

## CARDIOPROTECTIVE EFFECT OF INM-176 ON ISCHEMIA REPERFUSION INJURY USING RAT HEART MODEL

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### ABSTRACT

**Objective:** Cardioprotective effect of INM-176 in ischemia reperfusion (I/R) injury in rat heart was evaluated by measuring the extent of release of the enzyme lactate dehydrogenase (LDH) and creatine phosphokinase (CK) and by measuring the myocardial infarction (MI) size.

**Methods:** Five groups of animals were employed. Animals were sacrificed, and heart was mounted over the Langendorff's apparatus. Group I was employed as control group, Group II was the myocardial ischemic (MI) group, and Group III, IV, and V were pre-treated with different doses of INM-176 (150, 300, and 600 mg/kg). After this, global ischemia accompanied by reperfusion for 30 and 120 minutes was given, respectively. The effluent was collected from coronary vessel before the initiation of ischemia, immediately, after 5 and 30 minutes of reperfusion. The magnitude of myocardial injury was measured by LDH and CK release in coronary effluent. MI size was also evaluated macroscopically using triphenyl tetrazolium chloride staining.

**Results:** In the present study, INM-176 was found to produce a dose-dependent cardioprotective effect against I/R injury, as observed by a decrease in the level of LDH, CK, and infarct size. Pre-treatment with a dose of 600 mg/kg was found to be more effective as compared to 150 and 300 mg/kg dose of INM-176.

**Conclusion:** INM-176 pre-treatment at a dose of 600 mg/kg provides cardioprotection against I/R-induced myocardial injury.

**Keywords:** *Angelica gigas*, INM-176, Ischemia, Reperfusion, Langendorff's apparatus, Myocardial injury.

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### INTRODUCTION

Myocardial ischemia reperfusion (I/R) injury occurs due to the reduced blood supply to the myocardium, which further accompanied by the restoration of blood flow. Ischemia in any region of the body can initiate the tissue damage due to lack of oxygen and nutrients; however, the muscles of the heart are more susceptible as they demand more energy for their proper functioning. Therefore, immediate reflow of blood to the ischemic regions can prevent the further damage [1].

Ischemia accompanied by reperfusion can result into reversible or irreversible myocardial injury; however, it also depends on its duration as well as on severity [2]. If reperfusion does not occur, then ischemia leads to cell death. Therefore, reperfusion is the only treatment to save the myocardial cell from the expected death [3]. Delivery of O<sub>2</sub> and substrate for aerobic adenosine triphosphate generation are restored through prompt reperfusion, reperfusion after long duration itself appears to have detrimental consequences [4]. During reperfusion of ischemic tissue, the production of a number of toxic reactive oxygen species (ROS) occurs [5]. ROS may induce tissue injury through lipid peroxidation, protein oxidation, and also by producing damage to DNA [6,7]. The ROS is scavenged by endogenous antioxidants [8]. I/R injury induces the redox imbalance which makes the tissue more prone to oxidative damage [9].

Nowadays, researchers put more attention to develop or find out the medicine with more antioxidant activity and less side effects [10]. As per the traditional medicine system of China, Japan, and Korea, *Angelica gigas* Nakai (*A. gigas*) is used to promote blood flow in the brain and heart [10,11]. The various reported pharmacological activities of *A. gigas* are antiplatelet aggregation, antinematodal activity, antioxidant effects, anticancer, and antibacterial [11,12]. INM-176 is a standardized ethanolic extract of *A. gigas* Nakai roots belongs to family Umbelliferae.

It is pale-yellow powder. The various chemical constituents present in this plant are coumarins, chlorogenic acid, ferulic acid, essential oils, and polyacetylenes [13]. The coumarin includes decursinol, decursin, decursinol angelate, marmesin, and nodakenin [14,15]. As decursin and decursinol angelate are the main constituents of INM-176 have powerful antioxidant activity, so on the basis of its antioxidant properties, the present study has been designed to evaluate the cardioprotective effect of INM-176 in I/R injury.

### METHOD

#### Chemicals

INM-176 was provided by Mr. Jong Hoon Ryu, Professor at Kyung Hee University, Seoul, Republic of Korea, in the pale yellow-colored powdered form. The rest of the chemicals used in the present study were purchased from the Loba Chem Pvt., Ltd., CDH Pvt., Ltd., Molychem, Qualigens fine chemicals, and Himedia laboratories Pvt., Ltd., India.

#### Animals and approvals

Healthy adult albino Wister rats (250-450 g) were used in the study. The adult albino Wister rats were obtained from NIPER, Mohali. The animals were housed in the group of 5 animals each in clean acrylic cages. The animals were kept for 10 days for acclimatization in the central animal house, before the execution of protocol. The animals were kept under natural day and night cycle with temperature of 21±2°C. They were fed with the commercial pelleted animal feed and water ad libitum. The study design was approved by the Institutional Animal Ethics Committee (IAEC) with approval number (LPU/LSPA/IAEC/CPCSEA/MEETING NO. 1/SEPTEMBER 2015/2016 PROTOCOL NO. 7).

#### Grouping of animals

Five groups (n=6 per group) of animals were made. Drug was dissolved in physiological salt solution and administered through physiological

salt solution directly into the heart using Langendorff apparatus. For statistical evaluation, ANOVA one and Turkey test were applied, and p value ( $p \leq 0.005$ ) was used.

Group I served as control group. The isolated rat hearts, after 15 minutes of stabilization, were continuously perfused with K-H solution for 120 minutes. Group II served as myocardial injury group, and in this group, after the stabilization of 15 minutes, the hearts were subjected to global ischemia accompanied by reperfusion for 30 and 120 minutes, respectively. Group III, IV, and V served as treatment groups (INM-176 150, 300, and 600 mg/kg, respectively). After stabilization for 15 minutes, the hearts were perfused with K-H solution containing INM-176 (150, 300, and 600 mg/kg) for 20 minutes. Then, rat hearts were subjected to global ischemia accompanied by reperfusion with K-H solution containing INM-176 for 30 and 120 minutes, respectively (Table 1).

Isolated perfused rat heart preparation and assessment of the infarct was performed as mentioned in the study by Bhatti et al. 2008 [16]. Lactate dehydrogenase (LDH) and creatine phosphokinase (CK) were estimated in the samples of coronary effluent using 2, 4-dinitrophenylhydrazine method and Ochei method, respectively [17,18].

## RESULTS

### Effect of INM-176 on I/R-induced LDH release

Global ischemia accompanied by reperfusion for 30 and 120 minutes significantly increased the level of LDH instantly and 30 minutes after reperfusion when compared with control group. It was found that pre-treatment with INM-176 significantly reduced I/R-induced release of LDH when compared with myocardial injury group ( $p < 0.001$ ). Moreover, 600 mg/kg dose of INM-176 significantly attenuated the level of LDH when compared with 150 mg/kg and 300 mg/kg pre-treatment group (Fig. 1 and Table 2).

### Effect of INM-176 on I/R-induced CK release

It was found that pre-treatment with INM-176 (300 and 600 mg/kg) significantly reduced the release of CK due to I/R when compared with the myocardial injury group ( $p < 0.001$ ). However, pre-treatment with 150 mg/kg was not statistically significant when compared with the myocardial injury group ( $p > 0.05$ ). Moreover, a dose of 600 mg/kg of INM-176 significantly attenuated the level of CK when compared with 300 mg/kg pre-treatment group (Fig. 2 and Table 3).

### Effect of INM-176 on I/R-induced myocardial infarction (MI) size

MI size significantly increased with global ischemia measured by weight and volume method when compared with control group. It was found that pre-treatment with INM-176 (300 and 600 mg/kg) significantly reduced the MI size induced due to I/R when compared with myocardial injury group ( $p < 0.001$ ). However, pre-treatment with 150 mg/kg dose of INM-176 was not statistically significant ( $p > 0.05$ ) when compared with myocardial injury group. Moreover, 600 mg/kg dose of INM-176 significantly attenuated the infarct size when compared with 300 mg/kg pre-treatment group and therefore found to be more effective (Fig. 3 and Table 4).

## DISCUSSION

INM-176 is standardized ethanolic extracts of *A. gigas*, and chlorogenic acid, ferulic acid, nodakenin, decursinol, decursin, and decursinol angelate are its major constituents [10]. The multiple pharmacological activities reported for *A. gigas* include antibacterial, antiplatelet aggregation, antioxidant, antinematodal, and also anticancer [12]. INM-176 has been reported as neuroprotective agent as it reduces the lipopolysaccharide-induced neuronal injury and also improves the scopolamine-induced cognitive dysfunction through inhibiting the various proinflammatory mediators as well as by inhibiting the AchE (anticholinesterase). The constituents such as decursin, decursinol angelate, chlorogenic acid, and ferulic acid have been reported to have antioxidant activity [10,11,19]. Therefore, it may be possible

Table 1: Protocol design

Groups	Dosing schedule
I	Control
II	MI
III	MI+INM 176 (150 mg/kg)
IV	MI+INM 176 (300 mg/kg)
V	MI+INM 176 (600 mg/kg)

MI: Myocardial infarction

Table 2: Effect of INM 176 on I/R-induced LDH release

Groups	Basal (IU/L)	Immediate reperfusion (IU/L)	After 30 minute of reperfusion (IU/L)
I	54.25±1.51	63.5±0.72	60.27±0.95
II	59.66±0.70	405.5±4.33**	374.03±5.37**
III	55.5±1.65	370.26±7.52#	341±9.21#
IV	56.76±2.42	339.66±13.81##	280.27±8.89##, \$\$
V	53.5±0.96	266.3±5.01##, \$\$, ^^	216.5±7.17##, \$\$, ^^

Values are expressed as mean±SEM. \*\* $p < 0.001$ , II versus I, # $p < 0.05$ , III versus II, ## $p < 0.001$ , IV and V versus II, \$\$ $p < 0.001$ , IV and V versus III, ^^ $p < 0.001$ , V versus IV. SEM: Standard error mean, I/R: Ischemia reperfusion, LDH: Lactate dehydrogenase

Table 3: Effect of INM 176 on I/R-induced CK release

Groups	Basal (IU/L)	After 5 minutes of reperfusion (IU/L)
I	21.26±0.70	24.41±0.46
II	23.71±0.77	43.54±1.21**
III	22.30±0.58	40.48±0.95
IV	23.36±0.47	32.63±0.58##, \$\$
V	22.63±0.49	27.79±0.62##, \$\$, ^^

Values are expressed as mean±SEM. \*\* $p < 0.001$ , II versus I, ## $p < 0.001$ , IV and V versus II, \$\$ $p < 0.001$ , IV and V versus III, ^^ $p < 0.05$ , V versus IV. SEM: Standard error mean, CK: Creatine phosphokinase, I/R: Ischemia reperfusion

Table 4: Effect of INM 176 on I/R-induced infarct size

Groups	Volume (% age)	Weight (% age)
I	13.80±0.65	12.46±0.38
II	33.95±0.63**	29.65±0.77**
III	31.03±0.77	26.58±1.38
IV	23.03±0.85##, \$\$	17.58±1.16##, \$\$
V	18.53±0.62##, \$\$, ^^	15.25±0.68##, \$\$

Values are expressed as mean±SEM. \*\* $p < 0.001$ , II versus I, ## $p < 0.001$ , IV and V versus II, \$\$ $p < 0.001$ , IV and V versus III, ^^ $p < 0.05$ , V versus IV. SEM: Standard error mean, I/R: Ischemia reperfusion

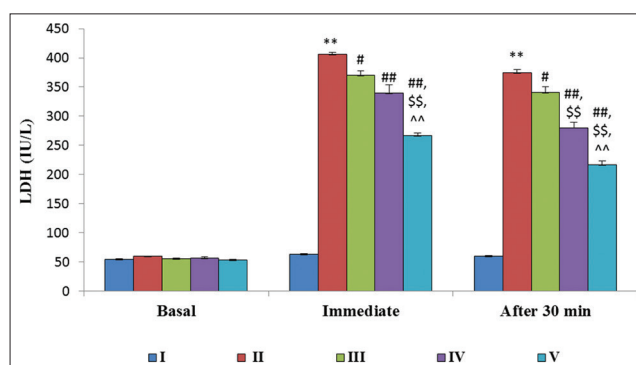
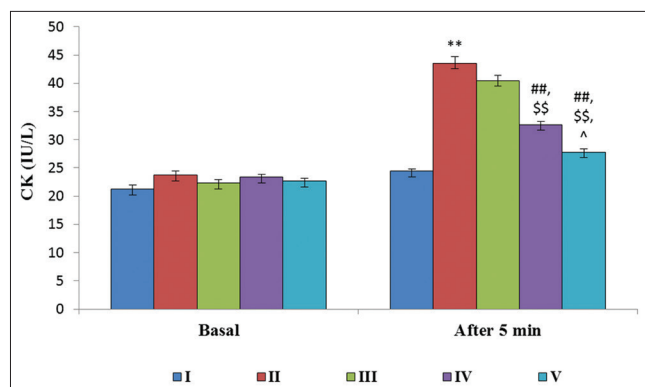
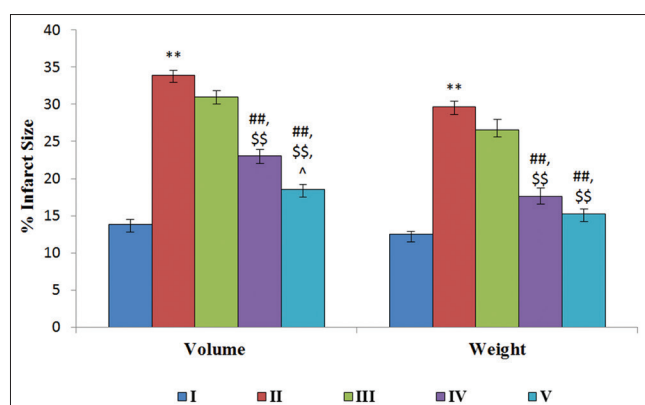


Fig. 1: Effect of INM-176 on ischemia reperfusion-induced lactate dehydrogenase release. Values are expressed as mean±standard error mean. \*\* $p < 0.001$ , II versus I, # $p < 0.05$ , III versus II, ## $p < 0.001$ , IV and V versus II, \$\$ $p < 0.001$ , IV and V versus III, ^^ $p < 0.001$ , V versus IV



**Fig. 2: Effect of INM-176 on ischemia reperfusion-induced creatine phosphokinase release. Values are expressed as mean±standard error mean. \*\*p<0.001, II versus I, ##p<0.001, IV and V versus II, \$\$p<0.001, IV and V versus III, ^p<0.05, V versus IV**



**Fig. 3: Effect of INM-176 on ischemia reperfusion-induced infarct size. Values are expressed as mean±standard error mean. \*\*p<0.001, II versus I, ##p<0.001, IV and V versus II, \$\$p<0.001, IV and V versus III, ^p<0.05, V versus IV**

to postulate that INM-176 may act as cardioprotective due to its antioxidant effect.

In the present study, global ischemia accompanied by reperfusion for 30 and 120 minutes, respectively, was given and it results into oxidative stress associated with increased in formation of various ROS which may be responsible for myocardial injury. Five groups of animals were employed. The first group was control group in which neither ischemia nor any drug treatment was given. The second group was the myocardial injury group, in which global ischemia accompanied by reperfusion for 30 and 120 minutes was given, respectively. The third, fourth, and fifth groups were the treatment groups, the hearts were pretreated with different doses of INM-176 (150, 300, and 600 mg/kg), and after this, 30 minutes of global ischemia accompanied by 120 minutes of reperfusion was given. LDH is an intracellular enzyme, which catalyzes the reversible reaction involving the conversion of lactate to pyruvate with the help of nicotinamide adenine dinucleotide. The level of this enzyme has been increased in case of myocardial injury. It was observed that immediately and after 30 minutes of reperfusion, LDH was at its peak release, whereas after 5 minutes of reperfusion, CK was at its peak release. A marked increase in release of LDH and CK as well as infarct size was observed in the myocardial injury group as compared to the control group. The reason includes as the myocardial cells containing CK and LDH get damaged due to insufficient supply of oxygen, and it may lead to increased permeability of membrane or rupture of the membrane. This further leads to the release of these enzymes. Furthermore, it also represents that the ischemia and reperfusion are responsible for myocardial injury [20].

In this study, it has been investigated that the administration of INM-176 shows a dose-dependent cardioprotective effect. The result suggested that INM-176 significantly attenuated the I/R-induced release of LDH, CK, and infarct size when compared with myocardial injury group. The pre-treatment with INM-176 at a dose of 150, 300, and 600 mg/kg significantly attenuated the release of LDH due to I/R when compared with the myocardial injury group. Similar effect was observed in case of release of CK at a dose of 300 and 600 mg/kg of INM-176. However, it was found that pretreatment with 150 mg/kg of INM-176 was not significantly reduces the release of CK due to I/R when compared with the myocardial injury group. Furthermore, global ischemia and reperfusion produce the myocardial injury assessed in terms of infarct size which was estimated by volume and weight method. It was observed that pre-treatment with INM-176 at dose of 300 and 600 mg/kg significantly attenuated the I/R-induced infarct size when compared with the myocardial injury group. However, the reduction in MI size at a dose of 150 mg/kg of INM-176 was not statistically significant when compared with the myocardial injury group. Therefore, in the present study, it has been observed that pre-treatment with INM-176 at a dose of 300 and 600 mg/kg resulted in decrease levels of LDH, CK as well as infarct size when compared with myocardial injury group and 150 mg/kg dose of INM-176. The results were more significant with INM-176 dose of 600 mg/kg, therefore producing greatest effect.

## CONCLUSION

On the basis of the findings of this study, it can be concluded that pre-treatment with INM-176 provides cardioprotection against I/R-induced myocardial injury. The effects included reduction in infarct size, reduction in LDH, and CK enzyme and improvement in heart function. Furthermore, it is observed that dose of 600 mg/kg is more effective to reduce the levels of cardiac parameters. The cardioprotective effect of INM-176 may be associated with its antioxidant activity. Hence, in the future, INM-176 can become the target compound for the development of the dosage form which can be used in the treatment of the I/R-induced injury.

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