

# Ameliorating Effects of Ginger on Isoproterenol-Induced Acute Myocardial Infarction in Rats and its Impact on Cardiac Nitric Oxide

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## Abstract

**Background:** Myocardial infarction is a major heart disease and is considered a significant reason for mortality and morbidity around the world. The model of Isoproterenol (ISO)-induced myocardial infarction provides a supported method for investigating the impacts of numerous possible cardioprotective bioactive substances. Nitric Oxide (NO) could react with reactive oxygen intermediates and free radicals to create harmful species. For several years, researchers have investigated the use of herbs and natural products as antioxidants to protect the body's organs against toxins and drug metabolites. However, studies on the antioxidant effects of ginger against cardiotoxicity induced by drugs and toxic agents remain insufficient, especially its effects on NO. **Aims and Objectives:** This study aimed to investigate the possible antioxidant and protective role of ginger in ISO-induced acute myocardial infarction in experimental rats. Special emphasis was given to the impact of ginger on NO levels. **Materials and Methods:** Forty adult male albino rats were used in this study. The animals were randomly divided into four equal groups. Group I served as control and received a normal mouse diet. Group II received ginger extract orally, Group III received normal diet for eight weeks, followed by ISO administration subcutaneously to induce myocardial infarction, Group IV received ginger extracts, followed by ISO. **Results and Conclusions:** The results of this study illustrated ginger's protective role against ISO-induced acute myocardial infarction. This role is mainly due to ginger's antioxidant and anti-inflammatory properties. We assume that sufficient intake of ginger by individuals who are regularly exposed to ISO would be beneficial in overcoming the cardiotoxicity of ISO. The effects of ginger may take place through inhibition of NOS enzymes, which needs further immunohistochemical and biochemical studies to reveal the underlying different mechanisms of the effects of ginger at the molecular and structural levels.

**Keywords:** Antioxidant, ginger, isoproterenol, myocardial infarction, nitric oxide

## INTRODUCTION

Myocardial infarction (MI) is a major heart disease and is considered a significant reason for mortality and morbidity around the world.<sup>[1]</sup> It is a serious condition that results in myocardial necrosis, due to inequality of coronary blood flow and myocardial demand. Clinical markers of MI might include blood pressure, heart rate, electrocardiogram, left ventricular disorder, and raised levels of cardiac-specific enzymes and proteins. Troponins are often related to MI, as are inflammation-related cytokines and proteins at raised levels.<sup>[2]</sup> The model of isoproterenol (ISO)-induced MI provides a

supported method for investigating the impacts of numerous possible cardioprotective bioactive substances.<sup>[3]</sup>

ISO [1-(3,4-dihydroxyphenyl)-2-(isopropylamino) ethanol hydrochloride] is a catecholamine b-adrenergic agonist used over maximal dosage to manage heart functions. However, the administration of ISO in overmaximal dosage might cause extreme stress in the heart via exhaustion of the cardiomyocytes' energy reserve, which leads to permanent cellular injury and to infarcts such as myocardial necrosis.<sup>[3]</sup> Numerous synthetic

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drugs are used for the treatment of myocardial attacks; however, they are not devoid of adverse effects, so a number of research studies have concentrated on determining new therapeutic strategies to avoid MI.<sup>[4]</sup>

Nitric oxide (NO) is a colorless, uncharged, relatively hydrophobic gas, which reacts in the gas phase with oxygen to form nitrogen dioxide. In aqueous media, it dissolves to a limited extent. NO is highly unstable with an apparent half-life of 6–60 s in a bioassay system that uses physiological salt solutions (NO binds to oxyhemoglobin and other heme-containing proteins, and its biological action is rapidly terminated by binding to oxyhemoglobin).<sup>[5]</sup> NO is synthesized from the substrate L-arginine by the NO synthase enzyme (NOS).<sup>[6]</sup>

Many isoforms of NOS have been recognized. The most important are the inducible NOS enzyme (iNOS) and the constitutive NOSs (cNOS), which include endothelial NOS (eNOS) and neuronal NOS (nNOS).<sup>[6]</sup> The activators of NOS consist of compounds released from nerves, such as bradykinin, acetylcholine, excitatory amino acids, and those released from platelets such as thrombin. Pharmacological activators are formylated peptides, calcium ionophores, and esters of phorbol.<sup>[7]</sup> iNOS continually releases large quantities of NO. The quantity of NO released per unit of time by fully induced macrophage enzymes is thousand times greater than that released by cNOS endothelial cells.<sup>[8]</sup>

The majority of NOS inhibitors are analogous of L-arginine or have a guanidino group. (N [gamma]-nitro-L-arginine) (L-NAME) as well as (N-monomethyl-L-arginine) (LNMA) are examples of L-arginine analogs.<sup>[9]</sup>

NO could react with reactive oxygen intermediates and free radicals to create even more toxic and harmful species.<sup>[10]</sup> NO's reaction with superoxide anion can generate peroxynitrite anions, which could break down to produce strong oxidants with reactivity comparable to hydroxyl radicals.<sup>[11]</sup> NO might be a mediator of endotoxin or medication-induced hepatotoxicity.<sup>[12]</sup> It might also influence drug-induced hepatotoxicity by modifying the activity of the cytochrome P450 enzyme, which plays a crucial role in drug metabolic processes as well as their toxicity. NO works as a cytotoxic macrophage effector molecule and as a modulator of neutrophil chemotaxis and of adhesion.<sup>[13]</sup>

The impacts of NO on the heart are a matter of controversy. It has beneficial impacts that depend on its modulation of cardiomyocyte contractility, the coronary arteries' tone, platelet aggregation, monocyte adhesion, smooth muscle cell proliferation, and fibroblasts. On the other hand, the activation of NOS enzymes, particularly iNOS, results in an extreme release of NO, which damages heart function through numerous mechanisms including endothelial disorder, the overgeneration of free radicals and reactive oxygen species, and the release of inflammatory cytokines and mediators.<sup>[14]</sup>

Ginger is a widely known herbaceous species used majorly worldwide. Antioxidants in ginger include shogaols

and gingerols, as well as some associated phenolic ketone by-products. Ginger's dried-out extract includes sesquiterpenes and monoterpenes. Ginger extract has antioxidant properties as it could scavenge superoxide anion as well as hydroxyl radicals.<sup>[15-17]</sup> Gingerols, at high concentration, prevent the formation of ascorbate ferrous complex that subsequently generates lipid peroxidation products.<sup>[18,19]</sup> Ginger has additionally been found to disrupt inflammatory procedures.<sup>[20]</sup> Ginger also works as a cholesterol-lowering factor in experimental animals fed with nutrients with high levels of cholesterol.<sup>[21,22]</sup> Feeding rats with ginger considerably raised the activity of enzymes related to cholesterol catabolism, especially hepatic cholesterol 7 $\alpha$ -hydroxylase, an important enzyme in bile acid biosynthesis that accelerates the conversion of cholesterol to bile acids, leading to greater excretion of cholesterol from the liver.<sup>[23,24]</sup>

For several years, researchers have investigated the use of herbs and natural products as antioxidants to protect the body's organs and tissues against toxins and drug metabolites.<sup>[25-31]</sup> However, studies on the antioxidant effects of ginger against cardiotoxicity induced by drugs and toxic agents remain insufficient, especially regarding its effects on NO and NOS enzymes. Thus, this study aimed to investigate and explore the possible antioxidant and protective role of ginger in ISO-induced acute MI in experimental rats. Special emphasis was given to the impact of ginger on NO levels.

## MATERIALS AND METHODS

### Chemicals

Kits for interleukin-6 (IL-6) (Cas No. 88-7064) and tumor necrosis factor alpha (TNF- $\alpha$ ) (Cas No. 88-7324) were purchased from eBioscience (San Diego, CA, USA). The highest analytical grade of ginger commercially available was purchased. Other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The procedures follows the guidelines laid down in Declaration of Helsinki (year 2013) and the ethical guidelines followed in college of pharmacy, King Abdulaziz University.

### Animals

This study conformed to the Bioethics in Research Regulations implemented by the Kingdom of Saudi Arabia. Forty adult male albino rats weighing 120–150 g were used in this study. The animals were obtained from the animal house of the Faculty of Pharmacy at King Abdulaziz University. They were housed under standard conditions (23°C  $\pm$  2°C and 12 h light/12 h dark cycles) and were given free access to food and drinking water.

### Experimental design

The animals were randomly divided into four equal groups: Group I served as control and received a normal mouse diet; Group II received ginger extract orally (200 mg/kg b.wt) through a gastric feeding tube for 8 weeks; Group III received normal diet for 8 weeks and on the 57<sup>th</sup> day, ISO (85 mg/kg b.wt)

was administered subcutaneously on 2 consecutive days with an interval of 24 h;<sup>[32]</sup> and Group IV received ginger extract (200 mg/kg b.wt) through a gastric feeding tube for 8 weeks, followed by ISO (85 mg/kg b.wt) subcutaneously on 2 consecutive days with an interval of 24 h.

At the end of the treatment period, the rats were fasted overnight and anesthetized with diethyl ether; blood samples were withdrawn from the heart and collected in heparinized tubes. The animals were subsequently sacrificed through cervical dislocation and then dissected to harvest their hearts. The blood samples were centrifuged at 3000 rpm for 10 min, and the sera were stored at  $-20^{\circ}\text{C}$  for determination of serum alanine transaminase. The heart samples were immediately washed in cold saline and then stored at  $-20^{\circ}\text{C}$  for determination of lipid peroxidation and NO products.

### Biochemical measurements

1. Determination of creatine kinase-myocardial bound (CK-MB) and lactate dehydrogenase (LDH): This was done by enzymatic colorimetric method<sup>[33]</sup>
2. Determination of IL-6 and TNF- $\alpha$ : This was done by enzyme-linked immunosorbent assay kits for rats according to the manufacturer's instructions. The kits for IL-6 (Cas No. 88-7064) and TNF- $\alpha$  (Cas No. 88-7324) were purchased from eBioscience
3. Determination of lipid peroxide levels in the heart tissue: The hearts were homogenized in ice-cold 0.15 M KCl (10% w/v). Malondialdehyde (MDA) levels were measured by a thiobarbituric acid test.<sup>[34]</sup> The breakdown product of 1,1,3,3-tetraethoxypropane was used as a standard
4. Determination of NO and nitrite and nitrate (NO $_x$ ) in the heart tissue: The presence of NO $_x$  was used as an indicator of NO synthesis. NO $_x$  was assayed just after the reduction of nitrate to nitrite by copperized cadmium granules in a glycine buffer with pH 9.7. Determination of nitrite was based on the Griess reaction, in which a chromophore with a strong absorbance at 540 nm is developed by a reaction of nitrite with a mixture of naphthyl ethylenediamine and sulfanilamide<sup>[35]</sup>
5. Histopathological study: The hearts were placed immediately in ice-cold physiological saline to wash them from blood. The left ventricle was sectioned into transverse slices and stained using 1.5% triphenyl tetrazolium chloride in a phosphate buffer for 10–15 min. Areas of necrosis did not stain, so the infarct size was calculated as the percentage of the infarct area to the surface area of all slices.

### Statistical analysis

Data were statistically analyzed using Minitab 19 for Mac State College, PA: Minitab, Inc. (www.minitab.com). All data were expressed as mean  $\pm$  standard deviation. The difference between the two means was analyzed using Student's *t*-test. For comparison of more than two means, the *F* value in analysis of variance (ANOVA) was calculated. When the *F* value was

found to be significant, Tukey's test was performed to compare two means. Statistical significance was indicated by  $P < 0.05$ .

## RESULTS

### Serum creatine kinase-myocardial bound level

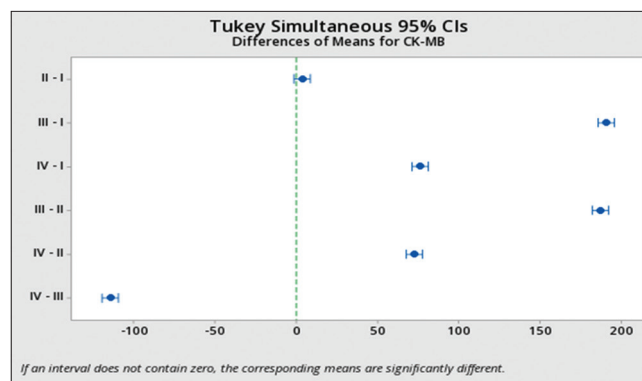
The mean serum CK-MB level (U/l) of the ginger-treated group ( $120.4 \pm 2.81$ ) did not significantly differ from that of the control group ( $117.1 \pm 2.81$ ). By contrast, the mean serum ginger level of the induced MI group treated by ISO group was significantly higher ( $52.300 \pm 2.312$ ) than that of the control group. However, ginger administration before ISO treatment resulted in reduced mean serum CK-MB level ( $41.4 \pm 2.119$ ) compared with that of rats with induced MI alone, although the value remained significantly higher than that of the control group [Table 1].

The mean serum CK-MB levels statistically significantly differed among the study groups ( $F = 4603.11$  at  $P = 0.001$ ). Moreover, within-group comparison showed significant differences in mean serum CK-MB levels between each pair of study groups, except in the pairing between control group and ginger-treated group [Table 1 and Figure 1].

### Serum lactate dehydrogenase level

There was no significant difference between the mean serum LDH level (U/l) of the ginger-treated group ( $69.9.6 \pm 1.91$ ) and that of the control group ( $72.6 \pm 1.95$ ). On the other hand, the mean serum LDH level of the ISO-induced MI group treated by ISO group was significantly higher ( $52.300 \pm 2.312$ ) than that of the control group. However, ginger administration before ISO treatment resulted in reduced mean serum LDH level ( $103.5 \pm 3.72$ ) compared with that of rats with induced MI alone, although the value remained significantly higher than that of the control group [Table 2].

The mean serum LDH levels significantly differed among the study groups ( $F = 2164.76$  at  $P = 0.001$ ). Moreover, within-group comparison showed significant differences in mean serum LDH levels between each pair of study groups, except in the pairing between control group and ginger-treated group [Table 2 and Figure 2].



**Figure 1:** Tukey's test simultaneously with 95% confidence interval differences for creatine kinase-myocardial bound

**Table 1: Comparison of mean values of serum CK-MB (U/l) between the studied groups, t-test, and analysis of variance**

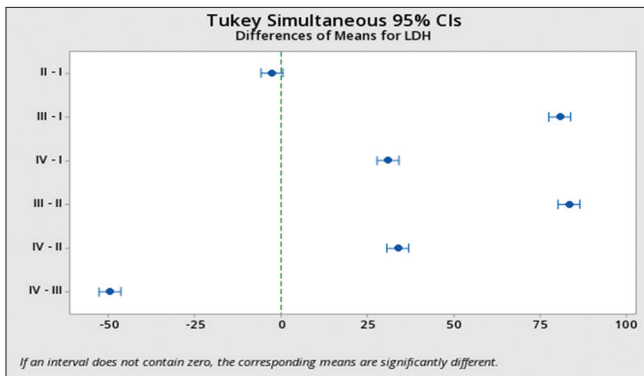
Group	Serum CK-MB				
	Mean ± SD	t-test		ANOVA	
		t	P	F	P
Group I (control group)	117.1±2.81				
Group II (ginger group)	120.4±2.67	2.69	0.015	4603.11	0.001**
Group III (isoproterenol-induced MI)	307.5±6.08	89.89	0.001*		
Group IV (ginger- and isoproterenol-induced MI)	193±4.14	47.97	0.001*		

\*Significant compared to the control group, \*\*Significant. MI: Myocardial infarction, ANOVA: Analysis of variance, SD: Standard deviation, CK-MB: Creatine kinase-myocardial bound

**Table 2: Comparison of mean values of serum LDH (U/l) between the studied groups, t-test, and analysis of variance**

Group	Serum LDH				
	Mean ± SD	t-test		ANOVA	
		t	P	F	P
Group I (control group)	72.6±1.95				
Group II (ginger group)	69.9±1.91	3.13	0.006	2164.76	0.001**
Group III (isoproterenol-induced MI)	153.2±2.53	79.79	0.001*		
Group IV (ginger- and isoproterenol-induced MI)	103.5±3.72	23.36	0.001*		

\*Significant compared to the control group, \*\*Significant. MI: Myocardial infarction, ANOVA: Analysis of variance, SD: Standard deviation, LDH: Lactate dehydrogenase



**Figure 2:** Tukey's test simultaneous with 95% confidence interval differences for lactate dehydrogenase

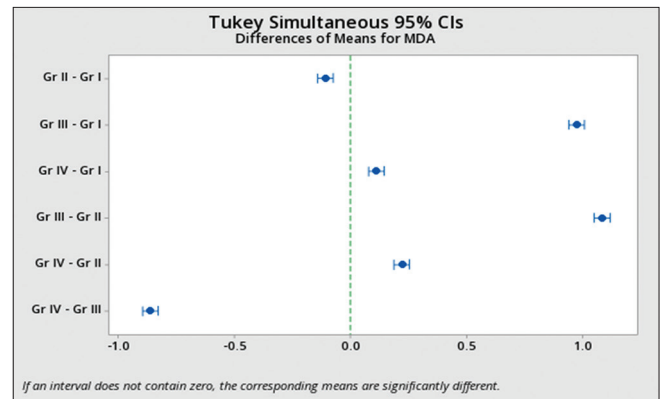
### Heart tissue malondialdehyde level

The mean heart tissue MDA levels (nmol/mg protein) in ginger-treated animals ( $0.84 \pm 0.023$ ) were significantly lower than those in the control animals ( $0.94 \pm 0.023$ ). By contrast, the ISO-induced MI group showed significantly increased MDA levels ( $1.92 \pm 0.038$ ) compared with the controls. However, ginger administration before ISO resulted in significant change in MDA levels ( $1.05 \pm 0.025$ ) of the treated rats compared with nontreated groups [Table 3].

One-way ANOVA revealed that the mean MDA level of the study groups significantly differed ( $F = 3168.64$  at  $P = 0.001$ ). In addition, within-group comparison revealed significant differences between each pair of study groups [Table 3 and Figure 3].

### Tumor necrosis factor-alpha

Table 4 shows that the mean serum TNF- $\alpha$  levels (pg/ml) were nonsignificantly lower in the ginger-treated rats ( $21.99 \pm 1.27$ )



**Figure 3:** Tukey's test simultaneously with 95% confidence interval differences for malondialdehyde

than in the controls ( $25.32 \pm 1.7$ ). On the other hand, TNF- $\alpha$  level significantly increased (mean value:  $89.73 \pm 2.82$ ) in the ISO-induced MI group compared with that in the control group. Ginger administration prior to ISO treatment caused significant decrease in the mean TNF- $\alpha$  levels ( $56.49 \pm 2.4$ ) in the treated rats compared to the nontreated groups [Table 4].

The mean serum TNF $\alpha$  levels in the study groups statistically significantly differed ( $F = 2198.23$  at  $P = 0.001$ ). Moreover, within-group comparison revealed significant differences between each pair of study groups [Table 4 and Figure 4].

### Interleukin-6

Administration of ginger resulted in nonsignificant decrease in mean serum IL-6 ( $36.45 \pm 1.14$ ) in the treated rats relative to that in the controls ( $38.78 \pm 1.14$ ). On the other hand, IL-6 level significantly increased (mean value:  $118.63 \pm 1.61$ ) in



**Table 3: Comparison of mean malondialdehyde values (nmol/mg protein) in heart homogenates between study groups using *t*-test and analysis of variance**

Group	MDA				
	Mean±SD	<i>t</i> -test		ANOVA	
		<i>t</i>	<i>P</i>	<i>F</i>	<i>P</i>
Group I (control group)	0.94±0.023				
Group II (ginger group)	0.84±0.023	8.77	0.001*	3168.64	0.001**
Group III (isoproterenol-induced MI)	1.92±0.038	78.32	0.001*		
Group IV (ginger- and isoproterenol-induced MI)	1.05±0.025	8.85	0.001*		

\*Significant compared to control group, \*\*Significant. MDA: Malondialdehyde, MI: Myocardial infarction, ANOVA: Analysis of variance, SD: Standard deviation

**Table 4: Comparison of the mean serum TNF-α levels (pg/ml) between study groups using *t*-test and analysis of variance**

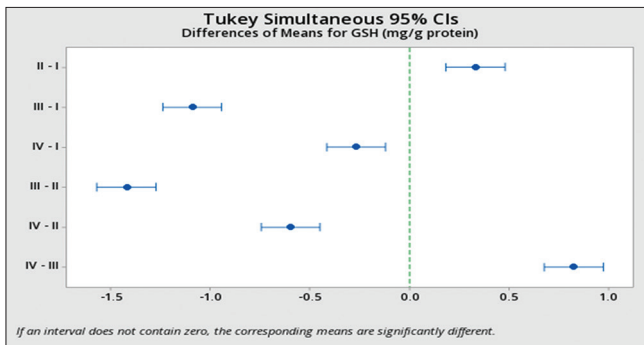
Group	TNF-α				
	Mean±SD	<i>t</i> -test		ANOVA	
		<i>t</i>	<i>P</i>	<i>F</i>	<i>P</i>
Group I (control group)	25.32±1.70				
Group II (ginger group)	21.99±1.27	3.49	0.007	2198.23	0.001**
Group III (isoproterenol-induced MI)	89.73±2.82	67.48	0.001*		
Group IV (ginger- and isoproterenol-induced MI)	56.49±2.40	32.66	0.001*		

\*Significant compared to control group, \*\*Significant. MI: Myocardial infarction, ANOVA: Analysis of variance, SD: Standard deviation, TNF-α: Tumor necrosis factor-alpha

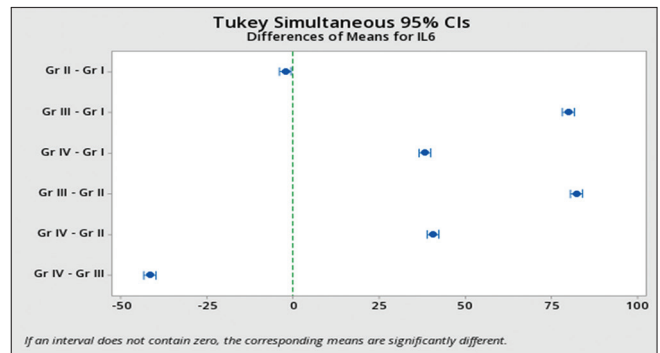
**Table 5: Comparison of mean serum IL-6 (pg/ml) between study groups using *t*-test and analysis of variance**

Group	IL-6				
	Mean±SD	<i>t</i> -test		ANOVA	
		<i>t</i>	<i>P</i>	<i>F</i>	<i>P</i>
Group I (control group)	38.78±1.14				
Group II (ginger group)	36.45±1.14	3.65	0.002	7362.62	0.001**
Group III (isoproterenol-induced MI)	118.63±1.61	125.19	0.001*		
Group IV (ginger- and isoproterenol-induced MI)	76.99±1.71	59.91	0.001*		

\*Significant compared to control group, \*\*Significant. MI: Myocardial infarction, ANOVA: Analysis of variance, SD: Standard deviation, IL-6: Interleukin-6



**Figure 4:** Tukey's test simultaneously with 95% confidence interval differences for tumor necrosis factor-alpha



**Figure 5:** Tukey's test simultaneously with 95% confidence interval differences for serum interleukin-6

the ISO-induced MI group compared with that in the control group. Ginger administration prior to ISO treatment caused significant decrease in the mean IL-6 levels ( $76.99 \pm 1.71$ ) in the treated rats compared to the nontreated groups [Table 5].

The mean IL-6 levels of all the study groups statistically significantly differed ( $F = 7362.62$  at  $P = 0.001$ ). Moreover, within-group comparison revealed significant differences between each pair of study groups, except for the

comparison between control group and ginger-treated group [Table 5 and Figure 5].

**Nitrite and nitrate**

The mean heart tissue NOx of the ginger-treated animals did not significantly differ ( $20.95 \pm 0.066$ ) from that of the control animals ( $21.88 \pm 1.11$ ). By contrast, the mean heart tissue of NOx level significantly increased (mean value:  $37.83 \pm 1.39$ ) in the ISO-induced MI group compared with that in the control group. Ginger administration prior to ISO treatment caused significant decrease in the mean heart tissue of NOx level ( $27.25 \pm 1.16$ ) in the treated rats compared to the nontreated groups [Table 6].

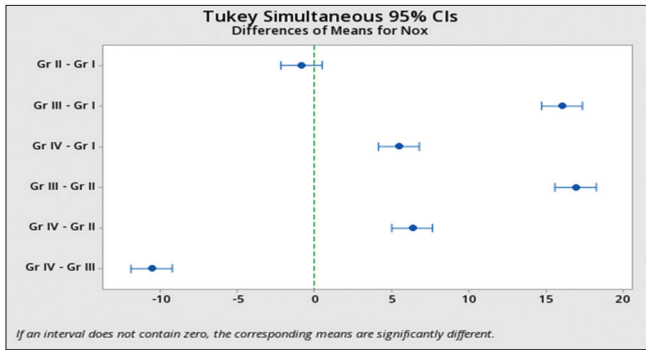
The mean heart tissue NOx of all the studied groups statistically significantly differed ( $F = 489.87$  at  $P = 0.001$ ). Moreover, within-group comparison revealed significant

differences between each pair of study groups, Except for the comparison between ginger and control groups [Table 6 and Figure 6].

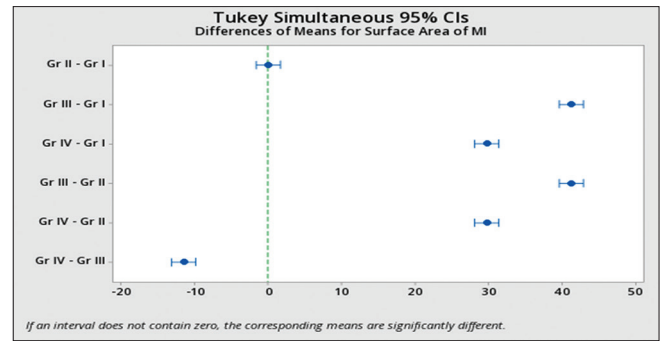
**Infarct surface area %**

Percentage surface of infarct size [Table 7 and Figure 7] shows the mean values of percentage surface area of infarct size among the studied groups. It shows significant increase in percentage surface area of ISO-treated group (mean value:  $41.23 \pm 1.9$  and  $P = 0.001$ ) when compared to ginger-treated group (mean value:  $29.72 \pm 1.95$ ).

The mean values of percentage surface area of infarct size among the studied groups statistically significantly differed ( $F = 2388.16$  at  $P = 0.001$ ). Moreover, within-group comparison revealed significant differences between each pair of study groups, except for non-ISO treated groups [Table 7 and Figure 7].



**Figure 6:** Tukey's test simultaneously with 95% confidence interval differences for heart tissue nitrite and nitrate



**Figure 7:** Tukey's test simultaneously with 95% confidence interval differences for infarct surface area %

**Table 6: Comparison of mean heart tissue (mmol/g tissue) between study groups using t-test and ANOVA**

Group	Mean±SD	Heart tissue NOx			
		t-test		ANOVA	
		t	P	F	P
Group I (control group)	21.81±1.11				
Group II (ginger group)	20.95±0.66	1.73	0.216	489.87	0.001**
Group III (isoproterenol-induced MI)	37.83±1.39	32.29	0.001*		
Group IV (ginger- and isoproterenol-induced MI)	27.25±1.16	10.98	0.001*		

\*Significant compared to control group, \*\* Significant. NOx: Nitrite and nitrate, MI: Myocardial infarction, ANOVA: Analysis of variance, SD: Standard deviation

**Table 7: Comparison of the mean infarct surface area percentage between study groups using t-test and analysis of variance**

Group	Mean±SD	Infarct surface area percentage			
		t-test		ANOVA	
		t	P	F	P
Group I (control group)	0.00±0.00				
Group II (ginger group)	0.00±0.00	0.00	1.00	2388.16	0.001**
Group III (isoproterenol-induced MI)	41.23±1.90	67.80	0.001*		
Group IV (ginger- and isoproterenol-induced MI)	29.72±1.95	48.88	0.001*		

\*Significant compared to control group, \*\*Significant. MI: Myocardial infarction, ANOVA: Analysis of variance, SD: Standard deviation

## DISCUSSION

The results of the current research study have shown that administration of ginger ameliorates ISO-induced MI in rats. The protective impact of ginger was evidenced by a decrease in the serum level of MI markers LDH and CK-MB, in addition to decreased surface area of infarct size by histopathological examination in treated animals compared to the nontreated group. Leaking of LDH and CK-MB into the circulation has been demonstrated by injury to cardiomyocytes.<sup>[36]</sup>

Serum cardiac cytosolic enzyme activities such as LDH and CK-MB are extremely sensitive, as are specific diagnostic markers for acute MI.<sup>[37,38]</sup> The results of the current study confirmed previous research studies demonstrating that ISO caused marked increases in the serum levels of LDH, CK-MB, and AST activities that reflect the extreme damage to myocardium cells. Ginger pre- and co-treatment decreased the activity of the serum marker enzymes of ISO-treated rats. This confirmed that ginger might preserve membrane integrity, thus limiting the leak of these enzymes.

ISO-treated rats demonstrated a significant increase in the levels of the measured inflammatory cytokine TNF- $\alpha$ , and it was found that these cytokines are elevated in cardiac toxicity conditions.<sup>[39]</sup> Ginger-treated rats showed significant decrease in the levels of TNF- $\alpha$  and IL-6, which could have contributed to its anti-inflammatory property.

The results of this study illustrate a link between NO and oxidative stress. This is evidenced by the raised cardiac NO that was accompanied by evidence of oxidative stress manifested by raised MDA. Such a raise in oxidative stress might influence NO by various mechanisms, including lowered synthesis through the uncoupling of NOS enzymes and the inactivation of NO into peroxynitrite. Exactly, in the same context, the rats treated with ISO demonstrated a significant increase in oxidative stress and inflammatory-mediated parameters. It was reported that the uncoupled eNOS might generate superoxide instead of NO.<sup>[40]</sup>

The impact of various NOS enzymes on cardiovascular disease pathogenesis is not completely recognized. The enzyme nNOS is expressed in coronary smooth muscles, cardiac myocytes, the autonomic nervous system, and cardiac ganglia, which are crucial resources of cardiac NO. Under conditions of cardiac insult such as MI, the enzyme nNOS releases NO, which protects against diastolic disorder; enhances the storage of  $\beta$ -adrenergic receptors; protects against heart hypertrophy; reduces infarct surface area; and safeguards the myocardium from dysrhythmia. At the cellular level, NO produced from nNOS lowers the generation of free radicals from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and assists in the relaxation of cardiac myocytes by means of the cyclic guanosine monophosphate/protein kinase G pathway.<sup>[41]</sup>

In conditions of acute MI, eNOS generates superoxide anion rather than NO; this is called the “uncoupling of eNOS” and

may contribute to the extreme generation of free radicals and the deficiency of cofactors crucial for the normal biosynthesis of NO.<sup>[42]</sup>

The outcomes of the present research study demonstrated high levels of NO in heart tissue with ISO-induced MI. In agreement with the current results, it has been reported that in MI, there is an overinduction of iNOS in various cardiac cells, resulting in overproduction of NO that mediates oxidative stress, inflammatory activity, and the development of myocardial injury.<sup>[43]</sup> This is shown by the significant increase of MDA as an indicator of oxidative stress and of IL-6 and TNF- $\alpha$  as inflammatory mediators.

In the current study, pre- and co-administration of ginger with ISO caused a significant decrease of NO in the cardiac tissue in comparison to the ISO group. These results assume the role of NOS enzymes in producing cardiac NO, as reported previously.<sup>[42]</sup> In addition, the present study signifies the impact of ginger in lowering NO through its effects on NOS enzymes. However, it is difficult to confirm from these results the specific effects of ginger on each isoform of NOS, the cNOS, eNOS, nNOS, and iNOS enzymes. This will require further investigation, including immunohistochemically staining cardiac tissues and using specific inhibitors of NOS isoforms, such as L-arginine or a guanidino group, L-NAME, and LNMMA, in combination with ginger to specify its modulating effects on different isoforms, if any.

## CONCLUSION

This study illustrated ginger's protective role against ISO-induced acute MI. This role is mainly due to ginger's antioxidant and anti-inflammatory properties. We assume that sufficient intake of ginger by individuals who are regularly exposed to ISO would be beneficial in overcoming the cardiotoxicity of ISO. The effects of ginger may take place through the inhibition of NOS enzymes, which needs further immunohistochemical and biochemical studies to reveal the underlying mechanisms of the effects of ginger on NO and NOS enzymes at the molecular and structural levels.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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