to the heart cannot be seen. Myophagocytosis is seen in the I/R control group photo-

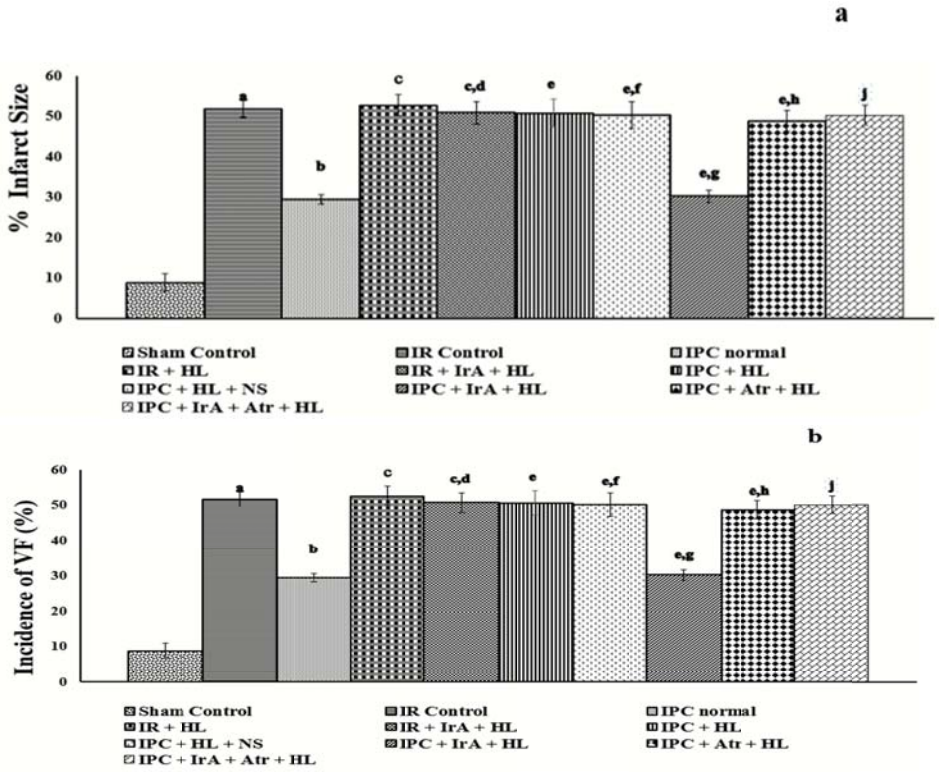

Figure 5. Pictured here is an infarct-heart-size and ventricular fibrillation-restoration study in HL rats using *Inula racemosa* as the modality of intervention. Unless otherwise noted, all data are presented as mean standard deviation (n = 6). I/R control was found to be more effective in terms of ^{a}p than sham, while I/R control was found to be more effective in terms of ^{c}p and ^{d}p than I/R control and I/R + HL. IPC control was found to be more effective in terms of anecdotal evidence, while IPC control and IPC + HL were found to be more effective in terms of objective evidence. When compared to IPC control and IPC + HL, $^{e,h,j}p < 0.05$ were found to be significantly lower than the IPC control and IPC + IrA + HL (one way ANOVA followed by Bon-ferroni post hoc test).

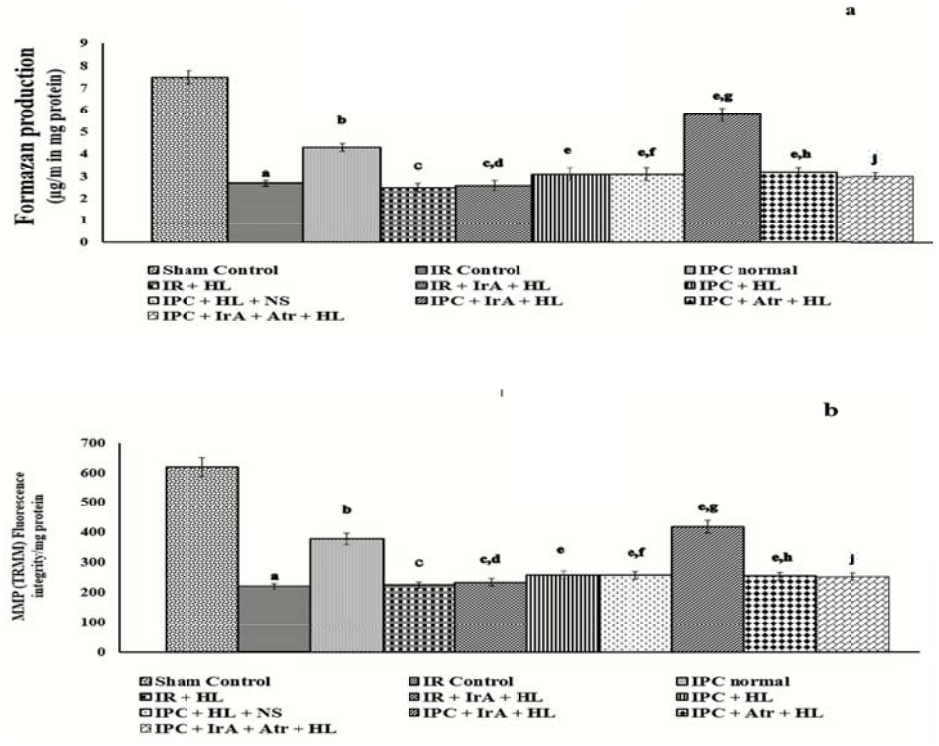

Figure 6. Mitochondrial function (Formazan production) and mitochondrial integrity were both restored by *Inula racemosa* in HL rat hearts via IPC-mediated changes. Unless otherwise noted, all data are presented as mean standard deviation (n = 6). IPC control and IPC + IrA + HL are both statistically significant, but IPC control and IPC + IrA are not, and IPC control and IPC + IrA are both statistically significant. $^{a}p < 0.05$ compared with the sham control; $^{b}p = 0.01$; $^{c}p = 0.01$; $^{d}p = 0.01$; $^{e}p = 0.01$; $^{f}p = 0.01$; $^{g}p = 0.01$; $^{h}p = 0.01$; $^{j}p = 0.01$; $^{k}p = 0.01$; $^{l}p = 0.01$; $^{m}p = 0.01$; $^{n}p = 0.01$; $^{o}p = 0.01$; $^{p}p = 0.01$; $^{q}p = 0.01$; $^{r}p = 0.01$; ^{st}p (one way ANOVA followed by Bon-ferroni post hoc test).

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Table 1. Myocardial tissue oxidative stress assessment

Oxidative stress markers	GSH level (units/min/mg protein)	SOD level (units/min/mg protein)	CAT level (units/min/mg protein)
Sham control	2.05 ± 0.091	0.56 ± 0.059	2.73 ± 0.26
I/R control	0.455 ± 0.069[a]	0.19 ± 0.09[a]	1.46 ± 0.27[a]
IPC control	1.03 ± 0.083[b]	0.55 ± 0.056[b]	2.38 ± 0.22[b]
I/R + HL	0.453 ± 0.056[c]	0.20 ± 0.024[c]	1.45 ± 0.21[c]
I/R + IrA + HL	0.453 ± 0.073[c,d]	0.22 ± 0.045[c,d]	1.48 ± 0.19[c,d]
IPC + HL	0.532 ± 0.06[e]	0.24 ± 0.023[e]	1.52 ± 0.23[e]
IPC + HL + NS	0.532 ± 0.075[e,f]	0.24 ± 0.056[e,f]	1.53 ± 0.24[e,f]
IPC + IrA + HL	1.21 ± 0.095[e,g]	0.56 ± 0.026[e,g]	2.4 ± 0.21[e,g]
IPC + Atr + HL	0.896 ± 0.086[e,h]	0.21 ± 0.051[e,h]	1.51 ± 0.22[e,h]
IPC + IrA + Atr + HL	0.851 ± 0.048[j]	0.201 ± 0.021[j]	1.52 ± 0.26[j]

All values are mean ± S.D. (n = 6); [a]$p < 0.05$ compared with sham control, [b]$p < 0.05$ compared with I/R control, [c]$p < 0.05$ compared with IR control, [c,d]$p < 0.05$ compared with I/R control and I/R + HL, [e]$p < 0.05$ compared with IPC control, [e,f]$p < 0.05$ compared with IPC control and IPC + HL, [e,g]$p < 0.05$ compared with IPC control and IPC + HL, [e,h]$p < 0.05$ compared with IPC control and IPC + HL, [j]$p < 0.05$ compared with IPC control and IPC + IrA + HL (one way ANOVA followed by Student-Newman-Keuls post hoc test).

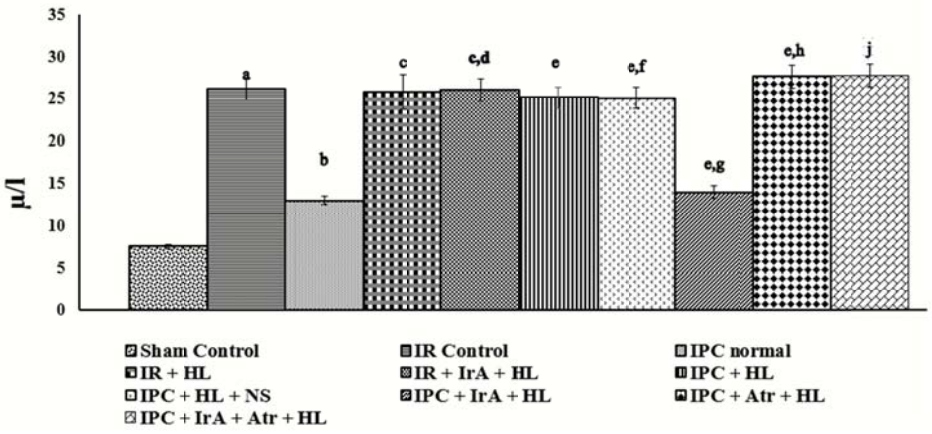

Figure 7. Troponin-T in the heart of HL rats was restored by *Inula racemosa* after it had been altered by IPC. [a]$p < 0.05$ compared with sham control, [b]$p < 0.05$ compared with I/R control, [c]$p < 0.05$ compared with IR control, [c,d]$p < 0.05$ compared with I/R control and I/R + HL, [e]$p < 0.05$ compared with IPC control, [e,f]$p < 0.05$ compared with IPC control and IPC + HL, [e,g]$p < 0.05$ compared with IPC control and IPC + HL, [e,h]$p < 0.05$ compared with IPC control and IPC + HL, [j]$p < 0.05$ compared with IPC control and IPC + IrA + HL (one way ANOVA followed by Bon-ferroni post hoc test).

micrograph, thereby confirming lymphocytic infiltration and localized necrosis. Cells with substantial chronic inflammation can be seen in the subendocardium under a microscope. In the IPC-treated group, myonecrosis decreased and the cells showed reduced inflammation. Sub-endocardial and myonecrotic alterations have been spotted under the microscope. Myonecrosis was more severe in the IPC + HL group, while inflammatory infiltration was less severe and cell myophagocytosis was reduced compared to those in the IPC group. Sub-endocardium vacuolar alterations can also be detected under a microscope. The IPC + IrA group displays reduced inflammation in cells and decreased myonecrosis, as can be seen using a microscope. Myonecrosis, myophagocytosis, and lymph vascular infiltration were less severe in the IPC + HL + IrA-treated group. Inflammatory cell infiltration and edema were also minimal. Compared to the IPC and IPC + HL + IrA groups, myonecrosis is visible, inflammation is infiltrated, and cell myophagocytosis is reduced in the IPC + HL + Atr-treated group.

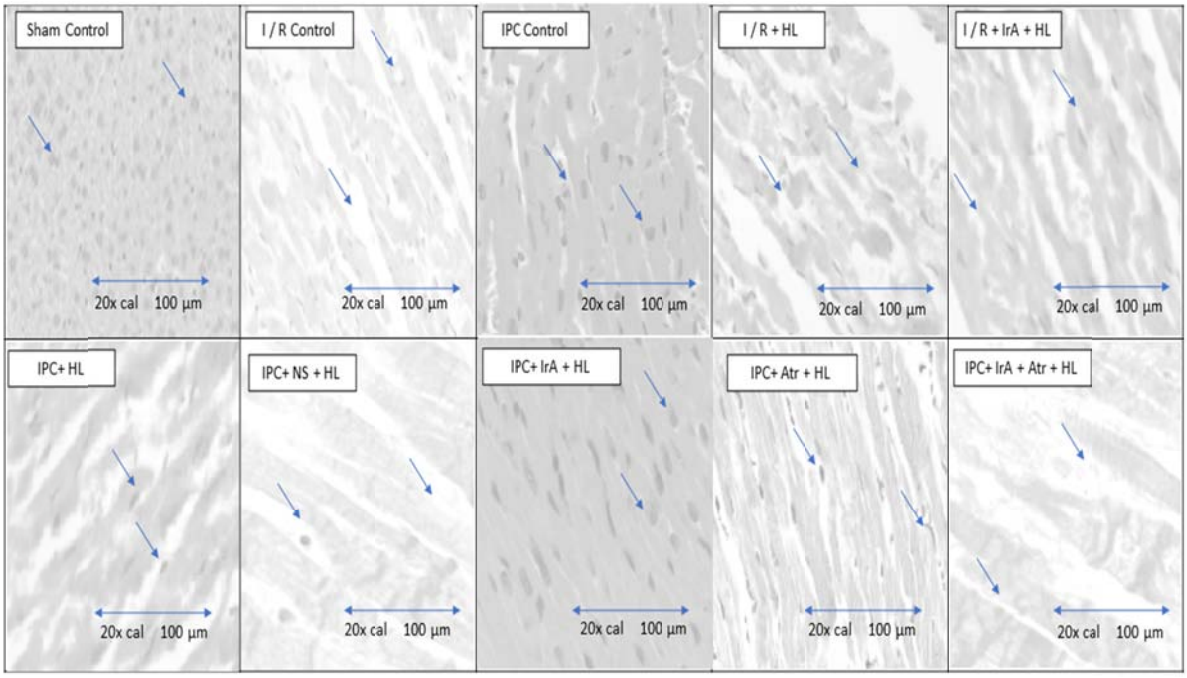

Figure 8. Normal, hyperlipidaemic, IrA-treated hyperlipidaemic, and I/R-treated heart histopathology Normal and hyperlipidemia heart histopathological changes, as a result of IPC. A histopathological report of the effects of Hyperlipidaemia on the heart of pretreated IrA, AtR or pretreated IrA and AtR. I/R control shows necrotic changes in myocardial tissue, while sham control shows normal myocardial cell architecture. Myocardial tissue has undergone a regenerative process, according to IPC control. I/R + HL shows necrotic change in tissue architecture, I/R + IrA + HL shows necrotic change in tissue architecture, IPC + HL shows less regenerative change in myocardial tissues, IPC + NS + HL shows less regenerative change in myocardial tissue, IPC + IrA + HL shows more regenerative change in myocardial tissue architecture, IPC + Atr + HL shows necrotic changes in myocardial tissues.

DISCUSSION

After 30 min of global ischemia and 120 min of reperfusion in an HL heart mounted on the Langendorff heart assembly, no significant cardioprotective phenomenon was observed in comparison to that exhibited by the I/R control group after 5 min of ischemia and KH buffer reperfusion. This study, however, found an important cardioprotective effect 30 days before isolating the heart of the animals provided with the *I. racemosa* extract. There was also strong evidence that the MPTP opener Atr diminished the cardioprotective effects of IPC in the HL rats provided with the *I. racemosa* extract [16]. This study showed that I/R-induced damage caused by the inhibition of the MPTP opening differs from the cardioprotection provided by *I. racemosa*, which was administered to sick hearts.

Administration of *I. racemosa* to normal rat hearts for 30 days did not influence the IPC cycle, although it significantly restored the IPC-induced cardioprotective effect in HL rat hearts. The LVDP, $+dP/dt_{max}$, and $-dP/dt_{max}$, coronary flow, and HR reduced, which weakened the cardiac dysfunction. We observed that *I. racemosa* sustains the hemodynamic parameters of HL rat hearts compared to that observed in the IR and IPC + HL groups.

The degree of CK-MB, LDH, myocardial infarct size, and VF release during ischemia reperfusion is closely related to the damage to the heart muscles. For HL rats, the pretreatment with *I. racemosa* extract reduces LDH, CK-MB, and the extent of myocardial infarction and VF. In contrast, pretreatment with Atr-perfused *I. racemosa* enhanced the CK-MB and LDH release, myocardial infarction size, and VF in HL rat hearts. Activation of KATP by inhibiting the MPTP opening and activation of IPC gives rise to the cardioprotective phenomenon [30, 31].

Reduced levels of oxidative stress markers such as GSH, SOD, and CAT are restored in IPC-treated HL rats, as are the levels of formazan and MMP. In contrast, pretreatment with the *I. racemosa* extract improved the IPC cardioprotective effect in HL rat groups. Because of the opening of MPTP, Atr + IPC pretreatment of the *I. racemosa* extract showed a reduced cardioprotective effect for HL rats. Hence, the inhibition of MPTP opening by the *I. racemosa* extract enhances the cardioprotec-

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tive effect afforded by IPC in HL rats. Suppression of MPTP opening is a protective mechanism.

The contractile force of myocytes is transferred to the ventricular lumen by the collagen, which keeps the ventricular structure intact. However, the cardioprotective effect afforded by the IPC was ineffective in several conditions. Various heart illnesses cause fibrosis of the myocardium with time [32]. Collagen-related data are collected for IPC in HL for high-level purposes. Myocardial infarction results in an extracellular matrix-rich scar on the myocardium [33]; however, the heart collagen is affected by HL. This study showed that cardioprotective effects in HL rat hearts reduce when Atr and *I. racemosa* are co-administered.

This study showed that *I. racemosa* could protect the heart from HL-induced cardiac damage by employing IPC. Consequently, the *I. racemosa* extract can benefit patients with acute myocardial infarction as it reduces the occurrence of additional ischemic events. Hence, *I. racemosa* treatment can be employed as a cardioprotective adjuvant therapy.

CONCLUSION

IPC may have a reduced cardioprotective impact on HL rat hearts because MPTP is inhibited by the hydroalcoholic extract from *I. racemose* Hook roots. The cardioprotective effects afforded by the IPC reduced in HL rat hearts treated with Atr (MPTP opener). Hence, *I. racemosa* could be used as an adjuvant to protect the heart from further damage after a recent myocardial infarction.

DECLARATION

The Institutional Animal Ethics Committee (KNIMT/PHAR/IAEC/18/05) approved all experimental techniques in conformity with national rules for animal experimentation.

AUTHENTICATION OF PLANT SPECIES

NIScPR/RHMD/Consult/2021/3881-82 is the reference number for the *Inula racemosa* roots that were obtained from a local supplier and authenticated by the Council for Scientific and Industrial Research (CSIR-NISCAIR) in New Delhi.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Researchers AKT, PSG, and MP are all involved in the design and conduct of the experiments. A research protocol was drafted by PM in conjunction with the first and co-authors after PM had participated in data processing and interpretation. It is a joint effort by all of the writers to design, evaluate, and analyse experimental research, as well as to write the manuscript.

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