

REPORT

TREATMENT OF *NIGELLA SATIVA* IN EXPERIMENTAL SEPSIS MODEL IN RATS

**OZLEM ALICI¹, HAVVA SAHIN KAVAKLI^{2*}, CEMILE KOCA³
AND NERIMAN DEFNE ALTINTAS⁴**

¹*Department of Infectious Diseases and Clinical Bacteriology, Medical Park Hospital, Istanbul, Turkey*

²*Department of Emergency Medicine, Ankara Atatürk Training and Research Hospital, Ankara, Turkey*

³*Department of Biochemistry, Faculty of Medicine, Fatih University Ankara, Turkey*

⁴*Department of Medical Intensive Care, Ankara Atatürk Training and Research Hospital, Ankara, Turkey*

ABSTRACT

This experimental study was designed to determine effects of *Nigella sativa* oil (NSO) on endothelin-1 (ET-1) level and oxidative stress parameters, superoxide dismutase (SOD) and malondialdehyde (MDA) in a rat sepsis model. Twenty four adult Wistar albino rats were divided randomly into three groups: sham group (group 1), sepsis group (group 2), sepsis group pretreated with NSO (group 3). Serum ET-1, tissue SOD and tissue MDA levels were measured in all groups. Compared to group 1, ET-1 and MDA levels were higher in group 2. ET-1 and MDA levels in NSO pretreated group 3 were lower with respect to group 2 ($p < 0.03$, and $p < 0.02$, respectively). Additionally, SOD levels in group 3 were found to be higher than group 2 ($p < 0.02$). Based on our results, it can be concluded that NSO may have a positive impact on ET-1 levels and oxidative stress induced by sepsis in experimental rat models.

Keywords: Sepsis, *Nigella sativa* oil, endothelin, superoxide dismutase, malondialdehyde.

INTRODUCTION

Sepsis, is an inflammatory process in response to an infectious stimulus, and it is the number one cause of mortality in the intensive care units other than coronary units (Dare *et al.*, 2009, Ribeiro *et al.*, 2009). It is characterized by systemic inflammation and multi-organ dysfunction which may eventually lead to death (Dare *et al.*, 2009). Despite advances in the understanding of its pathophysiology, there are limited pharmacotherapeutic options for sepsis, septic shock, and related complications (Gupta *et al.*, 2005). Several researchers have linked reactive oxygen species (ROS) with the onset, progression and outcome of sepsis, both in experimental and in clinical studies (Guo and Ward, 2007; Zapelini *et al.*, 2008). ROS are important signaling molecules but their overproduction results in detrimental oxidative stress and must be avoided by the organism (Andrades *et al.*, 2009b).

Endothelins (ET) are a family of powerful vasoconstrictor peptides that have numerous roles. ET-1 is the principal isoform produced by the endothelium and it acts locally to maintain the basal vascular tone. Its levels increase in a variety of clinical conditions involving the endothelium, including sepsis which has marked endothelial dysfunction (Piechota *et al.*, 2007; Shah, 2007).

Nigella sativa oil (NSO) is an agent that has been shown to have antioxidant and antiinflammatory activities in

different clinical conditions (Coban *et al.*, 2010; Hamdy and Taha, 2009; Yildiz *et al.*, 2010). In this study, we aimed to evaluate the effects of NSO on ET-1 levels and oxidative stress in a rat sepsis model.

MATERIALS AND METHODS

Study design

The study was approved by the Animal Research Ethics Committee of Fatih University, School of Medicine before 24 adult male Wistar rats (weight range, 250 to 270 g) were used for the experiment. All animals had access to food and water *ad libitum* throughout the study. The rats were divided randomly into three groups of eight animals each: control group (group 1), sepsis group (group 2), and sepsis induced group pretreated with NSO (group 3). Control group (group 1) received only 1 mL intraperitoneal (i.p.) injection of saline.

Sepsis in group 2 and group 3 were induced by intraperitoneal (i.p.) injection of 2×10^6 CFU of *Escherichia coli* ATCC 25922 which was grown in brain-heart infusion broth. In the logarithmic phase of the growth, the suspension was centrifuged at 1000 g for 15 minutes, the supernatant was discarded, and the bacteria were resuspended and diluted in sterile saline. The rats received an i.p. inoculum of 1 mL of saline containing 2×10^{10} CFU of *E. coli* ATCC 25922 (Ghiselli *et al.*, 2004). Rats in group 3 received NSO 50 g/kg orally 30 minutes before the bacterial challenge, whereas rats in group 2 received only saline.

*Correspondence author: e-mail: havvasahin1973@yahoo.com

Sample collection

24 hour after bacterial challenge, all animals were sacrificed using ketamine and cardiac puncture. Blood samples were drawn from vena cava inferior for the determination of endothelin levels, centrifuged at 3000g for 10 minutes and stored at -80°C . Liver tissue samples were subsequently removed and stored at -80°C for the determination of tissue associated malondialdehyde (MDA) activity and superoxide dismutase (SOD) levels, as the parameters of oxidative stress.

Quantitative determination of serum endothelin levels

Rat big ET-1 levels were measured using commercially available enzyme linked immunosorbent assay (ELISA) kits (Assay Designs, MI, USA) following the manufacturer's instructions. The results are presented as pg/ml.

Quantitative determination of tissue malondialdehyde levels

The tissue MDA level was determined based on pink color formation with thiobarbituric acid (TBA) at 532 nm (Okhawa *et al.*, 1979). After mixing with 10% (w/v) trichloroacetic acid to precipitate the protein, sample was reacted with 0.67% (w/v) TBA. At the end of incubation in a boiling water-bath for 10 min, sample was centrifuged and supernatant's absorbance was read at 532 nm. Results were expressed as nmol per gram wet tissue, according to the standard graphic prepared from measurements with a standard solution.

Quantitative determination of tissue superoxide dismutase levels

SOD activity was determined according to the method by Sun *et al.* (1988). One unit of SOD was defined as the amount causing 50% inhibition in the nitroblue tetrazolium (NBT) reduction rate. The SOD activity is expressed as U mg^{-1} protein.

STATISTICAL ANALYSIS

For statistical evaluation, we used the statistical software package SPSS 15.0 and a probability value of less than 0.05 was accepted as statistically significant. As the data were independent and showed normal distribution,

statistical analysis was performed using analysis of variance (ANOVA) followed by Tukey test when comparing groups. The results are given as the mean \pm standard deviation (SD).

RESULTS

ET-1, MDA and SOD levels for all groups are presented in table 1 as mean \pm SD.

Serum endothelin-1 levels

ET-1 levels were determined as it is one of the major peptides in the pathogenesis of sepsis (fig. 1). ET-1 levels in group 2 were significantly higher compared to group 1 (14.05 ± 4.26 vs. 7.26 ± 3.61 , $p=0.016$). When sepsis was induced in rats pretreated with NSO (group 3), ET-1 levels were found to be similar to group 1 ($p>0.05$) and significantly lower compared to group 2 (8.52 ± 2.70 vs 14.05 ± 4.26 , $p<0.033$).

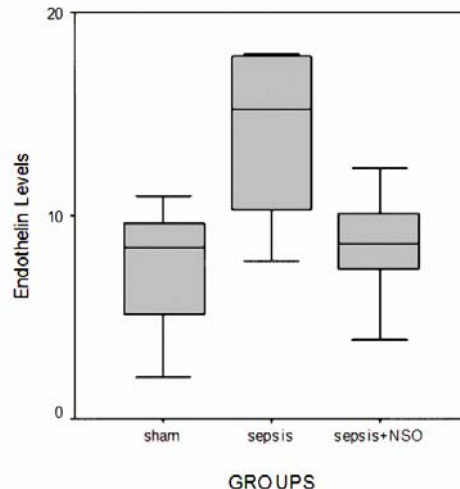


Fig. 1: Endothelin-1 levels in group 2 were significantly higher compared to group 1 ($p=0.016$). In group 3, endothelin-1 levels were significantly lower compared to group 2 ($p<0.033$).

Tissue malondialdehyde levels

Tissue MDA levels were determined as a product of lipid peroxidation, a result of oxidative stress (fig. 2). MDA

Table 1. Results of laboratory studies in group 1, group 2 and group 3, mean \pm SD (P results while comparing the groups have been presented beneath the table).

	ET(pg/mL)	MDA(nmol g^{-1} protein)	SOD(U mg^{-1} protein)
Group 1	7.26 \pm 3.61	6.89 \pm 1.31	6.92 \pm 2.11
Group 2	14.05 \pm 4.26	10.93 \pm 1.89	1.76 \pm 0.46
Group 3	8.52 \pm 2.70	7.76 \pm 2.54	3.65 \pm 0.43
<i>P values:</i>			
Group1-Group 2	$p=0.016$	$p=0.003$	$p<0.001$
Group1-Group3	$p>0.05$	$p>0.05$	$p<0.001$
Group2-Group3	$p=0.033$	$p=0.024$	$p=0.024$

levels in group 2 were significantly higher compared to group 1 (10.93 ± 1.89 vs. 6.89 ± 1.31 , $p=0.003$). When sepsis was induced in rats pretreated with NSO (group 3), MDA levels were found to be similar to control group (group 1) ($p>0.05$) and significantly lower compared to group 2 (7.76 ± 2.54 vs 10.93 ± 1.89 , $p<0.024$).

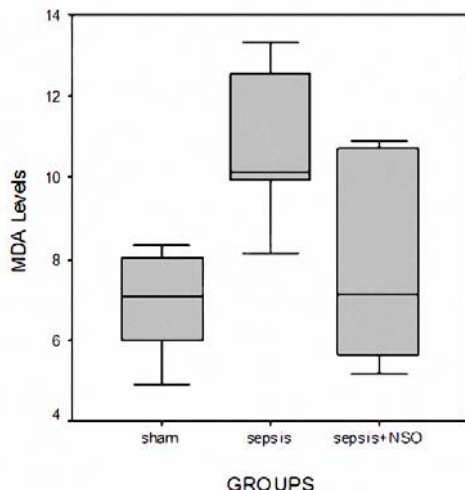


Fig. 2: Malondialdehyde levels in group 2 were significantly higher compared to group 1 ($p=0.003$). In group 3, malondialdehyde levels were significantly lower compared to group 2 ($p<0.024$).

Tissue superoxide dismutase levels

Tissue SOD levels were determined as a marker of antioxidant status (fig. 3). SOD levels were highest in group 1. SOD levels decreased significantly with induction of sepsis in group 2 (6.92 ± 2.11 vs 1.76 ± 0.46 , $p<0.001$). However, in rats pretreated with NSO (group 3), SOD levels were higher compared to group 2 (3.65 ± 0.43 vs. 1.76 ± 0.46 , $p=0.024$).

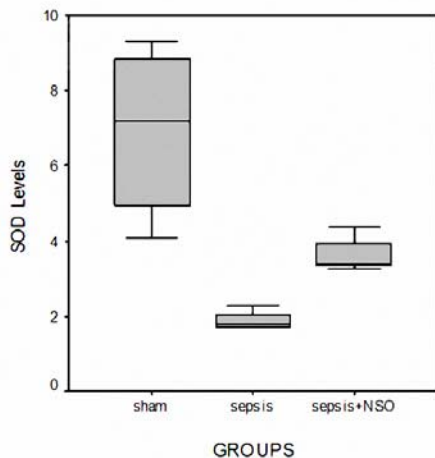


Fig. 3: Superoxide dismutase in group 1 was significantly higher compared to group 2 ($p<0.001$). In group 3, superoxide dismutase levels were higher compared to group 2 ($p=0.024$).

DISCUSSION

The results revealed that pretreatment with NSO significantly reduced serum ET-1 and MDA levels when sepsis was induced in a rat sepsis model. As well, SOD levels were found to be significantly higher in the NSO pretreated sepsis group when compared to the sepsis group.

Constituents of the *Nigella sativa* seed are known to possess potent antioxidant effects (Alenzi *et al.*, 2010; Terzi *et al.*, 2010). *Nigella sativa* (family: Ranunculaceae), which is also commonly known as Black Seed, Black Cumin, or Habbatul Barakah, have long been used in traditional medicine in the Arabian Gulf region, Far East Asia, and Europe. Its healing powers has been described by The Prophet Mohammad, the Holy bible, Hippocrates, Dioscorides and by Pliny. It was believed to be a remedy for all diseases. In the modern era, pharmacological studies have proved it to have multiple pharmacological effects among which are its analgesic, antimicrobial (against a wide range of organisms), antiinflammatory and antioxidant activities (Coban *et al.*, 2010; Hamdy and Taha 2009; Helal 2010; Tariq 2008; Terzi *et al.*, 2010). As well, thymoquinone, a constituent of NSO, has been reported to reduce acute liver dysfunction when endotoxemia was induced by LPS administration (Helal 2010).

Endothelin (ET)-1 is a potent vasoconstrictor peptide and it is involved in the pathogenesis of septic shock (Forni *et al.*, 2005). In sepsis, ET-1 levels are thought to correlate with sepsis severity and, especially with circulatory dysfunction (Figueras-Aloy *et al.*, 2004; Piechota *et al.*, 2007). Of remark, there are promising reports that ET-1 receptor antagonists may have beneficial effects on cardiovascular performance and survival in sepsis models (Iskit and Guc, 2004; Konrad *et al.*, 2007). Our results reveal that NSO may have an effect to decrease ET-1 levels which may be beneficial during sepsis.

Oxidative stress has been implicated as one of the causes of multiple organ dysfunction syndrome during the course of sepsis and it is the result of systemic inflammatory response (Andrades *et al.*, 2009a; Novak *et al.*, 2010; Rinaldi *et al.*, 2009). It is marked by increased levels of reactive oxygen species (ROS), resulting from both a decrease in antioxidant levels and from increased production of ROS (Andrades *et al.*, 2009 a; Ribeiro *et al.*, 2009). Oxidative stress has multiple deleterious effects on tissues. One of which is the lipid peroxidation of cellular membranes, characterised by lipid hydroperoxides that are decomposed to a variety of end products including MDA (Okhawa *et al.*, 1979). MDA levels were found to be increased with sepsis induction, however pretreatment with NSO resulted in lower levels of MDA. As well, NSO improved the antioxidant status

as shown by increased levels of SOD despite sepsis induction in the NSO pretreated group.

Enhanced generation of ROS in sepsis is suspected to be responsible of detrimental consequences and sepsis-related endothelium dysfunction (Andrades et al., 2009 a; Forceville et al., 2009; Kaymak et al., 2008; Rodriguez et al., 2009; Víctor et al., 2009). Modalities to counteract toxicity of ROS are being searched for. Major endogenous antioxidant systems include glutathione, vitamins A, C and E and several other enzymes such as SOD (Ribeiro et al., 2009). SOD is an antioxidant enzyme that scavenges the superoxide radical and catalyzes it to hydrogen peroxide and oxygen. Levels of SOD can provide information on the antioxidant status (Rabus et al., 2008). NSO may be a new agent to be studied as a supplement during sepsis as other antioxidants in the form of selenium, glutamine, eicosapentaenoic acid and micronutrients that have been shown to improve sepsis outcomes (Barichello et al., 2007; Berger and Chioléro, 2007).

In conclusion, the results of this study have shown that pretreatment with NSO decreased the ET-1 levels and improved antioxidant status as shown by SOD and MDA levels. Combined with the results of previous studies, our results indicate that further studies may establish NSO a role as an adjuvant therapeutic agent in sepsis.

REFERENCES

- Alenzi FQ, El-Bolkiny Yel-S and Salem ML (2010). Protective effects of *Nigella sativa* oil and thymoquinone against toxicity induced by the anticancer drug cyclophosphamide. *Br. J. Biomed. Sci.*, **67**(1): 20-28.
- Andrades M, Ritter C, deOliveira MR, Streck EL, Fonseca Moreira JC and Dal-Pizzol F (2009). Antioxidant Treatment Reverses Organ Failure in Rat Model of Sepsis: Role of Antioxidant Enzymes Imbalance, Neutrophil Infiltration, and Oxidative Stress. *J. Surg. Res.*, [Epub ahead of print]
- Andrades ME, Ritter C and Dal-Pizzol F (2009). The role of free radicals in sepsis development. *Front Biosci (Elite Ed)*, **1**: 277-287.
- Barichello T, Machado RA, Constantino L, Valvassori SS, Réus GZ, Martins MR, Petronilho F, Ritter C, Quevedo J and Dal-Pizzol F (2007). Antioxidant treatment prevented late memory impairment in an animal model of sepsis. *Crit. Care Med.*, **35**(9): 2186-2190.
- Berger MM and Chioléro RL (2007). Antioxidant supplementation in sepsis and systemic inflammatory response syndrome. *Crit. Care Med.*, **35**(9 Suppl): S584-S590.
- Coban S, Yildiz F, Terzi A, Al B, Aksoy N, Bitiren M and Celik H (2010). The effects of *Nigella sativa* on bile duct ligation induced-liver injury in rats. *Cell Biochem. Funct.*, **28**(1): 83-88.
- Dare AJ, Phillips AR, Hickey AJ, Mittal A, Loveday B, Thompson N and Windsor JA (2009). A systematic review of experimental treatments for mitochondrial dysfunction in sepsis and multiple organ dysfunction syndrome. *Free Radic. Biol. Med.*, **47**(11): 1517-1525.
- Figueras-Aloy J, Gómez-Lopez L, Rodríguez-Miguélez JM, Jordán-García Y, Salvia-Roiges MD, Jiménez W and Carbonell-Estrany X (2004). Plasma endothelin-1 and clinical manifestations of neonatal sepsis. *J. Perinat. Med.*, **32**(6): 522-526.
- Forceville X, Mostert V, Pierantoni A, Vitoux D, Le Toumelin P, Plouvier E, Dehoux M, Thuillier F, Combes A and Selenoprotein P (2009). Rather than glutathione peroxidase, as a potential marker of septic shock and related syndromes. *Eur. Surg. Res.*, **43**(4): 338-347.
- Forni M, Mazzola S, Ribeiro LA, Pirrone F, Zannoni A, Bernardini C, Bacci ML and Albertini M (2005). Expression of endothelin-1 system in a pig model of endotoxic shock. *Regul. Pept.*, **131**(1-3): 89-96.
- Ghiselli R, Giacometti A, Cirioni O, Mocchegiani F, Orlando F, D'Amato G, Sisti V, Scalise G, Saba V and Cecopin B (2004). Enhances betalactams activities in experimental rat models of gram-negative septic shock. *Ann. Surg.*, **239**(2): 251-256.
- Guo RF and Ward PA (2007). Role of oxidants in lung injury during sepsis. *Antioxid. Redox. Signal.*, **9**(11):1991-2002.
- Gupta A, Brahmabhatt S, Kapoor R, Loken L and Sharma AC (2005). Chronic peritoneal sepsis: myocardial dysfunction, endothelin and signaling mechanisms. *Front Biosci.* **10**: 3183-3205.
- Hamdy NM and Taha RA (2009). Effects of *Nigella sativa* oil and thymoquinone on oxidative stress and neuropathy in streptozotocin-induced diabetic rats. *Pharmacology*, **84**(3):127-134.
- Helal GK (2010). Thymoquinone supplementation ameliorates acute endotoxemia-induced liver dysfunction in rats. *Pak. J. Pharm. Sci.*, **23**(2): 131-137.
- Ilhan A, Koltuksuz U, Ozen S, Uz E, Ciralik H and Akyol O (1999). The effects of caffeic acid phenethyl ester (CAPE) on spinal cord ischemia/reperfusion injury in rabbits. *Eur. J. Cardiothorac. Surg.*, **16**: 458-463.
- Iskit AB and Guc MO (2004). A new therapeutic approach for the treatment of sepsis. *Med. Hypotheses*, **62**(3): 342-345.
- Kaymak C, Kadioglu E, Ozcaglu E, Osmanoglu G, Izdes S, Agalar C, Basar H and Sardas S (2008). Oxidative DNA damage and total antioxidant status in rats during experimental gram-negative sepsis. *Hum. Exp. Toxicol.*, **27**(6): 485-491.
- Konrad D, Haney M, Johansson G, Wanecek M, Weitzberg E and Oldner A (2007). Cardiac effects of endothelin receptor antagonism in endotoxemic pigs.

- Am. J. Physiol. Heart Circ. Physiol.*, **293**(2): H988-H996.
- Novak F, Vavrova L, Kodykova J, Novak F Sr, Hynkova M, Zak A and Novakova O (2010). Decreased paraoxonase activity in critically ill patients with sepsis. *Clin. Exp. Med.*, **10**(1): 21-25.
- Okhawa H, Ohishi N and Yagi K (1979). Assay of lipid peroxides in animal tissues by thiobarbituric reaction. *Anal. Biochem.*, **95**(2): 351-358.
- Piechota M, Banach M, Irzmanski R, Barylski M, Piechota-Urbanska M, Kowalski J and Pawlicki L (2007). Plasma endothelin-1 levels in septic patients. *J. Intensive Care Med.*, **22**(4): 232-239.
- Rabus M, Demirbağ R, Sezen Y, Konukoğlu O, Yildiz A, Erel O, Zeybek R and Yakut C (2008). Plasma and tissue oxidative stress index in patients with rheumatic and degenerative heart valve disease. *Turk. Kardiyol. Dern. Ars.*, **36**(8): 536-540.
- Ribeiro Nogueira C, Ramalho A, Lameu E, Da Silva Franca CA, David C and Accioly E (2009). Serum concentrations of vitamin A and oxidative stress in critically ill patients with sepsis. *Nutr. Hosp.*, **24**(3): 312-317.
- Rinaldi S, Landucci F and De Gaudio AR (2009). Antioxidant therapy in critically septic patients. *Curr. Drug Targets*, **10**(9): 872-880.
- Rodríguez ZZ, Guanche D, Alvarez RG, Rosales FH, Alonso Y and Schulz S (2009). Preconditioning with ozone/oxygen mixture induces reversion of some indicators of oxidative stress and prevents organic damage in rats with fecal peritonitis. *Inflamm. Res.*, [Epub ahead of print]
- Shah R (2007). Endothelins in health and disease. *Eur. J. Intern. Med.*, **18**(4): 272-282.
- Sun Y, Oberley LW and Li Y (1988). A simple method for clinical assay of superoxide dismutase. *Clin. Chem.*, **34**(3): 497-500.
- Tariq M (2008). *Nigella sativa* seeds: Folklore treatment in modern day medicine. *Saudi J. Gastroenterol.*, **14**(3): 105-106.
- Terzi A, Coban S, Yildiz F, Ates M, Bitiren M, Taskin A and Aksoy N (2010). Protective effects of *Nigella sativa* on intestinal ischemia-reperfusion injury in rats. *J. Invest. Surg.*, **23**(1): 21-27.
- Víctor VM, Espulgues JV, Hernández-Mijares A and Rocha M (2009). Oxidative stress and mitochondrial dysfunction in sepsis: A potential therapy with mitochondria-targeted antioxidants. *Infect. Disord. Drug Targets*, **9**(4): 376-389.
- Yildiz F, Coban S, Terzi A, Savas M, Bitiren M, Celik H and Aksoy N (2010). Protective effects of *Nigella sativa* against ischemia-reperfusion injury of kidneys. *Ren. Fail.*, **32**(1): 126-131.
- Zapelini PH, Rezin GT, Cardoso MR, Ritter C, Klamt F, Moreira JC, Streck EL and Dal-Pizzol F (2008). Antioxidant treatment reverses mitochondrial dysfunction in a sepsis animal model. *Mitochondrion*, **8**(3): 211-218.