

## The relative efficacy of aminoguanidine and pentoxifylline in modulating endotoxin-induced cardiac stress

Tarek K. Motawi, Hebatallah A. Darwish\* and Azza M. Abd El Tawab

Biochemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt

This study investigates the effect of aminoguanidine (AG), a selective inducible nitric oxide synthase (iNOS) inhibitor, and pentoxifylline (PTX), a tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) inhibitor, on lipopolysaccharide (LPS)-induced cardiac stress. Rats were divided into four groups: group I served as a control, group II (LPS) received a single intraperitoneal injection of LPS (10 mg·kg<sup>-1</sup>), group III (LPS+AG) and group IV (LPS+PTX) were injected with either AG (100 mg·kg<sup>-1</sup>) or PTX (150 mg·kg<sup>-1</sup>) intraperitoneally 10 days prior to LPS administration. Normalization of cardiac levels of nitrite/nitrate (NO<sub>x</sub>), malondialdehyde (MDA), glutathione (GSH), heme oxygenase-1 (HO-1), glutathione peroxidase (GPx) and Na<sup>+</sup>, K<sup>+</sup>-ATPase activities was evident in the AG group. Both AG and PTX decreased the elevated serum TNF- $\alpha$  levels, the activities of lactate dehydrogenase (LDH), creatine kinase (CK) and cardiac myeloperoxidase (MPO). The levels of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and phosphocreatine (PCr) were enhanced following AG and PTX pretreatments. Calcium (Ca<sup>2+</sup>) levels were altered, and the histopathological observations supported the described results. Conclusively, the study highlights the cardioprotective potential of AG and PTX with superior results from AG. These findings reveal the relative contribution of nitric oxide and TNF- $\alpha$  to oxidative stress and energy failure during endotoxemia. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS—endotoxin; aminoguanidine; pentoxifylline; nitric oxide; tumour necrosis factor- $\alpha$ ; heart

### INTRODUCTION

Over the last few decades, the incidence of heart diseases was progressively increased, contributing 16% of all registered death (Health statistics and health information systems, WHO, 2009). Bacteria or endotoxins are leading and growing causes of cardiovascular failure.<sup>1</sup> Diverse molecular mechanisms involving inflammation and cellular damage have been implicated during endotoxemia. The overt production of nitric oxide (NO), the expression of proinflammatory cytokines and oxidative stress have attracted intensive research because of their intimate roles in the regulation of cardiovascular function.<sup>2</sup>

It has been demonstrated that upon exposure to the bacterial endotoxin [lipopolysaccharide (LPS)], an unregulated isoform of NO synthase (NOS), inducible NOS (iNOS), often is expressed in cardiovascular tissues.<sup>3</sup> NO is produced in excess amounts by iNOS and may contribute significantly to the deleterious effects of endotoxin, such as hypotension, cardio-depression, organ injury and dysfunction in septic shock.<sup>4</sup> These findings led to the hypothesis that pharmacological inhibition of iNOS might be of therapeutic value for the treatment of LPS-induced complications.

Aminoguanidine (AG) has been described as a potential candidate for iNOS inhibition. Experiments from both aortic rings<sup>5</sup> and *in vivo* animal models<sup>6</sup> have demonstrated the highly selective inhibition of iNOS by AG without impairing the normal production of NO by the endothelial NOS. This latter enzyme is constitutively present in endothelial cells and produces physiological levels of NO, leading to the maintenance of vascular tone and normal blood pressure. Additionally, AG has antioxidant and free radical scavenger properties especially on peroxynitrite (ONOO<sup>-</sup>) production.<sup>7</sup> AG treatment has been reported to alleviate the adverse effects of doxorubicin in the heart<sup>8</sup> and to protect against the tissue damage associated with periodontitis.<sup>9</sup> Studies have shown that AG prevents gastric oxidative stress and NO formation as well as gastric haemorrhagic erosion.<sup>10</sup> AG also was effective in amelioration of cisplatin-induced nephrotoxicity<sup>11</sup> and radiation-induced lung toxicity by increasing the endogenous antioxidant defence mechanism in rats.<sup>12</sup> Abdel-Zaher *et al.*<sup>13</sup> have shown that AG markedly inhibits acetaminophen-induced hepatic and renal depletion of antioxidants as well as NO overproduction.

As cytokines are involved in the pathogenesis of endotoxemia, drugs that modulate cytokine synthesis also may serve as possible therapeutic tools. Pentoxifylline (PTX), a methyl xanthine derivative, specifically blocks the synthesis of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), among other cytokines, by inhibiting its gene transcription, thereby reducing the accumulation of TNF- $\alpha$  messenger RNA (mRNA).<sup>14</sup> Several lines of

\*Correspondence to: Hebatallah A. Darwish, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, Cairo 11562, Egypt. E-mail: darwishheba17@yahoo.com

evidence, suggesting that PTX has beneficial effects against LPS-induced damage, have been documented.<sup>15,16</sup> Moreover, PTX has been used in the attenuation of the inflammatory response after cardiopulmonary bypass in open-heart surgery and acute respiratory distress syndrome in neonates.<sup>17</sup> As a non-specific phosphodiesterase inhibitor, it also has been used clinically as an agent to improve peripheral circulation in intermittent claudication.<sup>18</sup>

Based on this background, the present study was conducted to further stretch the confirmatory role of these two agents against LPS-induced cardiac stress in rats. This study investigated the relative contribution of NO and TNF- $\alpha$  to oxidative stress and energy failure during endotoxemia and delineated their roles in the cardioprotective effects of AG and PTX.

## MATERIALS AND METHODS

### *Drugs and chemicals*

Endotoxin (LPS from *Escherichia coli* serotype 055: B5), AG and PTX were purchased from Sigma-Aldrich Chemicals Company Co. (St. Louis, MD). All other chemicals were of the highest purity and analytical grade. Rat TNF- $\alpha$  and rat heme oxygenase-1 (HO-1) enzyme-linked immunosorbent assay (ELISA) kits were obtained from Assaypro (St. Charles, MO) and Assay Design (Ann Arbor, MI), respectively.

### *Experimental design*

Male Wistar albino rats, weighing 170–200 g, were used in this study. The rats were obtained from the farm of the National Institute for Vaccination, Helwan, Egypt. The rats received *ad libitum* water and standard chow diet throughout the experimental period. After 1 week of acclimatization, the rats were randomly divided into four groups. Group I served as the saline-treated control; in Group II, animals were injected intraperitoneally with a single dose of LPS [10 mg·kg<sup>-1</sup> body weight (BW)] prepared in physiological saline.<sup>1</sup> Thus, Group II served as the stressed group. In Groups III and IV, rats were treated with LPS (as in Group II) and AG (100 mg·kg<sup>-1</sup> BW)<sup>19</sup> or PTX (150 mg·kg<sup>-1</sup> BW).<sup>20</sup> AG and PTX were administered intraperitoneally for 10 days prior to LPS injection.

The study was carried out according to The European Communities Council Directive of 1986 (86/609/EEC) and was approved by the Ethical Committee for Animal Experimentation at the Faculty of Pharmacy, Cairo University.

### *Tissue sampling*

Four hours after LPS injection, the rats were subjected to light ether anaesthesia and sacrificed by cervical dislocation. The blood was collected, and the separated serum was used for assaying lactate dehydrogenase (LDH) and creatine kinase (CK) activities as well as TNF- $\alpha$ , total nitrite/nitrate (NOx) and HO-1 protein levels.

Meanwhile, the abdominal cavity was dissected immediately, and the heart was separated, weighed and homogenized

in 10 volumes of ice-cold, double-distilled water using an Ultra Turrax homogenizer. A suitable aliquot of resultant homogenate (10% w/v) was centrifuged at 21 000 g for 15 min at 4 °C using a Dupont Sorvall Ultracentrifuge (United States). The supernatant was used to assay the cardiac NOx levels. Another aliquot of the homogenate was mixed with 2.5 ml of 10 mmol·l<sup>-1</sup> of Tris-HCl buffer, pH 7.6, containing 250 mmol·l<sup>-1</sup> of sucrose and 0.4 mmol·l<sup>-1</sup> of phenylmethylsulphonyl fluoride. The buffered homogenate was centrifuged at 800 g for 10 min and then at 13 500 g for 20 min at 4 °C. The resulting supernatant was used to assay HO-1 protein levels.

Glutathione (GSH) levels were estimated from a deproteinized aliquot of the homogenate, whereas another aliquot was mixed with 2.3% KCl, then centrifuged at 600 g for 15 min, and the resulting supernatant was used for the determination of malondialdehyde (MDA) levels.

Cardiac GSH peroxidase (GPx) activity and calcium (Ca<sup>2+</sup>) levels were determined from the heart cytosolic fraction prepared by mixing equal volumes of 10% aqueous homogenate and Tris-ethylenediaminetetraacetic acid (EDTA) buffer, pH 7.6 (100 mmol·l<sup>-1</sup> of Tris and 0.2 mmol·l<sup>-1</sup> of EDTA), followed by centrifugation at 105 000 g at 4 °C for 15 min using a Dupont Sorvall ultracentrifuge (USA). A sixth aliquot of the homogenate was mixed with an equal volume of ice-cold 100 mmol·l<sup>-1</sup> of potassium phosphate buffer, pH 6.0, containing 1% hexadecyltrimethylammonium bromide, after which, it was sonicated in an ice-bath for 1 min. The tubes were then centrifuged at 10 000 g for 20 min at 4 °C, and the supernatant was used to determine tissue myeloperoxidase (MPO) activity.

To determine the levels of high-energy phosphate (HEP) compounds [adenosine triphosphate (ATP), adenosine diphosphate (ADP) and phosphocreatine (PCr)], an appropriate volume of the homogenate was mixed with ice-cold 4.8 mol·l<sup>-1</sup> of perchloric acid. The tubes were centrifuged at 600 g for 15 min at 4 °C and were then neutralized with 0.33 ml of 2 mol·l<sup>-1</sup> of KHCO<sub>3</sub>. Again, the tubes were centrifuged at 600 g for 10 min at 4 °C to remove potassium perchlorate precipitates, whereas the supernatants were used for the estimation of ATP, ADP and PCr levels. The last aliquot of the homogenate was mixed with 2 ml of ice-cold 0.435 mol·l<sup>-1</sup> of sucrose and centrifuged at 600 g for 15 min. The obtained supernatant was used to determine sodium–potassium adenosine triphosphatase (Na<sup>+</sup>, K<sup>+</sup>-ATPase) activity.

### *Biochemical investigations*

*Cardiac markers.* Serum LDH and CK activities were assayed using commercially available kits (Stanbio, Texas) based on the method of Buhl and Jackson<sup>21</sup> and Rosalki.<sup>22</sup> Briefly, LDH and CK specifically catalyze the oxidation of lactate to pyruvate and the transphosphorylation of ADP to ATP, respectively, with subsequent formation of NADH. NADH production was monitored spectrophotometrically per min at 340 nm, which was proportional to the enzymatic activity.

**TNF- $\alpha$  and NO $_x$ .** Serum TNF- $\alpha$  levels were quantified with a commercially available rat TNF- $\alpha$  ELISA kit.<sup>23</sup> TNF- $\alpha$  in both standards and samples was sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for rat TNF- $\alpha$ , which was recognized by a streptavidin–peroxidase conjugate. All unbound material was then washed away, and a peroxidase enzyme substrate was added. The colour development was stopped with an acid stop solution, which converts the end point colour to yellow. The intensity of the colour was measured at 450 nm using a microtitration plate reader.

NO $_x$  was measured in serum and heart tissue according to the method described by Miranda *et al.*<sup>24</sup> with a minor modification that involved using zinc sulphate instead of ethanol for the protein precipitation. This method employs the reduction of any nitrate to nitrite by vanadium chloride followed by the detection of total nitrite by Griess reagent. The formed azo derivative was measured colourimetrically at 540 nm.

**Oxidative stress markers.** Malondialdehyde levels were measured according to the method of Uchiyama and Mihara<sup>25</sup> as an indicator of lipid peroxidation. MDA reacts with thiobarbituric acid, giving a coloured complex that can be measured spectrophotometrically.

GSH levels were determined using 5,5-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent). This reaction produces a stable yellow colour that is measured colourimetrically at 412 nm.<sup>26</sup> GPx activity was assessed by monitoring the rate of GSH oxidation by hydrogen peroxide in the presence of NADPH as a decrease in absorbance at 340 nm.<sup>27</sup> MPO activity was measured according to the method of Renlund *et al.*<sup>28</sup> This method is based on measuring the hydrogen peroxide-dependent oxidation of O-dianisidine catalysed by MPO, which results in the formation of a compound that exhibits an increased absorbance at 460 nm.

HO-1 expression was quantified according to the method of Katori *et al.*<sup>29</sup> using a commercially available rat HO-1 ELISA kit. In this method, a blue colour, in proportion to the amount of captured HO-1, is developed with tetramethyl benzidine substrate. The intensity of the colour was measured at 450 nm using a microtitration plate reader.

**Energy status.** The simultaneous determination of HEP compound concentrations (ATP, ADP and PCr) in myocardial tissue was achieved by high-performance liquid chromatography according to the method of Teerlink *et al.*<sup>30</sup> using an isocratic elution instead of a gradient elution. The peak areas of ATP, ADP and PCr were quantified using a Shimadzu CR501 Chromatopac integrator and Glass-VP5 integration software.

Na $^+$ , K $^+$ -ATPase activity was measured according to the method described by Rash.<sup>31</sup> Total ATPase activity was estimated from the amount of inorganic phosphorus (P $_i$ ) released after incubation of the sample with ATP disodium salt in the presence of Na $^+$ , K $^+$  and Mg $^{2+}$  ions and in the absence of ouabain. Mg $^{2+}$ -ATPase was estimated from the amount of P $_i$  released in the presence of ouabain. Na $^+$ , K $^+$ -

ATPase activity was calculated by subtracting the activity assayed in the presence of ouabain from that assayed in its absence. The amounts of liberated P $_i$  were estimated using the method of Weidman.<sup>32</sup>

**Cardiac calcium and protein.** Ca $^{2+}$  levels were assessed in the cytosolic fraction by the atomic absorption technique<sup>33</sup> using a Unicam 929 atomic absorption spectrophotometer. This method depends on the fact that free atoms of an element absorb light of a very specific wavelength ( $\lambda = 422$  nm).

The protein levels of different fractions, resulting from ultracentrifugation of the homogenate, were determined by the method of Lowry *et al.*,<sup>34</sup> using Folin–Ciocalteu reagent with bovine serum albumin as a standard.

#### *Histopathological studies*

Heart specimens were fixed with 10% formaldehyde and processed for embedding in paraffin. Sections of 5  $\mu$ m were stained with hematoxylin and eosin and examined under a light microscope.

#### *Statistical analysis*

The results are expressed as the mean  $\pm$  standard error of the mean. Differences among means were tested for statistical significance using one-way analysis of variance. When differences were significant, Tukey–Kramer's test was used for multiple comparisons between groups. Statistical significance was considered when  $P < 0.05$ .

## RESULTS

### *Effect of pretreatment with AG and PTX on LPS-induced changes in TNF- $\alpha$ and NO $_x$ levels*

Lipopolysaccharide injection caused a significant increase in serum TNF- $\alpha$  as well as serum and cardiac NO $_x$  levels. These increases reached 281, 250 and 295%, respectively, relative to normal levels. AG reversed the elevated levels of serum and cardiac NO $_x$  to nearly the levels found in the control group and significantly reduced the TNF- $\alpha$  levels by 48% compared with the stressed rats. Meanwhile, PTX pretreatment markedly restored elevated TNF- $\alpha$  levels, and it significantly decreased NO $_x$  levels by 15% in serum and by 17% in cardiac tissue, compared with the LPS group (Table 1).

### *Effect of pretreatment with AG and PTX on LPS-induced changes in MDA, GSH and HO-1 protein levels as well as GPx and MPO activities*

Compared with normal control rats, LPS injection induced a significant reduction of cardiac GSH levels (39%) in addition to a significant elevation of MDA levels (194%). AG administration clearly reversed the cardiac levels of GSH and MDA back to their normal values, demonstrating its protective effects, whereas early treatment with PTX did not significantly change cardiac levels compared with

Table 1. Effect of pretreatment with either aminoguanidine or pentoxifylline on lipopolysaccharide-induced changes in tumour necrosis factor- $\alpha$  and total nitrite/nitrate levels

Groups	Group I	Group II	Group III	Group IV
Parameters	(Control)	(LPS)	(LPS + AG)	(LPS + PTX)
<b>Serum TNF-<math>\alpha</math></b> ( $\text{pg}\cdot\text{ml}^{-1}$ )	3.9 $\pm$ 0.23	10.96 $\pm$ 0.6*	5.7 $\pm$ 0.19* <sup>†</sup>	4.72 $\pm$ 0.29 <sup>†</sup>
<b>Serum NO<math>_x</math></b> ( $\mu\text{mol}\cdot\text{l}^{-1}$ )	28.7 $\pm$ 0.84	71.9 $\pm$ 2.29*	25.7 $\pm$ 1.2 <sup>†</sup>	61.4 $\pm$ 1.9* <sup>†</sup>
<b>Cardiac NO<math>_x</math></b> ( $\mu\text{mol}\cdot\text{g}^{-1}\text{ tissue}$ )	0.23 $\pm$ 0.01	0.68 $\pm$ 0.03*	0.31 $\pm$ 0.01 <sup>†</sup>	0.57 $\pm$ 0.02* <sup>†</sup>

Values represent mean  $\pm$  standard error of the mean (SEM) for eight rats. AG, aminoguanidine; LPS, lipopolysaccharide; NO $_x$ , total nitrite/nitrate; PTX, pentoxifylline; TNF- $\alpha$ , tumour necrosis factor- $\alpha$ .

\*Significant difference from control group at  $P < 0.05$ .

<sup>†</sup>Significant difference from LPS group at  $P < 0.05$ .

the LPS-treated group. Moreover, LPS caused a significant increase in cardiac GPx (176%) and MPO (320%) activities relative to the normal control. AG normalized GPx and significantly reduced MPO activities by 35% compared with the LPS-treated group. PTX caused a significant reduction in the LPS-elevated MPO activity by almost twofold and failed to significantly alter the elevated GPx activity. Significant increases in cardiac and serum HO-1 protein levels also were detected after LPS injection. AG restored cardiac protein levels and significantly reduced serum HO-1 levels. However, PTX did not alter the LPS-elevated HO-1 level to a significant value (Table 2).

#### Effect of pretreatment with AG and PTX on LPS-induced changes in Ca<sup>2+</sup> levels as well as LDH and CK activities

Administration of LPS caused a 1.5-fold increase in Ca<sup>2+</sup> levels relative to the control rats. AG did not exert a pronounced change in cardiac Ca<sup>2+</sup> levels compared with the LPS-treated group. However, PTX nearly returned the cardiac Ca<sup>2+</sup> back to normal levels. A substantial increase in serum LDH and CK activities that reached 260 and 210%, respectively, relative to the control group also was observed in the LPS-treated group. AG effectively normalized the elevated CK activity while causing a significant decrease in the LDH activity compared with the stressed group. However,

PTX significantly decreased CK activity to approximately 67% of the stressed value, whereas only a slight, insignificant decrease in LDH activity was observed (Table 3).

#### Effect of pretreatment with AG and PTX on LPS-induced changes in energy status

A significant attenuation in Na<sup>+</sup>, K<sup>+</sup>-ATPase activity and in ATP, ADP and PCr levels was obvious in the LPS-treated rats. This attenuation caused a significant reduction of the ATP : PCr ratio along with an insignificant decrease in the ATP : ADP ratio when compared with control values (Table 3).

AG restored the LPS-reduced Na<sup>+</sup>, K<sup>+</sup>-ATPase activity in addition to the reduced ADP and PCr levels. AG also reversed the decrease in ATP and the ATP : PCr ratio by almost twofold and 1.5-fold, respectively, but it did not significantly alter the ATP : ADP ratio with respect to the LPS group. Treatment with PTX significantly enhanced the activity of Na<sup>+</sup>, K<sup>+</sup>-ATPase compared with both normal and stressed rats. This result was accompanied by increases in ADP and PCr to some extent rather than ATP levels. Such effects resulted in a marked decrease in the cardiac ATP : ADP ratio and an insignificant change in the ATP : PCr ratio as compared with the LPS group.

#### Histopathological examination

The histopathological examination supported the above biochemical analysis. Figure 1a shows a normal architecture of the control heart. Fibres are grouped into bundles with connective tissues in between. Each muscle fibre has an acidophilic cytoplasm and a central nucleus. In contrast, the animals receiving LPS were greatly affected. Focal inflammatory cell infiltration, haemorrhages, swelling, edema and degeneration with dilated blood vessels were detected in between the myocardial bundles (Figure 1b, c and d).

Animals that received AG displayed minimal negative effects in the cardiac muscles with an image similar to the control sections (Figure 1e and f). However, pretreatment with PTX showed minimal improvement in the histopathology. A few mononuclear leukocytes and inflammatory cell infiltration in a focal manner between the cardiac bundles were observed (Figure 1g). This result was associated with

Table 2. Effect of pretreatment with either aminoguanidine or pentoxifylline on lipopolysaccharide-induced changes in oxidative stress markers

Groups	Group I	Group II	Group III	Group IV
Parameters	(Control)	(LPS)	(LPS + AG)	(LPS + PTX)
<b>Cardiac MDA</b> ( $\text{nmol}\cdot\text{g}^{-1}\text{ tissue}$ )	43.05 $\pm$ 1.6	83.6 $\pm$ 3.6*	46.5 $\pm$ 2.81 <sup>†</sup>	71.7 $\pm$ 4.7*
<b>Cardiac GSH</b> ( $\mu\text{mol}\cdot\text{g}^{-1}\text{ tissue}$ )	2.63 $\pm$ 0.102	1.051 $\pm$ 0.076*	2.67 $\pm$ 0.06 <sup>†</sup>	1.41 $\pm$ 0.12*
<b>Cardiac GPx</b> ( $\text{mU}\cdot\text{mg}^{-1}\text{ tissue}$ )	137.4 $\pm$ 4.97	242.2 $\pm$ 16.2*	136.5 $\pm$ 5.75 <sup>†</sup>	224.7 $\pm$ 9.54*
<b>Cardiac MPO</b> ( $\text{U}\cdot\text{mg}^{-1}\text{ protein}$ )	0.69 $\pm$ 0.04	2.21 $\pm$ 0.08*	1.44 $\pm$ 0.06* <sup>†</sup>	1.24 $\pm$ 0.04* <sup>†</sup>
<b>Cardiac HO-1</b> ( $\text{ng}\cdot\text{mg}^{-1}\text{ tissue}$ )	28.2 $\pm$ 1.11	68.56 $\pm$ 3.5*	30.65 $\pm$ 1.59 <sup>†</sup>	61.9 $\pm$ 3.61*
<b>Serum HO-1</b> ( $\text{ng}\cdot\text{ml}^{-1}$ )	4.52 $\pm$ 0.35	14.3 $\pm$ 0.42	6.6 $\pm$ 0.29* <sup>†</sup>	13.4 $\pm$ 0.32*

Values represent mean  $\pm$  SEM for eight rats.

MDA, malondialdehyde; GPx, glutathione peroxidase; GSH, glutathione; HO-1, heme oxygenase-1.

\*Significant difference from control group at  $P < 0.05$ .

<sup>†</sup>Significant difference from LPS group at  $P < 0.05$ .

Table 3. Effect of pretreatment with either aminoguanidine or pentoxifylline on lipopolysaccharide-induced changes in  $Ca^{2+}$  levels, lactate dehydrogenase and creatine kinase activities and energy status

Groups	Group I	Group II	Group III	Group IV
Parameters	(Control)	(LPS)	(LPS + AG)	(LPS + PTX)
$Ca^{2+}$ ( $\mu g \cdot g^{-1}$ tissue)	78.5 $\pm$ 4.2	110.5 $\pm$ 3.9*	100 $\pm$ 3.4*	70.9 $\pm$ 3.2 <sup>†</sup>
LDH ( $U \cdot L^{-1}$ )	175.8 $\pm$ 3.3	456.6 $\pm$ 23*	315 $\pm$ 14.05* <sup>†</sup>	434 $\pm$ 26.2*
CK ( $U \cdot L^{-1}$ )	222.7 $\pm$ 9.97	466 $\pm$ 29.9*	231.3 $\pm$ 17.02 <sup>†</sup>	314 $\pm$ 18.1* <sup>†</sup>
Cardiac ATP ( $\mu mol \cdot g^{-1}$ tissue)	13.5 $\pm$ 0.3	5.78 $\pm$ 0.33*	10.8 $\pm$ 0.18* <sup>†</sup>	7.8 $\pm$ 0.59* <sup>†</sup>
Cardiac ADP ( $\mu mol \cdot g^{-1}$ tissue)	2.93 $\pm$ 1.12	1.41 $\pm$ 0.05*	2.66 $\pm$ 0.15 <sup>†</sup>	4.31 $\pm$ 0.12* <sup>†</sup>
Cardiac PCr ( $\mu mol \cdot g^{-1}$ tissue)	31.3 $\pm$ 0.44	25.7 $\pm$ 0.73*	33.9 $\pm$ 0.67 <sup>†</sup>	34.4 $\pm$ 0.98 <sup>†</sup>
Cardiac ATP : ADP ratio	4.6 $\pm$ 0.16	4.1 $\pm$ 0.2	4.1 $\pm$ 0.25	1.8 $\pm$ 0.12* <sup>†</sup>
Cardiac ATP : PCr ratio	0.43 $\pm$ 0.004	0.23 $\pm$ 0.01*	0.31 $\pm$ 0.004* <sup>†</sup>	0.23 $\pm$ 0.01*
Cardiac $Na^+$ , $K^+$ -ATPase ( $\mu mol \cdot Pi \cdot h^{-1} \cdot mg^{-1}$ of protein)	36 $\pm$ 2.8	18.8 $\pm$ 0.9*	31 $\pm$ 1.89 <sup>†</sup>	43.5 $\pm$ 3.4* <sup>†</sup>

Values represent mean  $\pm$  SEM for eight rats.

ADP, adenosine diphosphate; ATP, adenosine triphosphate; CK, creatine kinase; LDH, lactate dehydrogenase; PCr, phosphocreatine.

\*Significant difference from control group at  $P < 0.05$ .

<sup>†</sup>Significant difference from LPS group at  $P < 0.05$ .

impaction of the lumen of the dilated blood vessels by the leukocytes (Figure 1h). Such observations support our findings that AG was more potent than PTX in ameliorating LPS-induced cardiac stress in rats.

## DISCUSSION

It is widely accepted that endotoxin produces multiple organ failure, which is a frequent cause of death among patients who succumb to endotoxic shock.<sup>35</sup> NO emerged as a potentially important player in the pathogenesis of endotoxemia because it has major interactions with the pathways of gene expression controlled by the transcription factor NF- $\kappa$ B. When activated by various extracellular inflammatory triggers (e.g. stimulation of CD14 by LPS, stimulation of TNF or interleukin-1 receptors and oxidant stress), NF- $\kappa$ B translocates to the nucleus where it induces the transcription of numerous genes coding for proteins involved in inflammation, such as cytokines, leukocyte-endothelial adhesion molecules and iNOS.<sup>36</sup> In agreement with previous studies, the present study revealed elevated levels of NO<sub>x</sub> and TNF- $\alpha$  after administering LPS to rats. These levels were reversed by pretreatment with AG and PTX.

Aminoguanidine is regarded as a selective inhibitor of both iNOS activity and iNOS protein induction, which has been demonstrated *in vitro* and *in vivo*.<sup>37,38</sup> Tracey *et al.*<sup>39</sup> have shown that AG was able to elicit 95% inhibition of the LPS-induced increase in plasma NO<sub>x</sub> in addition to abolishing the LPS-caused mortality in mice. The mechanism of inhibition of NOS by guanidines is thought to be mediated either through their binding to the heme iron present at the catalytic site of NOS or through their competitive binding with L-arginine, thereby causing the observed decrease in NO levels.<sup>40</sup>

On the other hand, PTX has shown multiple beneficial effects in the inflammatory cascade through its ability to inhibit TNF- $\alpha$  synthesis.<sup>41</sup> As a phosphodiesterase inhibitor, PTX increases the intracellular 3,5-cyclic adenosine monophosphate (cAMP) levels in inflammatory and

immunocompetent cells. The elevated cAMP levels reduce the release of potent proinflammatory cytokines, such as TNF- $\alpha$  and interleukin-2. Certain proinflammatory cytokines are related to the generation of chemokines and the expression of cell adhesion molecules in addition to the production of other molecules related to inflammation such as NO.<sup>42</sup>

TNF- $\alpha$  levels have been formerly correlated to the production of NO. Studies by Rahat *et al.*<sup>43</sup> showed that NO increases the level of TNF- $\alpha$  mRNA in the lungs of rats submitted to intestinal ischaemia/reperfusion, suggesting a role for NO in regulating TNF transcription. Based on this finding, one can speculate that decreased NO<sub>x</sub> levels observed in both pretreated groups might account for the amelioration of their TNF- $\alpha$  levels (Table 1).

Myeloperoxidase plays a major role in a variety of inflammatory responses.<sup>44</sup> Increased MPO activity after LPS injection is an indication of the injurious state as a result of neutrophil infiltration. Enhanced neutrophil infiltration was attributed to the reported increases in TNF- $\alpha$  and leukotriene synthesis during endotoxemia.<sup>45</sup> These inflammatory mediators were capable of stimulating neutrophil adherence by the upregulation of intercellular adhesion molecule-1 (ICAM-1) expression. ICAM-1 has been shown to be essential for neutrophil recruitment into organs, and for their subsequent injury, after endotoxin exposure (Madjdpour *et al.*, 2000).<sup>46</sup> Suppression of MPO activity following pretreatment with PTX might be related to its ability to suppress ICAM-1 expression via inhibition of TNF- $\alpha$ .<sup>19,47</sup>

Importantly, Eiserich *et al.*<sup>48</sup> provided evidence indicating that MPO functions as an NO oxidase and that endothelial-associated MPO is responsible for endothelial dysfunction. Other studies also have demonstrated that MPO utilizes hydrogen peroxide and nitrite to generate nitrogen dioxide, resulting in nitrate death.<sup>49,50</sup> Thus, one of the beneficial effects of AG and PTX lies in their ability to diminish MPO activity and NO production, preventing cell death.

Oxidative stress resulting from increased oxidant production, reduced antioxidant levels or both contributes to a major mechanism of LPS-induced cardiac damage.<sup>2</sup> In the

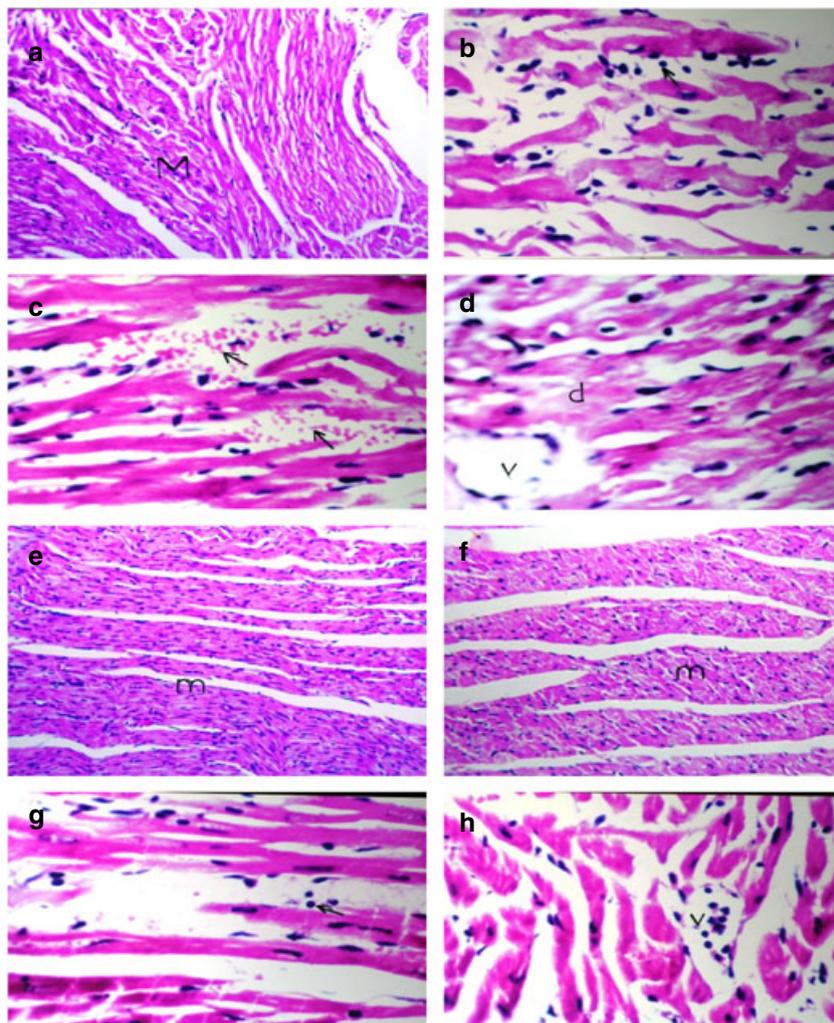


Figure 1. Histopathology of the heart in the four experimental groups (hematoxylin and eosin). (a  $\times$  40): Shows normal architecture of the control heart. (b, c and d  $\times$  160): Cardiac muscle fibres of the lipopolysaccharide group showing focal inflammatory cell infiltration (arrow) in between the cardiac bundles and degeneration of myocardium (d) with dilated blood vessels (v). (e  $\times$  40): Cardiac muscle fibres of aminoguanidine (AG)-pretreated group showing normal histological structure of the myocardium (m). (f  $\times$  64): Cardiac muscle fibers of AG-pretreated group with magnification to identify the normal histological structure of myocardium. (g and h  $\times$  160): Cardiac muscle fibres of pentoxifylline-pretreated group showing inflammatory cell infiltration (arrow) with impaction of blood vessels (v) by the leukocytes

present study, LPS induced a significant increase in cardiac GPx activity and MDA levels along with a significant decrease of GSH content. The elevated GPx activity observed in the LPS group implied a reduced redox status (GSH : GSSG ratio), which might be expected to guard against the encountered oxidative stress.

The primary targets of ROS-mediated attack are the polyunsaturated acids within the membrane lipids, causing lipid peroxidation that may lead to disorganization of cell structure and function.<sup>51</sup> The association between the elevated cardiac MDA and lowered cardiac GSH levels clearly indicated the oxidative damage caused by LPS. Depletion of GSH has been shown to markedly increase NO-dependent cytotoxicity.<sup>52</sup> S-nitrosothiols, including the S-nitroso-adduct of GSH itself (S-nitrosoglutathione), may inhibit several enzymatic pathways involved in the

maintenance of the GSH pool. It appears that nitrosative stress enhances the cellular susceptibility to oxidant-mediated damage in circumstances associated with high NO production, providing an important cycle of cytotoxic amplification in inflammatory conditions.<sup>53</sup>

The current study showed a significant amelioration of oxidative stress parameters following AG administration yet insignificant changes after PTX administration (Table 2). The protective effect of AG may be achieved through two mechanisms. First, AG reduces the formation of ONOO<sup>-</sup>. Most of the deleterious effects of NO are believed to be mediated by ONOO<sup>-</sup>, a potent oxidant that is produced by the reaction of NO with superoxide radical.<sup>54</sup> Peroxynitrite depletes the antioxidant defences, which include GSH, protein thiols and antioxidant enzymes.<sup>55</sup> Inadequacy of the protective antioxidant system results in enhanced lipid

peroxidation and tissue injury. However, when the production of NO is decreased by the administration of the iNOS inhibitor AG, the source of ONOO<sup>-</sup> is reduced, the oxidative damage to lipids and proteins is decreased and the cellular antioxidant systems are spared. AG also might reduce ROS production indirectly by inhibiting cyclooxygenase-2, resulting in reducing arachidonic acid metabolism and, subsequently, ROS generation.<sup>56</sup> This possible dual inhibition of ROS obtained with AG could account for its marked antioxidative stress effects. However, the insignificant PTX effect might be related to its mild amelioration of NO<sub>x</sub> levels that was insufficient to block ONOO<sup>-</sup> formation.

The heme oxygenase (HO) system is involved in the regulation of many physiological and pathological processes in stress.<sup>57</sup> HO-1 is an inducible isoform of the enzyme whose expression is upregulated in response to hypoxia, ROS, cytokines and endotoxin as an adaptive means to oxidative stress.<sup>57,58</sup> Lee *et al.*<sup>59</sup> demonstrated that the expression of HO-1 was completely inhibited by administration of antioxidative agents in rats subjected to ovariectomized stress. Our findings were consistent with this report, demonstrating that inhibition of HO-1 expression decreased cardiac stress in rats treated with AG.

Interestingly, the present study showed a positive correlation between NO and HO-1 levels in the LPS group. Indeed, NO is an HO-1 inducer.<sup>60</sup> Several studies have demonstrated the NO-mediated upregulation of HO-1 expression in macrophages stimulated with LPS.<sup>61,62</sup> AG, as a selective iNOS inhibitor, significantly reduced both NO and HO-1 levels. These data implied that LPS-elicited HO-1 expression might be mediated, at least in part, by NO. Nevertheless, proinflammatory cytokines also were reported to be involved in LPS-induced upregulation of HO-1.<sup>63</sup> However, this was not confirmed in this study as PTX normalized TNF- $\alpha$  levels but displayed little effect on the LPS-induced upregulation of HO-1.

Quantification of creatine compounds and adenine nucleotides in myocardial tissue is significant for determining changes in its energetic state.<sup>64</sup> A significant reduction of Na<sup>+</sup>, K<sup>+</sup>-ATPase, ATP, ADP and PCr levels as well as their ratios was observed in endotoxin-stressed rats relative to the control group. It is likely that the reduced energetic state in response to endotoxemia leads to a reduced maximal rate of sustainable ATP turnover in the heart. That is, the range of ATP turnover rates over which the CK reaction can temporally buffer ATP may be reduced by virtue of the lower PCr and adenine nucleotide pools.<sup>65</sup>

Reductions of HEP as a result of increased NO were clearly demonstrated in the present study. NO reversibly inhibits O<sub>2</sub> uptake by binding to cytochrome oxidase found in rat skeletal muscle and heart mitochondria, implying a likely transitory impairment of ATP available for muscle contraction.<sup>66</sup> Moreover, ONOO<sup>-</sup> also impairs cellular energetics through an indirect mechanism, implicating DNA damage and activation of the nuclear enzyme poly-(ADP-ribose) polymerase (PARP), a pathway increasingly recognized as a major mechanism of NO/ONOO<sup>-</sup>-mediated cytotoxicity. DNA single-strand breakage is the obligatory

trigger for the activation of PARP, which then catalyses the cleavage of its substrate, nicotinamide adenine dinucleotide, into ADP-ribose and nicotinamide. PARP covalently attaches ADP-ribose to various nuclear proteins and rapidly depletes the cellular nicotinamide adenine dinucleotide stores, thereby slowing the rate of glycolysis, electron transport and ATP formation, which results in cell dysfunction and death via the necrotic pathway.<sup>67,68</sup>

In the current study, AG significantly improved the LPS-induced energy depletion in cardiac tissue. The protective effect offered by AG was attributed to the decreased NO levels as a result of iNOS inhibition.<sup>19</sup> This result is in agreement with the report of Seven *et al.*<sup>69</sup> that found a negative correlation between iNOS and Na<sup>+</sup>, K<sup>+</sup>-ATPase activity in guinea pig kidney exposed to LPS, which suggested the possibility that NO may play a crucial role in energy depletion during endotoxemia. As with AG, PTX ameliorated almost all of the energy status parameters. However, the obvious decrease in ATP : ADP ratio could be related to the marked increase of Na<sup>+</sup>, K<sup>+</sup>-ATPase activity that, in turn, augmented the utilization of ATP and the production of ADP.<sup>70</sup>

Myocardial contractility is essentially dependent on Ca<sup>2+</sup> handling in cardiac myocytes and myofilament sensitivity to Ca<sup>2+</sup>. In this study, LPS induced a significant increase in cytosolic Ca<sup>2+</sup> concentration. A lack of energy has been shown to cause inhibition of Ca<sup>2+</sup>-ATPases and Na<sup>+</sup>, K<sup>+</sup>-ATPase present in the sarcoplasmic reticulum,<sup>71</sup> leading to the intracellular Ca<sup>2+</sup> overloads observed in our study. In addition, Sudharsan *et al.*<sup>72</sup> suggested the possibility that a decrease in Na<sup>+</sup>, K<sup>+</sup>-ATPase activity would lead to the accumulation of intracellular sodium, which favours an increase in levels of intracellular Ca<sup>2+</sup> owing to increased Na<sup>+</sup>/Ca<sup>2+</sup> exchange.

In view of the potential role of NO in Ca<sup>2+</sup> homeostasis and myocardial contractility, it has been shown that ONOO<sup>-</sup> can inactivate Ca<sup>2+</sup>-ATPase<sup>73</sup> and modify actin properties.<sup>74</sup> Nevertheless, NOS inhibition by AG failed to significantly affect Ca<sup>2+</sup> levels relative to the LPS group. These results suggested that other NO-independent pathways might be involved.<sup>75</sup> Conversely, PTX pretreatment inhibited the Ca<sup>2+</sup> increase triggered by endotoxin. The exact mechanisms of PTX on abnormal cardiac Ca<sup>2+</sup> levels are not fully known. It has been proposed that PTX possibly chelated Ca<sup>2+</sup>, causing a decrease of Ca<sup>2+</sup>-caused K<sup>+</sup> efflux and activation of ATPase in cardiac tissue membranes.<sup>70</sup>

Depending on the result of the present study, it could be concluded that sustained excessive NO production along with oxidative stress during endotoxemia caused mitochondrial dysfunction, resulting in myocardial energy depletion. The study highlights the efficacy of AG and PTX, but especially AG, in protecting the heart from the biochemical abnormalities caused by LPS administration. The histopathological data confirmed the cytoprotection rendered by AG. Eventually, the study warranted further research to support the finding that the use of a specific iNOS inhibitor, rather than TNF- $\alpha$  inhibitor, may be a good candidate for the treatment of cardiac stress.

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial assistance provided by the Faculty of Pharmacy, Cairo University, Cairo, Egypt. The authors are also thankful to Dr Adel Bakeer, Histology Department, Faculty of Veterinary Medicine, Cairo University, for performing the histopathological examination in this study.

## REFERENCES

- Chagnon F, Metz CN, Bucala R, Lesur O. Endotoxin-induced myocardial dysfunction. Effects of macrophage migration inhibitory factor neutralization. *Circ Res* 2005; **96**: 1095–1102.
- Liu YC, Chang AYW, Tsai YC, Chan JYH. Differential protection against oxidative stress and nitric oxide overproduction in cardiovascular and pulmonary systems by propofol during endotoxemia. *J Biomed Sci* 2009; **16**(8): 1–14.
- Kilbourn R. The discovery of nitric oxide as key mediator in septic shock. *Sepsis* 1998; **1**: 85–91.
- Iskit AB, Sungur A, Gedikoglu G, Guc MO. The effects of bosentan, aminoguanidine and L-canavanine on mesenteric blood flow, spleen and liver in endotoxaemic mice. *Eur J Pharmacol* 1999; **379**: 73–80.
- Griffiths MJ, Messent M, MacAllister RJ, Evans TW. Aminoguanidine selectively inhibits inducible nitric oxide synthase. *Br J Pharmacol* 1993; **110**: 963–968.
- Wu CC, Chen SJ, Szabo C, Thiemermann C, Vane JR. Aminoguanidine attenuates the delayed circulatory failure and improves survival in rodent models of endotoxic shock. *Br J Pharmacol* 1995; **114**: 1666–1672.
- Ihm SH, Yoo HJ, Park SW, Ihm J. Effect of aminoguanidine on lipid peroxidation in streptozotocin-induced diabetic rats. *Metabolism* 1999; **48**: 1141–1145.
- Abd El-Gawad HM, El-Sawalhi MM. Nitric oxide and oxidative stress in brain and heart of normal rats treated with doxorubicin: role of aminoguanidine. *J Biochem Mol Toxicol* 2004; **18**: 69–77.
- Ara C, Karabulut AB, Kirimlioglu H, Yilmaz M, Kirimlioglu V, Yilmaz S. Protective effect of aminoguanidine against oxidative stress in an experimental peritoneal adhesion model in rats. *Cell Biochem Funct* 2006; **24**: 443–448.
- Hung CR. Role of gastric oxidative stress and nitric oxide in formation of hemorrhagic erosion in rats with ischemic brain. *World J Gastroenterol* 2006; **12**: 574–581.
- Atasayar S, Güreç-Orhan H, Orhan H, Güreç B, Girgin G, Özgünes H. Preventive effect of aminoguanidine compared to vitamin E and C on cisplatin-induced nephrotoxicity in rats. *Exp Toxicol Pathol* 2009; **61**(1): 23–32.
- Eroglu C, Yildiz OG, Saraymen R, Soyuer S, Kilic E, Ozcan S. Aminoguanidine ameliorates radiation-induced oxidative lung damage in rats. *Clin Invest Med* 2008; **31**: E182–E188.
- Abdel-Zaher AO, Abdel-Rahman MM, Hafez MM, Omran FM. Role of nitric oxide and reduced GSH in the protective effects of aminoguanidine, gadolinium chloride and oleanolic acid against acetaminophen-induced hepatic and renal damage. *Toxicology* 2007; **234**: 124–134.
- Neuner P, Klosner G, Schauer E, et al. Pentoxifylline in vivo down-regulates the release of IL-1, IL-6, IL-8 and tumor necrosis factor- $\alpha$  by human peripheral blood mononuclear cells. *Immunology* 1994; **83**: 262–267.
- Lauterbach R, Pawlik D, Kowalczyk D, Ksycinski W, Helwich E, Zembala M. Effect of the immunomodulating agent, pentoxifylline, in the treatment of sepsis in prematurely delivered infants: a placebo-controlled, double-blind trial. *Crit Care Med* 1999; **27**: 807–814.
- Danjo W, Fujimura N, Ujike Y. Effect of pentoxifylline on diaphragmatic contractility in septic rats. *Acta Med Okayama* 2008; **62**(2): 101–107.
- Michetti C, Coimbra R, Hoyt DB, Loomis W, Junger W, Wolf P. Pentoxifylline reduces acute lung injury in chronic endotoxemia. *J Surg Res* 2003; **115**: 92–99.
- Porter JM, Cutler BS, Lee BY, et al. Pentoxifylline efficacy in the treatment of intermittent claudication: Multicenter controlled double-blind trial with objective assessment of chronic occlusive arterial disease patients. *Am Heart J* 1982; **104**: 66–72.
- Yaylak F, Canbaz H, Caglikulekci M, et al. Liver tissue inducible nitric oxide synthase (iNOS) expression and lipid peroxidation in experimental hepatic ischemia reperfusion injury stimulated with lipopolysaccharide: The role of aminoguanidine. *J Surg Res* 2008; **148**: 214–223.
- Irie K, Fujii E, Ishida H, et al. Inhibitory effects of cyclic AMP elevating agents on lipopolysaccharide (LPS)-induced microvascular permeability change in mouse skin. *Br J Pharmacol* 2001; **133**: 237–242.
- Buhl SN, Jackson KY. Optimal conditions and comparison of lactate dehydrogenase catalysis of the lactate-to-pyruvate and pyruvate-to-lactate reactions in human serum at 25, 30 and 37 degrees C. *Clin Chem* 1978; **24**: 828–831.
- Rosalki SB. An improved procedure for serum creatine phosphokinase determination. *J Lab Clin Med* 1967; **69**: 696–705.
- Taylor PC. Anti-TNF therapy for rheumatoid arthritis and other inflammatory diseases. *Mol Biotechnol* 2001; **19**: 153–168.
- Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide* 2001; **5**: 62–71.
- Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem* 1978; **86**: 271–278.
- Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med* 1963; **61**: 882–888.
- Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; **70**: 158–169.
- Renlund DG, Macfarlane JL, Christensen RD, Lynch RE, Rothstein G. A quantitative and sensitive method for measurement of myeloperoxidase. *Clin Res* 1980; **28**: 75A.
- Katori M, Tamaki T, Tanaka M, et al. Nitric oxide donor induces up-regulation of stress proteins in cold ischemic rat hearts. *Trans Proceed* 1999; **31**: 1022–1023.
- Teerlink T, Hennekes M, Bussemaker J, Groeneveld J. Simultaneous determination of creatine compounds and adenine nucleotides in myocardial tissue by high-performance liquid chromatography. *Anal Biochem* 1993; **214**: 278–283.
- Rash R. Kidney Na, K-ATPase activity in streptozotocin-diabetic rats. *Scand J Clin Lab Invest* 1986; **46**: 59–62.
- Weidman SW. In *Gradwohl's Clinical Laboratory Methods and Diagnosis*. Sonnenwirth AC, Jarett L (eds.) vol. 1. CV Mosby company, 1980. Missouri, USA. p. 272.
- Tsalev DL, Razboinikova FM. Use of atomic absorption spectrometry for blood and serum analysis. *Vutr Boles* 1978; **17**: 111–114.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RG. Protein measurement with folin reagent. *J Biol Chem* 1951; **193**: 265–272.
- Mehanna A, Vitorino DC, Panis C, Blanco EEA, Pinge-Filho P, Martins-Pinge MC. Cardiovascular and pulmonary effects of NOS inhibition in endotoxemic conscious rats subjected to swimming training. *Life Sci* 2007; **81**: 1301–1308.
- Janssen-Heininger YMW, Poynter ME, Baeuerle PA. Recent advances towards understanding redox mechanisms in the activation of nuclear factor kappa B. *Free Radic Biol Med* 2000; **28**: 1317–1327.
- Corbett JA, McDaniel ML. Does nitric oxide mediate autoimmune destruction of beta-cells? Possible therapeutic interventions in IDDM. *Diabetes* 1992; **41**(8): 897–903.
- Numata M, Suzuki S, Miyazawa N, et al. Inhibition of inducible nitric oxide synthase prevents LPS induced acute lung injury in dogs. *J Immunol* 1998; **160**(6): 3031–3037.
- Tracey WR, Tse J, Carter G. Lipopolysaccharide-induced changes in plasma nitrite and nitrate concentrations in rats and mice: pharmacological evaluation of nitric oxide synthase inhibitors. *J Exp Ther* 1993; **272**(3): 1011–1015.

40. Dambrov M, Kirjanova O, Baumann L, et al. EPR investigation of *in vivo* inhibitory effect of guanidine compounds on nitric oxide production in rat tissues. *J Physiol Pharmacol* 2003; **54**(3): 339–347.
41. Coimbra R, Melbostad H, Hoyt DB. Effects of phosphodiesterase inhibition on the inflammatory response after shock: role of pentoxifylline. *J Trauma* 2004; **56**: 442–449.
42. Sanz MJ, Cortijo J, Morciollo EJ. PDE4 inhibitors as new anti-inflammatory drugs: Effects on cell trafficking and cell adhesion molecules expression. *Pharmacol Ther* 2005; **106**: 269–297.
43. Rahat MA, Lahat N, Smollar J, Brod V, Kinarty A, Bitterman H. Divergent effects of ischemia/reperfusion and nitric oxide donor on TNF alpha mRNA accumulation in rat organs. *Shock* 2001; **15**: 312–317.
44. Klebanoff SJ. Myeloperoxidase: friend and foe. *J Leukoc Biol* 2005; **77**: 598–625.
45. Raeburn CD, Calkins CM, Zimmerman MA, et al. Vascular cell adhesion molecule-1 expression is obligatory for endotoxin induced myocardial neutrophil accumulation and contractile dysfunction. *Surgery* 2001; **130**: 319–325.
46. Madjdpour C, Oertli B, Ziegler U, Bonvini JM, Pasch T, Beck-Schimmer B. Lipopolysaccharide induces functional ICAM-1 expression in rat alveolar epithelial cells in vitro. *Am J Physiol Lung Cell Mol Physiol* 2000; **278**: L572–L579.
47. Abdin AA, Abd El-Hamid MA, Abou El-Seoud SH, Balaha MFH. Effect of pentoxifylline and/or alpha lipoic acid on experimentally induced acute pancreatitis. *Eur J Pharmacol* 2010; **643**: 289–296.
48. Eiserich JP, Baldus S, Brennan ML, et al. Myeloperoxidase, a leukocyte-derived vascular NO oxidase. *Science* 2002; **296**: 2391–2394.
49. Eiserich JP, Hristova M, Cross CE, et al. Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. *Nature* 1998; **391**: 393–397.
50. Hazen SL, Zhang R, Shen Z, et al. Formation of nitric oxide-derived oxidants by myeloperoxidase in monocytes: pathways for monocyte-mediated protein nitration and lipid peroxidation in vivo. *Circ Res* 1999; **85**: 950–958.
51. Skibska B, Jozefowicz-Okonkwo G, Goraca A. Protective effects of early administration of alpha-lipoic acid against lipopolysaccharide-induced plasma lipid peroxidation. *Pharmacol Rep* 2006; **58**: 399–404.
52. Walker MW, Kinter MT, Roberts RJ, Spitz DR. Nitric oxide-induced cytotoxicity: involvement of cellular resistance to oxidative stress and the role of glutathione in protection. *Pediatr Res* 1995; **37**: 41–49.
53. Feihl F, Waeber B, Liaudet L. Is nitric oxide overproduction the target of choice for the management of septic shock? *Pharmacol Therap* 2001; **91**: 179–213.
54. Radi R, Beckman JS, Bush KM. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch Biochem Biophys* 1991; **288**: 481–485.
55. Olas B, Nowak P, Wachowicz B. Resveratrol protects against peroxynitrite-induced thiol oxidation in blood platelets. *Cell Mol Biol Lett* 2004; **9**: 577–587.
56. Nogawa S, Zhang F, Ross ME, Iadecola C. Cyclooxygenase-2- gene expression in neurons contributes to ischemic brain damage. *J Neurosci* 1997; **17**: 2746–2755.
57. Maines MD. The heme oxygenase system: A regulator of second messenger gases. *Annu Rev Pharmacol Toxicol* 1997; **37**: 517–554.
58. Iwasashi H, Suzuki M, Unno M. Inhibition of heme oxygenase ameliorates sepsis-induced liver dysfunction in rats. *Surg Today* 2003; **33**: 30–38.
59. Lee YM, Cheng PY, Hong SF. Oxidative stress induces vascular heme oxygenase-1 expression in ovariectomized rats. *Free Radic Biol Med* 2005; **39**: 108–117.
60. Datta PK, Lianos EA. Nitric oxide induces heme oxygenase-1 gene expression in mesangial cells. *Kidney Int* 1999; **55**: 1734–1739.
61. Srisook K, Cha YN. Super-induction of HO-1 in macrophages stimulated with lipopolysaccharide by prior depletion of glutathione decreases iNOS expression and NO production. *Nitric Oxide* 2005; **12**: 70–79.
62. Srisook K, Kim C, Cha YN. Role of NO in enhancing the expression of HO-1 in LPS-stimulated macrophages. *Methods Enzymol* 2005; **396**: 368–377.
63. Song Y, Shi Y, Ao LH, Harken AH, Meng XZ. TLR4 mediates LPS-induced HO-1 expression in mouse liver: role of TNF- alpha and IL-1 beta. *World J Gastroenterol* 2003; **9**: 1799–1803.
64. Volonte MG, Yuln G, Quiroga P, Consolini AE. Development of an HPLC method for determination of metabolic compounds in myocardial tissue. *J Pharm Biochem Anal* 2004; **35**: 647–653.
65. Arthur PG, Hogan MC, Bebout DE, Wagner PD, Hochachka PW. Modeling the effects of hypoxia on ATP turnover in exercising muscle. *J Appl Physiol* 1992; **73**: 737–742.
66. Milatovic D, Gupta RC, Dettbarn WD. Involvement of nitric oxide in kainic acid-induced excitotoxicity in rat brain. *Brain Res* 2002; **957**: 330–337.
67. Szabo C, Dawson VL. Role of poly (ADP-ribose) synthetase in inflammation and ischaemia-reperfusion. *Trends Pharmacol Sci* 1998; **19**: 287–298.
68. Szabo C, Ohshima H. DNA damage induced by peroxynitrite: subsequent biological effects. *Nitric Oxide* 1997; **1**: 373–385.
69. Seven I, Turkozhan N, Cimen B. The effects of nitric oxide synthesis on the Na<sup>+</sup>, K<sup>+</sup>-ATPase activity in guinea pig exposed to lipopolysaccharides. *Mol Cell Biochem* 2005; **271**(1–2): 107–112.
70. Porsche E, Stefanovich V. The effect of pentoxifylline on the Ca<sup>2+</sup>-induced potassium efflux and on the ATPase-activity of erythrocytes. *Arzneimittelforschung* 1981; **31**(5): 825–828.
71. Levitsky DO, Levchenko TS, Saks VA, Sharov VG, Smirnov VN. The role of creatine phosphokinase in supplying energy for the calcium pump system of the heart sarcoplasmic reticulum. *Membr Biochem* 1978; **2**: 81–9.
72. Sudharsan PT, Mythili Y, Selvakumar E, Varalakshmi P. Lupeol and its ester inhibit alteration of myocardial permeability in cyclophosphamide administered rats. *Mol Cell Biochem* 2006; **292**(1–2): 39–44.
73. Viner RI, Hamer AF, Bigelow DJ, Schoneich C. The oxidative inactivation of sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase by peroxynitrite. *Free Rad Res* 1996; **24**: 243–259.
74. Hantler PD, Gratzler WB. Effects of specific chemical modification of actin. *Eur J Biochem* 1975; **60**: 67–72.
75. Wu CC, Chen SJ, Yen MH. Nitric oxide-independent activation of soluble guanylyl cyclase contributes to endotoxin shock in rats. *Am J Physiol* 1998; **275**: H1148–H1157.