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# Effects of adenosine N1-Oxide and pioglitazone on inflammatory and antioxidant state in sepsis caused by experimental cecal puncture in rat

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## Abstract

Sepsis is an acute condition caused by the systemic inflammatory response syndrome to an infection. There are very few drugs that could improve the severe conditions in patients with sepsis. Hence, it is important to consider different treatment options. In this survey, we studied the effect of adenosine N1-oxide (ANO) and pioglitazone on rats with cecal ligation and perforation (CLP). They were randomly divided to four groups (n = 10) including Group A: as control group receiving normal saline, Group B: the rats with CLP as surgical control group, Group C: the rats receiving ANO, and Group D: the rats receiving pioglitazone. Interleukin (IL) -6, IL-1β, tumor necrosis factor alpha (TNF-α), nitric-oxide (NO) in serum blood and superoxide dismutase (SOD), catalase (CAT) malondialdehyde, (MDA) and myeloperoxidase (MPO) in liver and spleen homogenates were measured. The amount of antioxidant enzymes in the spleen and liver, and finally cell viability and rats' survival were investigated. The measurement of blood serum nitric-oxide and survival of all groups of rats were also performed. The results indicated that both drugs could cause a decrease in IL-1β, IL-6, TNF-α and NO in rat blood serum and MDA and MPO in the liver and spleen homogenates, however, a significant increase in SOD and CAT in the liver and spleen homogenates in rats that received ANO and pioglitazone was observed compared to rats with CLP group. Cell viability and rats' survival were significantly improved in rats that received ANO and pioglitazone compared to rats with CLP group. Adenosine N1-oxide showed stronger and more effective effects.



## Introduction

Sepsis is an acute condition caused by the systemic inflammatory response syndrome (SIRS) to an infection that is exacerbated by organ dysfunction.[1] In the presence of products of bacterial origin in the bloodstream and at the beginning of the immune system's response to infection, the innate immune system produces and releases several pro-inflammatory cytokines, such as interleukin (IL)-1, tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and IL-6. Sometimes various factors such as a high burden of infection, super antigens presence, bacterial resistance against opsonization and phagocytosis, and antibiotic resistance do not allow the host to prevent infection, resulting in increased synthesis and release of secondary mediators and other pro-inflammatory cytokines by mesenchymal cells and macrophages as the immune system respond to infection and produces more pro-inflammatory cytokines, leading to the absorption of inflammatory cells such as macrophages and neutrophils, and production of nitric oxide (NO). Such a condition eventually causes a cytokine storm that eventually leads to sepsis.[2,3]

In addition to the apparent production of cytokines, several molecular inflammatory mechanisms and cellular damage are involved in the pathogenesis of sepsis and septic shock including the production of eicosanoids and reactive oxygen species (ROS)[4] that are mainly produced by phagocytes during phagocytosis and could lead to the damage of the endothelial cell, formation of chemotactic factors, utilization of neutrophils, DNA damage, the liberation of IL-1β, and TNF-α, and the formation of peroxynitrite.[5,6]

Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) are proteins that are involved in the catalytic modification of reactive oxygen species and their by-products to stable non-toxic molecules. Hence, they are the most important defense mechanism against

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cell damage caused by oxidative stress and it has been found that antioxidant protection is decreased under sepsis condition that increases the ROS production.[7]

Malondialdehyde (MDA) is a final product created during oxidative stress that is formed especially during lipid peroxidation[8] and a high level of MDA occurs in people who have lost their lives due to sepsis compared to the remaining patients.[9] During the activation of neutrophils, superoxide ($O^{-2}$) is formed by membrane-associated oxidase nicotinamide adenine dinucleotide phosphate (NADPH) complex and subsequently[10] produces hydrogen peroxide, and these oxygen-reactive species are used by myeloperoxidase (MPO) to produce a strong oxidant of hypochlorous acid, thereby, increasing the antimicrobial ability of neutrophils.[10] However, hypochlorous acid and other oxidants produced by MPO of active neutrophils react with host tissue and can increase the inflammatory response by causing cell damage.[11,12]

Pioglitazone is a peroxisome proliferator-activated receptor-gamma (PPARγ) that is involved in the regulation of inflammation caused by sepsis. The treatment with PPARγ activator reduces inflammation and improves survival in laboratory animals.[13] On the other hand, adenosine N1-oxide (ANO) is an oxidized product of adenosine in position N1 from the basic part of adenine and it has been reported that it inhibits the secretion of inflammatory mediators by active macrophages, reduces mortality from septic shock and suppresses the production of pro-inflammatory cytokines due to lipopolysaccharide.[14]

Currently, sepsis treatment includes cardiac resuscitation and infection immediate risk management. Vasopressor medications, intravenous fluid therapy and oxygen therapy are the main resuscitation options. The only currently usable immunotherapeutic treatment is the hydrocortisone short-term use in septic shock patients. However, previous studies showed that high doses of corticosteroids were harmful and devoid of beneficial helpful effects and many of these treatments did not improve survival rate in patients with septic shock.[9,11] Therefore, the present study was aimed to investigate the effects of adenosine in preventing the harmful effects of septic shock. The pioglitazone drug was chosen as the approved drug with positive effects on septic shock to compare the effects of the two drugs side by side. Therefore, in this study, we investigated the effect of these two drugs on serum blood inflammatory cytokines, antioxidant enzymes in the spleen and liver, blood serum nitric oxide, and survival of all groups of rats.

## Materials and Methods

**Animal.** In the study, 40 Sprague-Dawley rats weighing approximately 250 g were purchased from the Faculty of Veterinary Medicine Laboratory Animal Breeding Center, University of Tabriz, and randomly divided them into four groups (n = 10), including Group A: as control group receiving normal saline, Group B: The rats with cecal ligation and perforation (CLP) as surgical control group, Group C: The rats receiving the adenosine N1-oxide, and Group D: The rats receiving pioglitazone. Each group was kept in a separate cage and clean environment with a constant temperature of 25.00 ˚C and a 12 hr cycle of light and dark (L:D), and free access to adequate water and food. All experiments were based on the ARRIVE guidelines (https All://arriveguidelines.org), approved by the University of Tabriz Animal Ethics Committee (Approval ID: IR.TABRIZU.REC.1398.008), and conducted in accordance with the relevant guidelines and regulations.

**Experimental sepsis and treatment.** General anesthesia was performed with intra-peritoneal injection of 80.00 mg $kg^{-1}$ ketamine (Alfasan, Woerden, The Netherlands) and 10.00 mg $kg^{-1}$ xylazine (Alfasan) in rats. Celiotomy was performed. For cecal ligation and perforation the cecum was identified and exteriorized from the peritoneal cavity. Then, a simple ligature was placed below the ileocecal valve using 3-0 silk (Supa, Tehran, Iran) suture material. The cecum was punctured twice by an 18-G needle. Slight pressure was applied to the perforated cecum to ensure leakage of its contents. The cecum was returned into the peritoneal cavity and the abdominal wall and skin were sutured.[15] Finally, the animals were returned to the cage and regained their consciousness in a warm environment with suitable conditions. After regaining consciousness, water and food were fully provided for the animals. Pioglitazone (Sigma Aldrich, St. Louis, USA). Composition of 1:1 dimethyl-sulfoxide (DMSO; Merck, Darmstadt, Germany):Phosphate-buffered saline (PBS; Merck) at dose of 20.00 mg $kg^{-1}$,[13] and adenosine N1-oxide (Aobious, Shanghai, China) were dissolved in 0.50% DMSO at dose of 18.00 mg $kg^{-1}$ and injected intraperitoneally four times a day to the treated group for 3 days saline.[16] Animals were closely checked for general condition. The survived ones were weighed 3 days after CLP and then approximately 1.50 mL of blood sample was taken from their heart and finally, they were euthanized under deep general anesthesia via cervical dislocation. The livers and spleens of rats were removed 3 days later or until the animals' death. The blood samples were also taken from the rats immediately after death.

**Cell viability assay.** After 3 days, the animals were euthanized and the peritoneal macrophages were acquired by injecting and repeated flushing of ice-cold sterile PBS, inside the peritoneal cavity. The spleens were excised and lightly crushed. The suspension was centrifuged at 2,500 rpm for 5 min. Red blood cells were lysed using ammonium-chloride-potassium (Merck) buffer. The cells were then washed and suspended in the RPMI-1640 medium (Gibco, New York, USA) with 10.00% fetal

bovine serum (FBS; Gibco). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Merck) staining was used for calculating the cell viability. The formazan crystals formed in living cells were dissolved using DMSO, then the absorbance was measured at 570 nm (model MPR4 plus; Hiperion, Neuss, Germany).[14] Cell viability was calculated using the following equation:

*Cell viability (%) = (OD test – OD control)/(OD control) × 100*

**Measurement of nitric oxide.** Nitric oxide level was estimated by reagent Greiss (Merck) using the protocol originally described by Grace.[17]

**Measurement of cytokines by enzyme-linked immunosorbent assay (ELISA).** We analyzed the serum samples collected from rats to determine levels of pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6) according to Sandwich ELISA and kit manufacturer's instructions (Bender Med Co., Vienna, Austria). The reaction stopped by adding sulfuric acid 2.00N and absorbance was measured at 450 nm (Hiperion).

**Measurement of MPO.** The MPO is a marker of leukocyte migration and accumulation. Liver and spleen tissue samples were homogenized in ice-cold potassium phosphate buffer (50.00 mM, pH 6.00) containing 0.50% hexadecyltrimethylammonium bromide (Merck). The resulting homogenate was frozen and thawed three times followed by centrifugation for 25 min at 3,000 *g* at 4.00 ˚C. The MPO activity was measured in the sample using the O-dianisidine method. The absorbance was measured at 460 nm. The enzyme activity data was indicated in U $mg^{-1}$ of protein.[16]

**Measurement of lipid peroxidation.** Liver and spleen MDA levels was measured as a marker of lipid per-oxidation.[18] In short, each supernatant (500 µL) was mixed with a reaction mixture containing 0.67% thiobarbituric acid (Merck), 0.22% sulfuric acid (Merck) and distilled water. The mixture was placed in a boiling water bath at 95.00 ˚C for 30 min and cooled to room temperature. The samples were then centrifuged at 1,000 *g* for 15 min and the supernatant absorbance was measured at 540 nm (Hiperion). Data were reported in nmol $mg^{-1}$ of protein.

**Antioxidant enzymes estimation.** The SOD activity was determined in homogenates of liver and spleen by checking the enzymatic ability to inhibit nitroblue tetrazolium dye (Merck) reduction through phenazine methosulfate (Merck).[19] An increase in absorbance was recorded at 560 nm for 5 min. Enzyme activity data were expressed as a U $mg^{-1}$of protein. Catalase activity was measured (Hiperion) by determining the rate of $H_2O_2$ decomposition at 240 nm.[20] The absorbance decrease was checked for 180 sec and the enzyme activity was defined as µM of $H_2O_2$ decomposed $mg^{-1}$ of protein per sec.

**Statistical analysis.** Minitab software (version 16.2.0; Minitab Inc., Boston, USA) was used for statistical analysis. Data were evaluated by one-way analysis of variance (ANOVA) and Tukey's post hoc test. Chi-Square method was used to analyze Survival. The results were presented as percent (%) for survival rate and mean ± standard error of mean (SEM) for other data. $p < 0.05$ was considered as statistical significance.

## Results

**Evaluation of cytokine and nitric oxide levels.** The blood serum samples indicated that cytokines IL-1β, IL-6 and TNF-α levels were significantly decreased in rats that received ANO compared to rats with CLP (surgical control), ($p < 0.05$) and they were also significantly decreased in rats receiving pioglitazone compared to the affected group ($p < 0.05$, Fig. 1A). Blood serum sample indicated that the amount of nitric oxide in rats, which received ANO, was significantly decreased compared to rats with CLP (surgical control) ($p < 0.05$), and there was a significant reduction in rats receiving pioglitazone compared to the affected group ($p < 0.05$, Fig. 1B).

**Evaluation of oxidative stress and antioxidant enzymes levels in the liver and spleen.** The results indicated that the levels of SOD, CAT in liver and spleen were significantly increased in rats that received ANO compared to rats with CLP (surgical control), ($p < 0.05$),

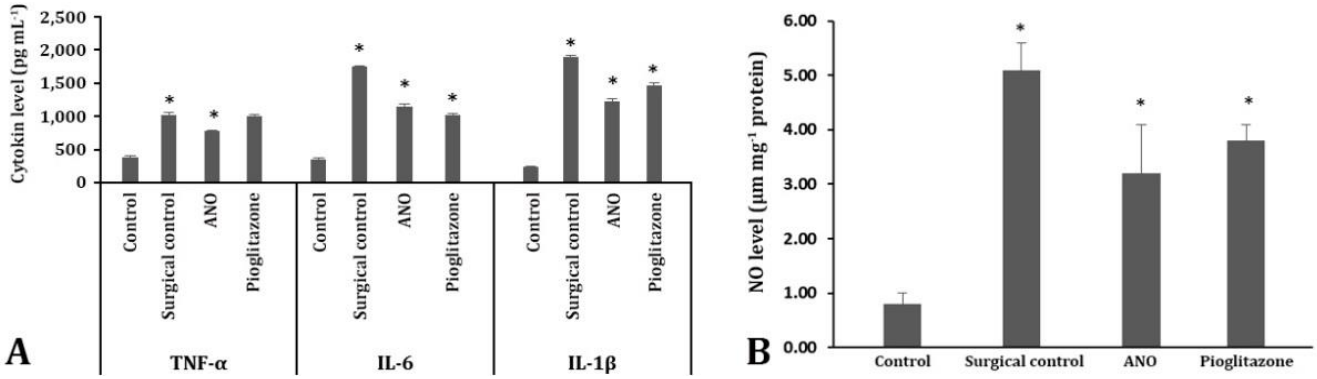


**Fig. 1.** Cytokines and nitric oxide levels changes in serum of rats that received ANO and pioglitazone. * indicates significant differences in the level of $p < 0.05$ between treated and non-treated groups.

however, the increase was not significant for spleen enzymes. There was a significant increase in rats receiving pioglitazone compared to the affected group ($p < 0.05$, Figs. 2A and 2B). However, the results indicated that levels of MPO in the liver and spleen of rats that received ANO were significantly decreased compared to rats with CLP (surgical control), ($p < 0.05$). There was a significant decrease in rats receiving pioglitazone compared to the affected group ($p < 0.05$), however, in the MDA for the both drugs, the reduction was significant only in the spleen ($p < 0.05$, Figs. 2C and 2D).

**Effect of ANO and pioglitazone on cell viability.** Statistical analysis of data did not show any cytotoxic effect on cell viability (Table 1).

**Viability of rats.** None of the rats of the control and pioglitazone groups (0.00%) died during 3-days period. In this study, 10.00% of the surgical control group died one day after surgery and 50.00% died after two days, so only 40.00% were survived after 3 days in the group. The remaining rats were secluded and lethargic. Only 10.00% of the ANO group died on day 2 post-surgery and 80.00% of them survived. The survivors were alert and ambulatory. There was not any significant difference between the control and pioglitazone and ANO groups, however, there was a significant difference between the pioglitazone group with the control surgery group, and also a significant difference between control surgery and ANO groups ($p = 0.04$), (Fig. 3).

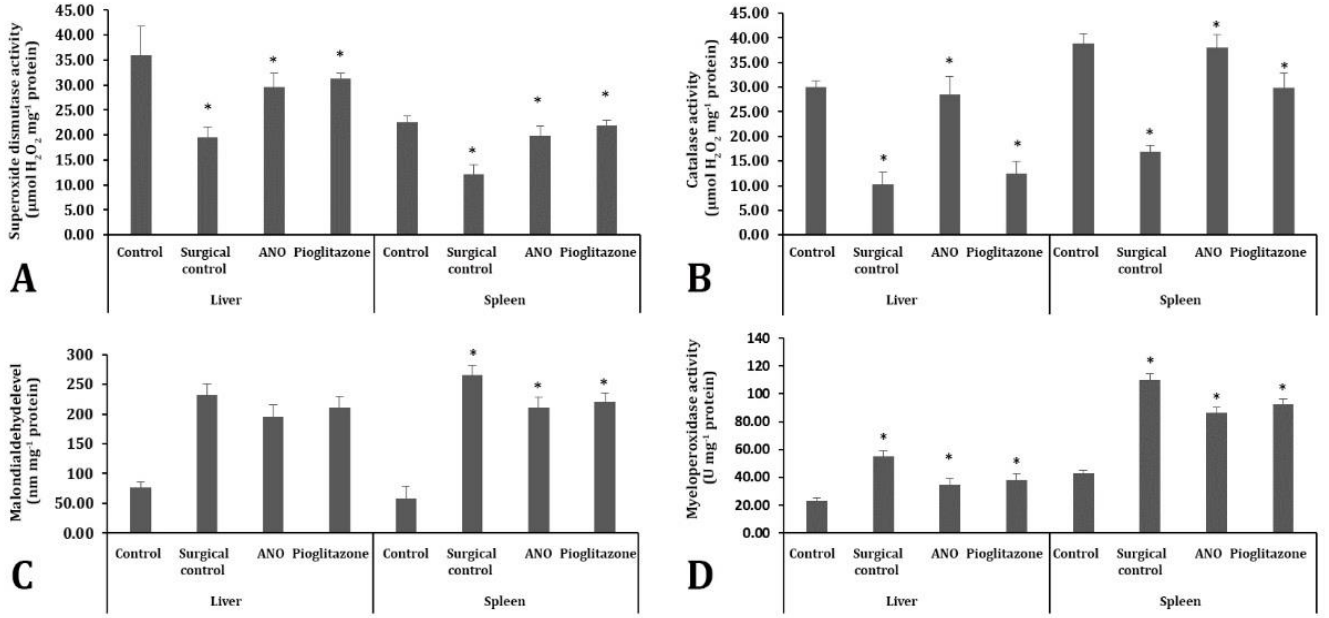


**Fig. 2.** Changes in enzymes levels of liver and spleen in rats receiving ANO and pioglitazone.
* indicates significant differences in the level of $p < 0.05$ between treated and non-treated groups.

**Table 1.** Cell viability test results after drug treatment with adenosine N1 oxide and pioglitazone in peritoneal macrophages and cultured splenocytes. Data are presented as mean ± SEM. The data were normalized (= 100%) to the untreated cells.

| Source | Cell viability (%) | |
|---|---|---|
| | Control | drug (*ex vivo*) |
| ***Peritoneal macrophage*** | | |
| **Control** | 82.00 ± 0.03 | 85.00 ± 0.00 |
| **Surgical control** | 148.00 ± 0.04 | 93.00 ± 0.08 |
| **Adenosine N1 oxide** | 109.00 ± 0.00 | 99.00 ± 0.00 |
| **Pioglitazone** | 127.00 ± 0.03 | 106.00 ± 0.02 |
| ***Splenocytes*** | | |
| **Control** | 98.00 ± 0.05 | 96.00 ± 0.00 |
| **Surgical control** | 198.00 ± 0.22 | 101.00 ± 0.03 |
| **Adenosine N1 oxide** | 113.00 ± 0.11 | 116.00 ± 0.32 |
| **Pioglitazone** | 153.00 ± 0.00 | 161.00 ± 0.00 |

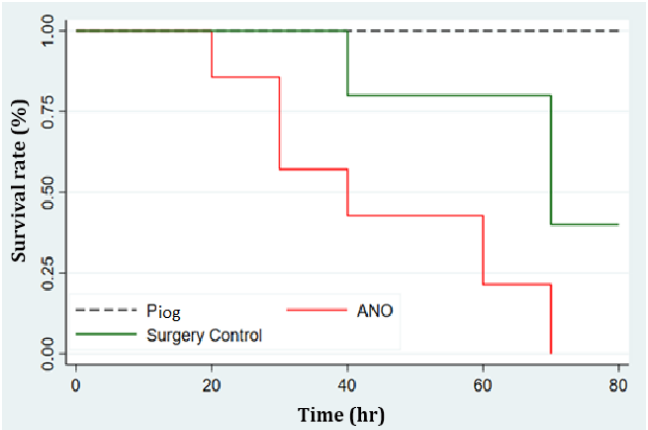


**Fig. 3.** Rat viability chart that shows the rats viability of the Surgery control, adenosine N1-Oxide, and pioglitazone groups (0.00%) during 3-days period.

## Discussion

For the first time, the present study examined the effect of pioglitazoneadenosine N1-oxide (ANO) on sepsis caused by an experimental cecal puncture in rat. It is worth noting that there were not any comprehensive work on the effects of ANO on septic shock and inflammatory diseases. Various reports have presented the roles of pro-inflammatory cytokines in the pathogenesis of sepsis so that the production and release of inflammatory cytokines such as TNF-α and IL-1 to the systemic circulation would occur due to the entry of bacterial endotoxin[21,22] and the two cytokines cause the secretion and production of more pro-inflammatory cytokines including IL-6, IL-8, and macrophage migration inhibitory factor (MIF), lipid mediators, and reactive species of oxygen and nitrogen, which ultimately lead to organ dysfunction during sepsis by activating macrophages and creating inflammatory cascades.[23,24]

Ohashi *et al*. reported that ANO inhibited inflammatory mediators secreted by active macrophages and reduced mortality from endotoxemic shock, and also suppressed the production of pro-inflammatory cytokines including IL-1, IL-6, and TNF-α, and their results were consistent with the results of the present study.[14] Consistent with the results of the present study, Kohno *et al*. found that ANO had an anti-inflammatory effect *in vitro* and *in vivo*, and adenosine N1-oxide significantly decreased inflammatory cytokines such as TNF-α and IL-6 and inhibited the secretion of IL-6 by peritoneal macrophages stimulated by toll like receptors (TLRs) agonists.[16] Even though ANO and adenosine are structurally similar, the capacity of ANO is much stronger than adenosine to inhibit the production of pro-inflammatory cytokines, and ANO has more superiority than adenosine in anti-inflammatory functions, however, ANO more effectively inhibits inflammatory cytokines than adenosine under the same stimulatory conditions. On the contrary to adenosine, the ANO anti-inflammatory effect is not limited to a specific anti-inflammatory cytokine or to a condition in which a particular pathogen is involved. ANO significantly reduces septic shock mortality,[25] however, adenosine fails to protect rats against endotoxin-induced mortality possibly due to the rapid metabolism of adenosine in the body. In confirmation of the results of the present study, it was found that oral administration of ANO before and after injection of lipopolysaccharide reduced mortality of rats. It could be concluded that ANO through oral administration could systematically exert its anti-inflammatory effects and the resistance of ANO against adenosine deaminase that may explain the strong anti-inflammatory activity of ANO compared to adenosine and other drugs.[26]

About pioglitazone and its role as an activator of PPARγ, the data of previous studies indicated that endogenous ligands of PPARγ had anti-inflammatory effects on activation of nuclear factor of kappa binding (NF-κB). Furthermore, PPAR-γ inhibits the expression of anti-inflammatory genes such as inducible nitric oxide synthase (iNOS), TNF-α or IL-1β by inhibiting NF-κB activity. In animal models of sepsis and many other inflammatory diseases, treatment with ligands and agonists of PPARγ reduced the penetration of neutrophils and many inflammatory mediators all of which indicated the anti-inflammatory properties of the drug.[27] The results of other study indicated that treatment with pioglitazone reduced pro-inflammatory cytokines and exerted its anti-inflammatory effects by reducing plasma adiponectin levels.[13] Consistent with the results of the present study, Gao *et al*. examined the protective effect of pioglitazone on sepsis induced by CLP in the rodents model and they found that CLP caused a significant increase in expression of TNF-α and IL-6 in the intestines of the rats, and the pioglitazone pretreatment reduced local production of TNF-α and IL-6.[28] Shafaroodi *et al*. found that administration of pioglitazone positively affected the improvement of survival in a model of rat sepsis with CLP and rats with CLP-induced sepsis under the treatment with pioglitazone showed a significant reduction in serum levels of TNF-α, 12 and 24 hr after induction of sepsis by CLP and also a significant reduction in the concentration of serum IL-1β in pioglitazone-treated groups. The results were consistent with the findings of the present study.[27]

Shafaroodi *et al*. found that injection of N-Nitroarginine methyl ester (L-NAME) as a selective inhibitor of iNOS improved survival in pioglitazone-treated animal groups and reduced serum levels of pro-inflammatory cytokines.[27] It is suggested that the mechanism of increasing survival by pioglitazone is possibly by inhibiting nitric oxide release through iNOS and reducing the production of pro-inflammatory cytokines. Furthermore, pioglitazone suppresses the expression of iNOS, TNF-α, Gelatinase B, and induction of cyclooxygenase-2 in active macrophages[29] and reduces the hyper-inflammatory response and inhibits the sepsis development during sepsis activation by activating PPAR-γ.[30] The role of oxidative stress has been widely reported in the pathogenesis of this process and the overproduction of reactive oxygen is highly toxic to the host tissue, and their interactions with a variety of biological macromolecules could lead to severe pathophysiological consequences that eventually lead to the organ dysfunction syndrome in sepsis.[31] Consistent with the results of the present study, Kumar *et al*. found that plasma oxidant levels in sepsis patients were higher than the control groups and various factors such as higher activity of NADPH oxidase in response to inflammation and increased activity of MPO in plasma during sepsis compared to the control group could play a role in increasing levels of oxidants in sepsis.[32]

Furthermore, high activity of MPO in sepsis was due to the penetration of neutrophils in the site of inflammation under the influence of secretion of pro-inflammatory cytokines. MPO is a marker of neutrophil uptake to assess intestinal damage caused by CLP, and it could cause significant damage to inflamed tissues indirectly by absorbing neutrophils in the infected area despite its important role in causing host defense reactions.[33] Gao *et al*. conducted a study consistent with the results of the present study and found that pretreatment with pioglitazone significantly increased the level of MPO and reduced the intestinal infiltration of neutrophils into sepsis.[34] In another study, Haraguchi *et al*. found that pioglitazone improved the survival of rats after the onset of septic shock by suppressing inflammatory responses, and oral administration of pioglitazone for five days increased the survival rate in CLP-operated rats.[35] Kumar *et al*. found that the activity of plasma SOD had a significant reduction in rats with sepsis compared to the control group and their results were consistent with the findings of the present study. The reduction in SOD indicated that the organs were not protected from oxidative damage of $O_2$ during sepsis.[32] In this regard, CAT was another important antioxidant enzyme that led to the destruction of $H_2O_2$, and thus protected tissues and cells from toxicity mediated by $H_2O_2$, and a reduction process similar to SOD was seen for CAT in patients with sepsis that was similar to the results of the present study.

In confirmation of our data, Gao *et al*. indicated that sepsis induced by CLP caused a significant increase in the level of MDA, while the activity of SOD was greatly decreased. Pioglitazone pretreatment regulated the SOD activity and led to a decrease in the level of MDA. Therefore, it is quite clear that PPARγ agonists bear anti-inflammatory and antioxidant effects.[28]

It could be concluded that both ANO and pioglitazone were effective in treating sepsis, however, ANO had stronger and more effective effects according to the results of the present study. It is necessary to prove this by examining the extensive effects of this drug in terms of immunopathology in all vital organs and comparing these effects with routine drugs used in the treatment of sepsis.

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## Conflict of interest

The authors declare no conflict of interest.

## References

1. Chaudhry H, Zhou J, Zhong Y, et al. Role of cytokines as a double-edged sword in sepsis. In Vivo 2013; 27(6): 669-684.
2. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. Semin Immunopathol 2017; 39(5): 517-528.
3. Janeway CA Jr, Travers P, Walport M, et al. Induced innate responses to infection. New York, USA: CRC Press 2001; 77-137.
4. Salvemini D, Cuzzocrea S. Oxidative stress in septic shock and disseminated intravascular coagulation. Free Radic Biol Med 2002; 33(9): 1173-1185.
5. Zimmerman JJ. Defining the role of oxyradicals in the pathogenesis of sepsis. Crit Care Med 1995; 23(4): 616-617.
6. Víctor VM, Espulgues JV, Hernández-Mijares A, et al. Oxidative stress and mitochondrial dysfunction in sepsis: a potential therapy with mitochondria-targeted antioxidants. Infect Disord Drug Targets 2009; 9(4): 376-389.
7. Ritter C, Andrades M, Frota Júnior ML, et al. Oxidative parameters and mortality in sepsis induced by cecal ligation and perforation. Intensive Care Med 2003; 29(10): 1782-1789.
8. Dalle-Donne I, Rossi R, Colombo R, et al. Biomarkers of oxidative damage in human disease. Clin Chem 2006; 52(4): 601-623.
9. Lorente L, Martín MM, Abreu-González P, et al. Prognostic value of malondialdehyde serum levels in severe sepsis: a multicenter study. PLoS One 2013; 8(1): e53741. doi: 10.1371/journal.pone.0053741.
10. Winterbourn CC, Kettle AJ, Hampton MB. Reactive oxygen species and neutrophil function. Annu Rev Biochem 2016; 85: 765-792.
11. Weiss SJ. Tissue destruction by neutrophils. N Engl J Med 1989; 320(6):365-376.
12. Gaweł S, Wardas M, Niedworok E, et al. Malondialdehyde (MDA) as a lipid peroxidation marker [Polish]. Wiad Lek 2004; 57(9-10): 453-455.
13. Kaplan J, Nowell M, Chima R, et al. Pioglitazone reduces inflammation through inhibition of NF-κB in poly-microbial sepsis. Innate Immun 2014; 20(5): 519-528.
14. Ohashi E, Kohno K, Arai N, et al. Adenosine N1-oxide exerts anti-inflammatory effects through the PI3K/Akt/GSK-3β signaling pathway and promotes osteogenic and adipocyte differentiation. Biol Pharm Bull 2019; 42(6): 968-976.
15. Hubbard WJ, Choudhry M, Schwacha MG, et al. Cecal ligation and puncture. Shock 2005; 24(Suppl 1): 52-57.
16. Kohno K, Ohashi E, Sano O, et al. Anti-inflammatory effects of adenosine N1-oxide. J Inflamm (Lond) 2015;12(1): 2. doi: 10.1186/s12950-014-0045-0.

17. Griess P. Remarks on the treatise of HH. Weselsky and Benedikt "On some azo compounds" [German]. Angew Chem Int Ed 1879; 12(1), 426-428.
18. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95(2): 351-358.
19. Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 1972; 46(2): 849-854.
20. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105: 121-126.
21. Cannon JG, Tompkins RG, Gelfand JA, et al. Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. J Infect Dis 1990; 161(1): 79-84.
22. Frimpong A, Owusu EDA, Amponsah JA, et al. Cytokines as potential biomarkers for differential diagnosis of sepsis and other non-septic disease conditions. Front Cell Infect Microbiol 2022; 12: 901433. doi: 10.3389/fcimb.2022.901433.
23. Cohen J. The immunopathogenesis of sepsis. Nature 2002; 420(6917): 885-891.
24. Schulte W, Bernhagen J, Bucala R. Cytokines in sepsis: potent immunoregulators and potential therapeutic targets - - an updated view. Mediators Inflamm 2013; 2013: 165974. doi: 10.1155/2013/165974.
25. Parmely MJ, Zhou WW, Edwards CK 3rd, et al. Adenosine and a related carbocyclic nucleoside analogue selectively inhibit tumor necrosis factor-alpha production and protect mice against endotoxin challenge. J Immunol 1993; 151(1): 389-396.
26. Zingarelli B, Cook JA. Peroxisome proliferator-activated receptor-gamma is a new therapeutic target in sepsis and inflammation. Shock 2005; 23(5): 393-399.
27. Shafaroodi H, Hassanipour M, Mousavi Z, et al. The effects of sub-chronic treatment with pioglitazone on the septic mice mortality in the model of cecal ligation and puncture: involvement of nitric oxide pathway. Acta Med Iran 2015; 53(10): 608-616.
28. Gao BT, Lee RP, Jiang Y, et al. Pioglitazone alters monocyte populations and stimulates recent thymic emigrants in the BBDZR/Wor type 2 diabetes rat model. Diabetol Metab Syndr 2015; 7: 72. doi: 10.1186/s13098-015-0068-6.
29. Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature 1998; 391(6662): 82-86.
30. Feihl F, Waeber B, Liaudet L. Is nitric oxide overproduction the target of choice for the management of septic shock? Pharmacol Ther 2001; 91(3): 179-213.
31. Sakaguchi S, Furusawa S. Oxidative stress and septic shock: metabolic aspects of oxygen-derived free radicals generated in the liver during endotoxemia. FEMS Immunol Med Microbiol 2006; 47(2): 167-177.
32. Kumar S, Gupta E, Kaushik S, et al. Evaluation of oxidative stress and antioxidant status: correlation with the severity of sepsis. Scand J Immunol 2018; 87(4): e12653. doi: 10.1111/sji.12653.
33. Grommes J, Vijayan S, Drechsler M, et al. Simvastatin reduces endotoxin-induced acute lung injury by decreasing neutrophil recruitment and radical formation. PLoS One 2012; 7(6): e38917. doi: 10.1371/journal.pone.0038917.
34. Gao M, Jiang Y, Xiao X, et al. Protective effect of pioglitazone on sepsis-induced intestinal injury in a rodent model. J Surg Res 2015; 195(2): 550-558.
35. Haraguchi G, Kosuge H, Maejima Y, et al. Pioglitazone reduces systematic inflammation and improves mortality in apolipoprotein E knockout mice with sepsis. Intensive Care Med 2008; 34(7): 1304-1312.