

# Natural Product Research

Formerly Natural Product Letters


ISSN: (Print) (Online) Journal homepage: <https://www.tandfonline.com/loi/gnpl20>


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**To cite this article:** Kakarla Ramakrishna & Sairam Krishnamurthy (2022) Indole-3-carbinol ameliorated the isoproterenol-induced myocardial infarction via multimodal mechanisms in Wistar rats, *Natural Product Research*, 36:23, 6044-6049, DOI: [10.1080/14786419.2022.2041632](https://doi.org/10.1080/14786419.2022.2041632)


**To link to this article:** <https://doi.org/10.1080/14786419.2022.2041632>

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SHORT COMMUNICATION



## Indole-3-carbinol ameliorated the isoproterenol-induced myocardial infarction via multimodal mechanisms in Wistar rats

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### ABSTRACT

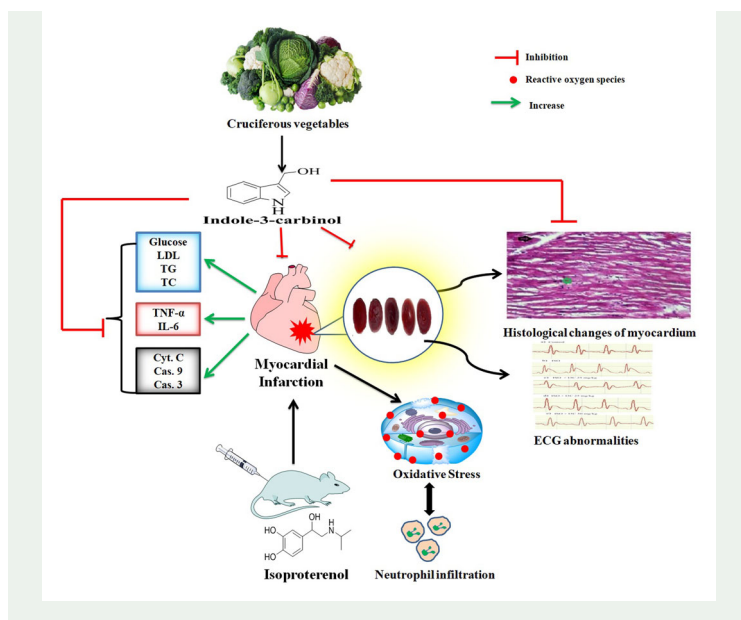
The present study investigated the cardioprotection of Indole-3-carbinol on isoproterenol (ISO)-induced myocardial infarction in Wistar rats. I3C treatment significantly reduced the prolongation of the QRS complex, QT interval, and ST-segment elevation. I3C was also able to normalise blood pressure (SBP, DBP, and MAP) and HR. I3C significantly decreased heart weight, cardiac troponin I (cTn I), CK-MB, LDH, AST and ALT. I3C ameliorated acute hyperglycaemia, hyperlipidemia, and myocardial infarction (%) in ISO rats. I3C treatment significantly elevated the antioxidant enzymes like SOD, CAT, and GSH and attenuated the MDA levels. I3C reduced the inflammatory cytokines (TNF- $\alpha$  and IL-6) and increased the anti-inflammatory cytokine 1 L-10. Furthermore, I3C significantly recovered myocardial structure by inhibiting neutrophil infiltration and oedema. Moreover, I3C attenuated apoptotic markers (cytochrome C, caspase 9 and caspase 3). Consequently, I3C restored cardiac function in MI rats by alleviating oxidative stress, inflammation, and apoptosis, and I3C could be used to treat myocardial infarction.

### ARTICLE HISTORY

Received 9 October 2021  
Accepted 9 February 2022

### KEYWORDS

Cruciferous vegetable; indole-3-carbinol; isoproterenol; myocardial infarction; inflammation; apoptosis



## 1. Introduction

Acute myocardial infarction (MI) is occurred due to an imbalance between coronary blood supply and blood demand to myocardial muscles (Aronow 2006, Shukla et al. 2010). Myocardial ischemia is often recognised by alterations in electrocardiography (ECG), blood pressure, and up-regulation of cardiac enzymes (Raish 2017), cardiac membrane alterations (Vijayakumar et al. 2012), elevation in reactive oxygen species (ROS) (Prasad et al. 2017, Zhang et al. 2019), inflammation (interleukin-6, IL-6), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), and decreased in anti-inflammatory cytokine (interleukin-10, IL-10) is observed (Ong et al. 2018). Oxidative stress and inflammation further aggravate apoptosis and necrosis, significantly affecting cardiac function (Saranya et al. 2019). Additionally, oxidative stress and inflammation cause vascular damage and recruit the platelets by binding to damaged subendothelial cells and stimulating platelet aggregation (Picker 2013). The activated platelets release several platelet aggregating agents, including adenosine diphosphate (ADP), which further aggravates the enhanced platelet aggregation and leads to secondary myocardial tissue damage, resulting in myocardial cell death (Gawaz 2004).

Natural compounds are promising approaches for treating cardiovascular diseases (Enayati et al. 2021). Indole-3-carbinol (I3C), an anticancer agent (Aggarwal and Ichikawa 2005) found in cruciferous vegetables reported as neuroprotective (El-Naga et al. 2014), anti-nephrotoxic (El-Naga and Mahran 2016), antioxidant, and anti-inflammatory (Choi et al. 2018), antiplatelet, and antithrombotic (Paliwal et al. 2018, Park et al. 2008) activities in rodents. I3C prevents cardiac remodelling and hypertrophy (Deng et al. 2014), ameliorates the cardiotoxicity induced by doxorubicin by mitigating oxidative stress and inflammation (Adwas et al. 2016), and salt-induced cardiac hypertrophy (Akkiraju et al. 2021). Therefore, we hypothesised that I3C intervention

simultaneously inhibits oxidative stress, inflammation, and platelet aggregation to restore cardiac function in MI. Hence, in the present study, we have investigated the cardioprotective role of I3C on isoproterenol (ISO) induced myocardial infarction.

## 2. Results and discussion

I3C showed cardioprotective activity in the rat model of ISO-induced myocardial infarction. Our research highlighted that I3C treatment preserved the structural, biochemical, and functional integrity of the myocardium. This protective activity of I3C is due to the amelioration of oxidative stress, inflammation, platelet aggregation, and apoptosis.

ISO is a dual adrenoreceptor agonist ( $\beta_1$  and  $\beta_2$ ) which elevates heart rate and decreases blood pressure, alters the ECG (Balea et al. 2018). ISO administration to Wistar rats has been reported to produce significant haematological, biochemical, and histopathological abnormalities similar to MI (Allawadhi et al. 2018, Lobo Filho et al. 2011). Similarly, these anomalies were found in the current experiment. I3C treatment significantly reduced the heart rate and normalised the blood pressure (SBP, DBP and MAP), indicating that I3C improved the hemodynamic functions of the heart (Table S1). I3C treatment significantly normalised the R-R interval (Figure S1E). Further, I3C ameliorated the prolongation of the QRS complex, indicating the inhibition of ventricular hypertrophy and the normalisation of dilation of the ventricles, which eventually causes regular ventricular depolarization (Figure S1B). I3C attenuated the prolongation of the QT interval, which is a sign of decreased ventricular arrhythmias (Figure S1C). I3C significantly ameliorated the ST-segment elevation, indicating the minimization of electric potential changes and inhibition of necrosis (Figure S1D). I3C treatment also ameliorated the ISO-induced elevation of CK-MB and cTnI, which are biomarkers for MI. Moreover, I3C also mitigated the LDH, AST, ALT (Table S2), and heart weight (Figure S2) in MI rats, indicating that I3C could conserve the myocardial cell membrane integrity, thereby limiting the leakage of these enzymes. I3C pre-treatment ameliorated the total cholesterol, LDL, triglycerides and augmented the HDL, suggesting that I3C inhibited lipid synthesis (Table 3). Our results indicated that I3C inhibited glucose levels, suggesting that I3C can mitigate acute hyperglycaemia after MI (Table S3).

ISO undergoes auto-oxidation and produces cardiac muscle cytotoxicity by elevating lipid peroxidation, further aggravating myocardial necrosis (Akila et al. 2017). I3C pre-treatment attenuated the myocardial MDA and nitric oxide (NO) levels in ISO treated rats suggesting that I3C hamper the lipid peroxidation (Table S4) and NO-mediated oxidative stress, respectively. Further, I3C augmented the SOD and CAT levels in ISO-treated rats, suggesting that I3C is capable of scavenging superoxide and hydrogen peroxide free radicals (Table S4). Moreover, I3C treatment significantly improved the GSH levels, implying that I3C decreased the utilisation of the protective-SH group of cardiac proteins (Table S4). Moreover, I3C attenuated the ISO-induced myocardial inflammation by reducing TNF- $\alpha$ , IL-6 and enhancing the anti-inflammatory cytokine, IL-10 (Figure S5A-C). Hyperlipidaemia is one of the major risk factors for myocardial infarction (Nelson 2013). I3C treatment significantly ameliorated the triglycerides and cholesterol in ISO-induced MI in rats. These results indicate that

I3C lipid-lowering capability may also be responsible for cardioprotection. It also reduced hyperglycaemia associated with myocardial infarction. Moreover, hyperglycaemia, oxidative stress, and inflammation trigger platelet aggregation (Fan 2019), further aggravating ischemic heart diseases. I3C pre-treatment significantly ameliorated the ADP and collagen-induced platelet aggregation, which indicates that I3C mediated cardioprotection could be due to mitigation of platelet aggregation. In accordance with other observations (Park et al. 2008, Paliwal et al. 2018), we have also identified the inhibition of ADP and collagen-induced platelet aggregation in ISO rats, suggesting that I3C protected the myocardium from ischemic injury (Figure S3A and B).

Further, TTC staining identified the pale patches (white colour), which indicate the necrosis of myocardial tissue. I3C treatment ameliorated the myocardial infarction, showing that I3C protected the heart from necrosis (Figure S4A and S4B). ROS and inflammation induce cytochrome C release from the mitochondrial membrane, further activating caspase 9 and caspase 3, resulting in cell death (Li et al. 2012). I3C ameliorated cytochrome C, caspase9, and caspase3, thereby protecting the cardiac tissue from apoptosis (Figure S6A–C). Furthermore, our histopathological analysis stressed that control rats exhibited the normal myocardium. In contrast, ISO-treated rats were found to have irregular myocardial architecture, neutrophil infiltration, oedema, vacuoles, and necrotic cells. However, I3C treatment shows the approximate architecture of normal myocardium, which is due to the prevention of necrosis (Figure S7). Our results indicate that I3C protected the cardiac tissue from ISO-induced cell death.

### 3. Conclusions

Indole-3-carbinol was explored for cardioprotective activity in ISO-induced myocardial infarction. I3C was able to recover cardiac function by altering the ECG, hemodynamics, and leakage of cardiac enzymes, lipids, and glucose levels. Furthermore, I3C treatment preserved the myocardial architecture by attenuating the platelet aggregation, oxidative stress, inflammation, and apoptosis. The present study demonstrated that I3C could be beneficial to treat myocardial infarction.

### Acknowledgements

Kakarla Ramakrishna is thankful to MHRD, India, for student fellowship and teaching assistantship through the Indian Institute of Technology (Banaras Hindu University), Varanasi. The authors also acknowledge Dr. A.N. Sahu for providing the semi-automated biochemical analyzer.

### Disclosure statement

The authors declare no conflict of interest.

### Funding

The author(s) reported there is no funding associated with the work featured in this article.

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