

## Dose Response Effect of Nitroglycerin on Cardiac Hemodynamic Functions and Myocardial Infarction in a Rat Model of Ischemia Reperfusion

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### ABSTRACT

**Background:** Millions of people suffer from acute coronary syndrome, angina, acute myocardial infarction (MI), or related illnesses. These patients are prescribed nitroglycerin (NG) for the management of these diseases. Accordingly, any risk associated with the use of nitroglycerin can potentially affect those people despite of the undeniable benefit in relieving acute angina. **Aim of the present work:** was to study the potential of treatment with 3 different doses of NG (25, 50, 100 mg/kg) to develop nitrate tolerance.

**Materials and Methods:** 50 Male Albino rats were randomly divided into 2 main groups: sham group and ischemia/reperfusion (I/R) groups, which was further subdivided into 4 groups, Untreated group; NG 25, 50 and 100 mg groups; rats received nitroglycerin three times daily for 3 days before induction of ischemia reperfusion. the effect of NG on cardiac functions and infarction size and severity score were assessed.

**Results:** Rats exposed to I/R exhibited a significant decrease in their cardiac hemodynamic functions ( $\uparrow$  left ventricular end diastolic pressure; LVEDP and  $\downarrow$ dp/dt<sub>max</sub>) and development of a measurable cardiac infarction area. Regarding pretreatment with NG; NG 25 mg/kg improved cardiac functions and myocardial infarction size & severity score. NG 50 mg/kg significantly decreased myocardial contractility and increased myocardial infarction size. Meanwhile NG 100 mg/kg produced significant decrease in cardiac functions and significant increase in myocardial infarction size & severity score.

**Conclusion:** NG pretreatment dose dependently decreased cardiac hemodynamic functions and increased myocardial infarction size & severity score in a rat model of ischemia reperfusion.

**Key words:** Nitroglycerin, cardiovascular system, LVEDP, LV dp/dt<sub>max</sub>, ischemia/reperfusion.

### INTRODUCTION

Cardiovascular diseases (CVDs) is the leading cause of morbidity and mortality, accounting for 17.3 million deaths globally each year and it is expected to grow to 23.6 million by the year 2030<sup>1</sup>. 80 percent of these deaths occur in lower and middle-income countries<sup>1</sup>.

CVDs comprise many conditions, including coronary heart disease (CHD), heart failure, rheumatic fever/rheumatic heart disease, stroke, and congenital heart disease. Ischemic heart disease, consisting principally of CHD, is the predominant manifestation of CVDs<sup>2</sup>.

Organic nitrates and particularly nitroglycerin (NG), has long been one of the key therapies for CVDs including coronary artery disease, acute myocardial infarction and congestive heart failure<sup>3</sup>. The ability of mammalian cells to convert NG to vaso-relaxant nitric oxide (NO) played a significant part in the discovery of the unique role of both NO and NG in acute angina and congestive heart disease<sup>4</sup>.

It is worthy to mention that most of the studies that used organic nitrates for the treatment of heart failure and coronary artery disease (CAD) demonstrated improvement in symptoms, but

mostly failed to demonstrate an improvement in prognosis<sup>5</sup>. An old retrospective meta-analysis study performed in post-infarct patients revealed that the nitrate use was associated with an unfavorable prognosis<sup>6</sup>. Moreover, it was demonstrated that sustained treatment with NG resulted in an increase in infarct size and cardiac dysfunction after myocardial infarction in rats<sup>7</sup>. Acute high-dose of organic nitrates also is likely to cause a similar loss of potency in the form of tachyphylaxis<sup>5</sup>.

The aim of the present work was to study the effect of treatment with 3 different doses of NG (25, 50, 100 mg/kg, three times daily for three successive days on cardiac hemodynamics and myocardial infarction size in a rat model of ischemia/reperfusion.

### MATERIALS AND METHODS

**Experimental animals:** All animal procedures were approved by the Institutional Animal Ethics Committee for Ain Shams University, Faculty of Medicine. Male albino rats (weighing 250 to 300 g) were purchased from National Research Institute (Cairo, Egypt) and housed in an animal room with temperature (22-24°C) and lighting (12

h light–dark cycles). Rats received standard rat chow and tap water. An adaptation period of one week was allowed. **The study was approved by the Ethics Board of Ain Shams University.**

**Drugs & chemicals:** Nitroglycerin (NG, Nitro MAK Retard 5mg Capsules, October Pharma, Egypt). Rat chow (was purchased from Meladco for Animal Food, Egypt) in the form of pellets.

#### **Experimental procedures:**

##### **1. Induction of myocardial ischemia reperfusion (I/R):**

Left anterior descending coronary artery (LAD) ligation was made as follows: Albino rats were anesthetized with urethane ip (1.2g/kg) dissolved in distilled water. An incision in the neck was made to expose the trachea, then a small incision was made in the trachea to connect it with the ventilator at 70-80 breaths/min. Body temperature was maintained at 37°C using blanket<sup>8</sup>.

Ligature was placed around the LAD coronary artery, using a 5-0 polyethylene suture. Coronary occlusion was achieved by tightening a suture for 35 min. Occlusion was confirmed by blanching of the infarcted area and ECG recording. Reperfusion was achieved by release of the ligature for 60 min<sup>8</sup>.

##### **2. Study design:** 50 male albino rats were divided into 2 groups:

- a) **Sham group (10 rats):** receive standard rat chow and tap water for 3 days, then chest wall was open without induction of I/R.
- b) **I/R groups (10 rats in each group):** These groups underwent induction of I/R and are divided into:
  - i. **Untreated I/R group:** rats received standard rat chow and tap water for 3 days, then induction of I/R.
  - ii. **NG 25mg group:** rats received nitroglycerin 25mg/kg three times daily for 3 days, then induction of I/R.
  - iii. **NG 50mg group:** rats received nitroglycerin 50mg/kg<sup>9</sup> three times daily for 3 days, then induction of I/R.
  - iv. **NG 100mg group:** rats received nitroglycerin 100mg/kg<sup>10</sup> three times daily for 3 days, then induction of I/R.

**Parameters measured:** At the end of the experiment the following parameters were measured

##### **1. Hemodynamic parameters** (using power lab AD instrument):

- a. Invasive left ventricular end diastolic pressure (LVEDP).
- b. LV dP/dt<sub>max</sub>.

##### **2. Histopathological examination:**

- a. The heart was taken and fixed in 10% formaldehyde for a few days and dehydrated with graded concentrations of alcohol for embedding in paraffin. Blocks were done from which 5µm thick sections were cut and stained for light microscopic examination by Hematoxylin-Eosin stain (HE). The severity and extent of MI were observed for each case grossly and microscopically<sup>11</sup>.
- b. The severity and extent of MI were observed for each case grossly and microscopically. The findings were classified into the following degrees, to compose a range of histologic myocardial injury (0): No change (1): Mild - focal myocyte damage or small multifocal degeneration with slight degree of inflammation, (2): Moderate - extensive myofibrillar degeneration and/or diffuse inflammatory process, (3): Severe - necrosis with diffuse inflammatory process<sup>12</sup>.

**Statistical analysis:** was carried out using Graphpad prism, software program, version 5.0. (2007). Inc., CA, USA. All values in the results were expressed as means ± SD. Statistical difference among groups were determined using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. P values < 0.05 were considered statistically significant.

## **RESULTS**

### **Effect of nitroglycerin (NG) at different doses (25 mg, 50 mg, 100 mg/kg) on cardiac hemodynamics [LVEDP and dP/dt<sub>max</sub>] in an ischemia reperfusion (I/R) rat model:**

**Table (1)** showed that I/R untreated rats exhibited significant increase of the LVEDP and significant decrease of dP/dt<sub>max</sub>. NG when administered in a dose of 25mg/kg produced significant decrease of the LVEDP and significant increase of the dP/dt<sub>max</sub>. NG effect on cardiac hemodynamics functions was in a dose dependant manner as NG 50mg group showed insignificant increase of LVEDP and significant decrease of dP/dt<sub>max</sub>. Meanwhile NG 100mg produced significant increase of the LVEDP and significant decrease of the dP/dt<sub>max</sub>.

**Table (1): Effect of nitroglycerin (NG) at different doses (25mg, 50mg, 100 mg/kg) on cardiac hemodynamics [LVEDP and dP/dt<sub>max</sub>] in an ischemia reperfusion (I/R) rat model:**

Animal Groups	LVEDP(mmHg) Mean± SD	dP/dt <sub>max</sub> (mmHg s <sup>-1</sup> ) Mean± SD
Sham	6.12 ± 0.43	14418 ± 1905
Untreated I/R	13.41 ± 0.23 <sup>#</sup>	5026 ± 958.2 <sup>#</sup>
NG 25mg/kg	3.58 ± 1.54 <sup>*</sup>	10191 ± 1119 <sup>*</sup>
NG 50mg/kg	18.15 ± 3.62	2588 ± 721.4 <sup>*</sup>
NG 100mg/kg	39.51 ± 2.70 <sup>*</sup>	1654 ± 183.9 <sup>*</sup>

Data are means ± SD; n = number of rats (n=5-6). <sup>#</sup>P < 0.05 vs. sham group; <sup>\*</sup>P < 0.05 vs I/R group by one-way ANOVA followed by tukey's posttest. NG=nitroglycerin, LVEDP=left ventricular end diastolic pressure, dP/dtmax=delta pressure/delta time.

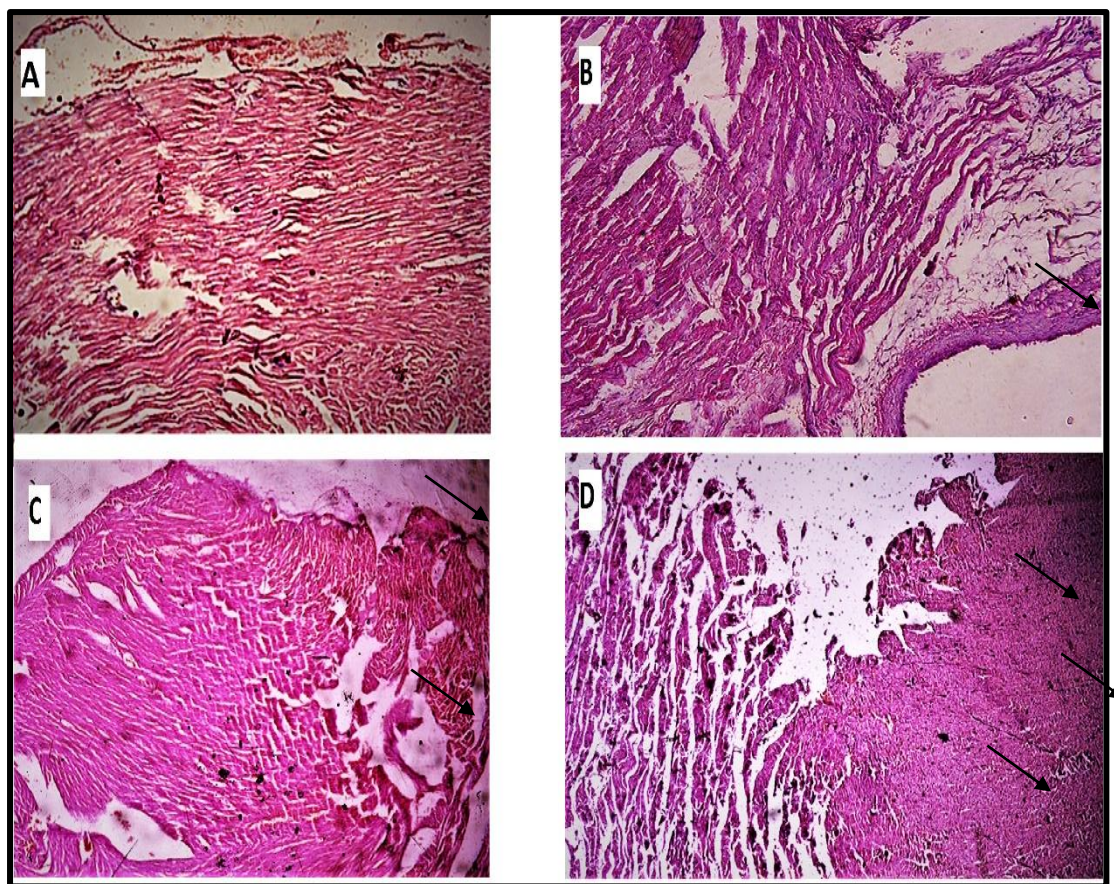
**Effect of nitroglycerin (NG) at different doses (25mg, 50mg, 100mg/kg) on infarct size and MI (myocardial infarction) severity score in an ischemia reperfusion (I/R) rat model:**

Table (2) showed that NG 25 mg produced significant decrease of infarct size and MI severity score. Meanwhile, NG 50 mg group showed significant increase of infarct size but showed insignificant increase on MI severity score. NG in a dose of 100mg produced significant increase of infarct size and MI severity score.

**Table (2): Effect of nitroglycerin (NG) at different doses (25mg, 50mg, 100mg/kg) on infarct size and MI (myocardial infarction) severity score in an ischemia reperfusion (I/R) rat model:**

Groups of rats (n=5)	Infarction size (ml) Mean± SD	MI severity score Mean± SD
Sham	0	0
Untreated I/R	4.75 ± 0.5 <sup>#</sup>	2 ± 0 <sup>#</sup>
NG 25mg/kg	1.25 ± 0.5 <sup>*</sup>	1 ± 0 <sup>*</sup>
NG 50mg/kg	6.6 ± 0.89 <sup>*</sup>	2.25 ± 0.50
NG 100mg/kg	9.25 ± 0.96 <sup>*</sup>	2.75 ± 0.50 <sup>*</sup>

Data are means ± SD; n = number of rats in each group. <sup>#</sup>P < 0.05 vs. sham group; <sup>\*</sup>P < 0.05 vs I/R group by one-way ANOVA followed by tukey's posttest. The severity and extent of MI were observed for each case grossly and microscopically. The findings were classified into the following degrees, (0): No change (1): Mild - focal myocyte damage or small multifocal degeneration with slight degree of inflammation, (2): Moderate - extensive myofibrillar degeneration and/or diffuse inflammatory process, (3): Severe - necrosis with diffuse inflammatory process<sup>12</sup>.



**Figure (1):** histopathological (H&E) examination of heart (200× magnification) showing MI severity score:

**A:** showing myocardial tissue with no histological changes (Grade 0), **B:** showing myocardial tissue with mild histological changes (arrows refer to: focal myocyte damage or small multifocal degeneration with slight degree of inflammation) (Grade 1), **C:** showing myocardial tissue with moderate histological changes (arrows refer to: extensive myofibrillar degeneration and/or diffuse inflammatory process) (Grade 2), **D:** showing severe histological changes (arrows refer to: necrosis with diffuse inflammatory process) (Grade 3).

## DISCUSSION

The present work was conducted to investigate the effect of nitroglycerin administration using three different doses (25 mg, 50 mg, and 100 mg/kg) three times daily for three successive days on cardiac hemodynamics, and infarction size & severity score in a model of coronary I/R. The results of the present study showed that I/R groups produced significant decrease in cardiac hemodynamic functions in addition to development of a measurable myocardial infarction area. Similar results obtained in previous studies<sup>13-14</sup>.

The present work showed that pretreatment with 3 doses with nitroglycerin showed a dose dependent effect, NG 25 mg/kg improved cardiac hemodynamics as well as myocardial infarction, NG 50 mg showed insignificant effect on LVEDP and myocardial infarction score while, it significantly decreased

cardiac contractility and myocardial infarction size. On the other-hand, NG 100mg/kg showed significant deterioration of cardiac functions and myocardial infarction size & severity score.

These results are ongoing with<sup>15</sup> who found that chronic intake of a low dose of nitrate (100 mg/L for 2 months) in male Wistar rats improved the recovery of the cardiac function after ischemia, as indicated by a lower LVEDP during reperfusion in heart samples of rats in comparison to control group. Meanwhile in male Wistar rats (300–360 g) that were given subcutaneous (s.c) 100 mg/kg NG three times a day for 3 days to induce vascular tolerance it was found that 10 min coronary occlusion showed increase LVEDP and decreased  $dP/dt_{max}$  in NG treated group<sup>10</sup>.

On-going with our result in which NG 25mg/kg decrease both infarct size and MI severity score, it was reported that nitrite infusion in rats (0.7–7 mg/L) during ischemia reduced

infarct size and improved left ventricular function<sup>16</sup>. An additional investigation has documented a similar protection in an in vivo canine model<sup>17</sup>. Meanwhile, it was found that in male Sprague Dawley rats that were randomized to receive nitroglycerin (60 µg/kg/h) or saline for 12 h followed by 40 min of MI and 4 h of reperfusion enlarged infarct size, and impaired cardiac functional recovery after ischemia<sup>18</sup>.

Nitroglycerin's effects on CVDs can be divided into hemodynamic and anti-ischemic effect. The hemodynamic effect of nitroglycerin is based on the reversal of ischemic left ventricular dysfunction and reductions in end-systolic and diastolic ventricular dimensions and pressure, increased left ventricular delta pressure/delta time (dp/dt) at rest and during exercise<sup>19</sup>.

The anti-ischemic effect of NG is believed to be based on the drug-induced inhibition of platelet aggregation and decrease in preload and afterload. This result in improvement of coronary collateral flow and dilatation of stenotic coronary arteries<sup>3</sup>.

Despite of the clear benefits of NG treatment, yet it rapidly loses its hemodynamic and anti-ischemic clinical effectiveness when used as long-term therapy and this phenomenon is referred as nitrate tolerance<sup>20</sup>. Moreover, acute high-dose of organic nitrates is likely to cause a similar loss of potency in the form of tachyphylaxis<sup>5</sup>.

A cross talk between reactive oxygen species (ROS), mitochondrial and endothelial dysfunction was documented in nitrate tolerance process. In this scenario, substantial interplay occurs between sources generating ROS including uncoupled mitochondrial respiratory chain<sup>21</sup>, activated nicotinamide adenine dinucleotide phosphate (NADPH) oxidases<sup>22</sup> and uncoupled NO synthase<sup>23</sup>.

Prolonged exposure to nitroglycerin has been shown to shift the physiologic balance between the sympathetic and vagal nervous systems in the modulation of cardiac heart rate and heart rate variability towards a prevalence of the sympathetic system impairing baroreflex function and heart rate variability<sup>24</sup>. Of note, such modifications should be considered relevant because they have been associated with an increased incidence of arrhythmias and a worse prognosis when observed in patients with coronary artery disease and/or heart failure<sup>25</sup>.

It was found that I/R is associated with a defect in complex I and complex III activity which may account for the enhanced production of H<sub>2</sub>O<sub>2</sub> in mitochondria<sup>26-13</sup>. Additionally, the activity of

mitochondrial complex I is decreased following continuous incubation with NG adding more harmful effect to I/R<sup>27</sup>.

## CONCLUSION

From the above results it could be concluded that NG showed a dose dependent manner. NG in a dose 25 mg/kg showed a beneficial effect on cardiac function and infarction, while increased doses of NG result in harmful effect on cardiac function and add more deleterious effect on myocardial infarction.

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