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# Oxytocin Effect on Sepsis-induced Experimental Rat Renal Failure Model: A Histological and Biochemical study

# Sepsis Kaynaklı Deneysel Sıçan Böbrek Hasarı Modeli Üzerine Oksitosin Etkisi: Histolojik ve Biyokimyasal Bir Çalışma

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## Abstract

**Objective:** We aimed to investigate the therapeutic role of Oxytocin (OT) in sepsis induced rats and its effects on kidneys. Sepsis was induced in 24 of them by cecal ligation-perforation (CLP) method.

**Material-Method:** One of the groups of CLP did not recieve any treatment while either saline 1 ml/kg or OT 0.4 mg/kg was applied intraperitoneally to the other groups within the first hour of surgery. 8 rats were sham-operated and 8 rats were spared as control group. Plasma TNF-α α (Tumor necrosis factor- α), CRP (C reactive Protein), MDA (Malondialdehyde) levels, total antioxidant capacity (TAC), creatinine level and peripheral leukocyte (WBC) counts were measured.

**Results:** TNF-α, CRP, MDA, TAC levels and WBC counts were significantly increased in CLP and CLP-saline groups, on the contrary they are decreased in CLP-OT group. Histopathologically loss of brush border, tubular dilatation and mononuclear cell infiltration was significantly decreased in CLP-OT group but there was no significant difference in erytrocyte extravasation and cast formation when compared to CLP and CLP-saline groups.

**Conclusions:** Our results indicate that OT may have a therapeutic value in limiting sepsis induced inflammation and organ damage.

**Keywords:** Sepsis model, Kidney, Oxytocin, Rat

## Özet

**Amaç:** Bu çalışmada, sepsis ile indüklenen sıçanlarda oksitosinin (OT) terapotik rolü ve bunun böbrekler üzerindeki etkisi araştırılmıştır. 24 adet sıçanda çekal ligasyon-perforasyon (CLP) yöntemi ile sepsis oluşturulmuştur.

**Materyal-Metot:** Cerrahi girişimin ilk saati içerisinde, CLP oluşturulan gruplarından birine herhangi bir tedavi uygulanmamış diğer gruba 1 ml/kg salin ve 0.4 mg/kg OT intraperitoneal olarak uygulanmıştır. 8 sıçan sham opere, 8 sıçan da kontrol grubu olarak ayrılmıştır. Plazmada TNF-α (Tümör Nekrozis Faktör-α), CRP (C-Reaktif Protein), MDA (Malondialdehit) düzeyleri, toplam antioksidan kapasite (TAK), kreatinin seviyesi ve periferik lökosit (WBC) sayıları ölçülmüştür.

**Sonuç:** CLP ve CLP-salin gruplarında TNF-α, CRP, MDA düzeyleri ve WBC sayılarında anlamlı artma saptanırken CLP-OT grubunda anlamlı düşüş görülmüştür. CLP ve CLP-salin gruplarıyla kıyaslandığında; CLP-OT grubunda serum MDA, CRP ve WBC seviyeleri anlamlı olarak düşerken, TAC anlamlı derecede yüksek çıkmıştır. CLP-OT gruplarında, histopatolojik olarak fırçamsı kenar kaybı, tübüler dilatasyon ve mononükleer hücre infiltrasyonu anlamlı düzeyde azalmıştır. Ancak, CLP ve CLP-salin grupları karşılaştırıldığında ekstravazasyon ve kast bulguları açısından anlamlı fark bulunamamıştır.

**Sonuç:** OT'nin sepsis kaynaklı inflamasyon ve organ hasarı açısından terapotik değeri olabileceğini göstermektedir.

**Anahtar kelimeler:** Sepsis modeli, Böbrek, oksitosin, Sıçan

## Introduction

Sepsis is a severe clinical syndrome that results from systemic host response to infection. Besides the infection, the intensity of immunoinflammatory response also influences the outcome (1). If this response is uncontrolled it can lead to multiple organ dysfunction syndrome. This final result is responsible of a mortality rate exceeding 50% (1). Infection contributes to the pathogenesis of severe sepsis, which is characterized by an overwhelming production of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β). These cytokines trigger a beneficial inflammatory response that promotes local coagulation to confine tissue damage.

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However, the excessive production of these proinflammatory cytokines can be even more dangerous than the original stimulus, overcoming the normal regulation of the immune response and producing pathological inflammatory disorders (2). This is especially notable in severe sepsis, in which the excessive production of proinflammatory cytokines causes capillary leakage, tissue injury and lethal organ failure (3,4). The mechanisms involved in shock and organ injury induced in septic shock are multifactorial. Diverse molecular mechanisms of inflammation and cellular damage have been implicated in the pathogenesis of septic shock and multiple organ failure, including those related to the over generation of cytokines, eicosanoids and of reactive oxygen species (5). Oxygen-derived free radicals generated by activated neutrophils, are mediators of tissue injury. Free radical injury occurs via lipid peroxidation in a variety of disease processes, including shock. Lipid peroxidation is the oxidative deterioration of polyunsaturated lipids. The changes induced in the apolar side residues of the membrane phosphoglycerides are thought to bring about some structural alterations. Therefore, biomembranes and subcellular organelles are the major sites of lipid peroxidative damage.

Oxytocin (OT), synthesized in the supraoptic and paraventricular nuclei of the hypothalamus is a nonapeptide hormone which has diverse actions on many target tissue. While its main effect is on uterine contraction and milk ejection in the postpartum women, it also effects cardiovascular and hydroelectrolitic regulation (6,7). OT receptors have been identified not only in the uterine and myeopithelial tissues, but also in the kidney, heart, adipocytes, pancreas and thymus (8). Based on the evidence that OT and OT receptors are located in the thymus, recent studies have focused on the antiinflammatory, immune-modulatory and wound healing effects of OT (9,10). It was shown that OT protects against sepsis-induced multiple organ damage and acetic-acid induced colonic inflammation (11).

The present study was designed to investigate the effects of OT on oxidative tissue damage especially on kidneys in a rat model of sepsis.

## Material-Methods

### Animals

In this study 40 male Sprague Dawley rat weighing 200-220 g, were used. Animals were fed ad libitum and housed inpairs in steel cages having a temperature-controlled environment (22 ± 2°C) with 12-h light/dark cycles. The experimental procedures were approved by the Committee for Animal Research of Tokat Gaziosmanpaşa University. All animal studies are strictly confirmed to the animal experiment guidelines of the Committee for Human Care.

### Experimental procedures

Rats were randomly assigned into 5 groups. Study groups were designed as follows: Group 1: normal (nonoperative and orally fed control, (n=8); Group 2: sham-operated (n =8); Group 3: CLP (untreated group, n = 8); Group 4: CLP and 1 ml/kg 0.9 NaCl (saline) intraperitoneal (i.p.) (n = 8); Group 5: CLP and 0.4 mg/kg oxytocin (Pituisan®, Ege Vet, Alfasan International B.V., Holland ) i.p. (n = 8). For the surgical procedure, rats were anesthetized by intraperitoneal injection of a combination of ketamine hydrochloride at a dose of 50 mg/kg and 7 mg/kg xylazine hydrochloric (Alfazyne; Alfasan International BV, Woerden, Holland).

Under aseptic conditions, a 3 cm midline laparotomy was performed to allow exposure of the cecum with adjoining intestine. The cecum was ligated tightly with a 3.0 silk suture at its base under the iliocecal valve and punctured once with a 22-gauge needle. The cecum was then gently squeezed to extrude a small amount of feces from the perforation site. The cecum was returned to the peritoneal cavity, and the laparotomy incision was closed with 4-0 polyglactin 910 sutures. Following surgery, a recovery period was allowed to the animals and then they were placed in their cages. In the sham group, under aseptic conditions, only laparotomy was performed on rats, but their cecum was neither ligated nor punctured. In this model, rats were accepted as septic 5 h following CLP (11). All treatments were performed within the first hour of surgical procedure. The study was finished after 24 hours. At the end of the study, the animals were euthanized and blood samples were collected by cardiac puncture for biochemical analysis and bilateral nephrectomy was performed for histopathological examination.

### Measurement of plasma TNF-α and CRP (C-reactive protein) levels

Plasma TNF-α and CRP levels were measured using commercially available enzyme-linked immuno-sorbent assay (ELISA) kit (eBiosciences, Inc, San Diego, CA).

### Measurement of lipid peroxidation

Lipid peroxidation was determined in plasma samples by measuring malondialdehyde (MDA) levels as thiobarbituric acid reactive substances (TBARS) (12). Briefly, trichloroacetic acid and TBARS reagent were added to the plasma samples, then mixed and incubated at 100 °C for 60 min. After cooling on ice, the samples were centrifuged at 3000 rpm for 20 min and the absorbance of the supernatant was read at 535 nm. MDA levels were expressed as nM and tetraethoxypropane was used for calibration.

### Determination of serum creatinine

The plasma creatinine levels were determined with an autoanalyzer (Synchron LX 20, Ireland) using commercial Beckman Coulter diagnostic kits (Beckman Coulter Inc., CA, USA).

### Histopathological examination of kidney

For histological study, all animals were anesthetized by ketamine (40 mg/kg, Alfamine®, Alfasan International B.V., Holland) and xylazine (4 mg/kg, Alfazyne®, Alfasan International B.V., Holland) i.p. and perfused with 200 ml of 4% formaldehyde in 0.1 M phosphate-buffer saline (PBS). Formalin-fixed kidney sections (5 μm) were stained with

hematoxylene & eosine. All sections were photographed with Olympus C-5050 digital camera mounted on Olympus BX51 microscope.

### Peripheral Neutrophil Count

Peripheral blood smears obtained at 24th hour were stained with Wright-Giemsa to evaluate the circulating neutrophil counts. Five fields per slide on high-power-field (X100) magnification were randomly selected and the number of neutrophils was manually counted. A circulating neutrophil count calculated as 1000 times the average value was obtained.

### Evaluation of Plasma total antioxidant capacity (TAC)

Plasma TAC was measured by the ferric reducing antioxidant power (FRAP) assay according to Benzie and Strain (13). Briefly, the FRAP reagent (sodium-acetate, tripiridiltriazyne in hydrochloric acid, and ferric chloride) prewarmed to 37◦C was mixed with the plasma; the absorbance was read after 4 min at 593 nm. A calibration curve was prepared by substituting the sample with freshly prepared ascorbic acid solution (100-1000 μM).

### Statistical Analysis

Data analyses were performed using SPSS version 15.0 for Windows. Nonparametric variables (histopathology) were analyzed by the Mann-Whitney U test. Parametric variables (biochemistry data) were evaluated by one-way ANOVA, followed by Tukey's HSD test. $p<0.05$ was accepted as statistically significant.

### Biochemical Findings

There was no significant difference in MDA, TAC, TNF-α, WBC and CRP levels between sham-operated and normal groups. MDA, TNF, WBC and CRP levels were significantly increased and TAC levels significantly decreased in CLP and CLP+saline groups when compared to sham-operated and normal groups. MDA, TNF, WBC and CRP levels were significantly decreased and TAC levels significantly increased in CLP+oxytocin group when compared to CLP and CLP+saline groups. Creatinine level was significantly decreased in CLP+oxytocin group when compared to CLP group (Table 1).

### Histological Findings

Renal sections of the normal (non-operative) group revealed normal differentiation of medulla and cortex and an intact fibrous capsule around cortex. The glomerular structures were intact and the parietal and visceral leaflets of the Bowman capsule were normal histologically. The proximal and distal tubules, collector duct of Henle and the collector tubules showed normal characteristics of epithelial cells and structure. Renal sections of the sham-operated group showed similar findings. There was no significant difference in loss of brush-border, tubular dilatation, erytrocyte extravasation and cast formation between sham-operated and normal groups. Mononuclear histological index (MNHI) was significantly increased in sham-operated group compared to normal group. (Table 2). CRP is a protein thats level rises in plasma to response to inflammation. Sections of the CLP group demonstrated that cortex and medulla could be well differentiated but Bowman's capsular spaces in cortex were widened. In cortical and medullar regions MNHI and erytrocyte extravasation was seen; being most prominent in peritubular regions and around the glomerules. Loss of brush border in proximal tubule cells around the glomerular structure, dilatation of proximal and distal tubules, a proteinous matter accumulation, some vacuolisation and cellular debris was observed. CLP+saline group showed similar findings. In CLP+ OT group, loss of brush border in proximal tubule cells around the glomerular structure, dilatation of proximal and distal tubules and MNHI were significantly less; but cast formation in tubules, change in glomerular structure and erytrocyte extravasation were similar when compared to CLP and CLP+ saline group (Fig 1).

**Table 1.** Comparison between the groups according to the level of Plasma TNF- α, MDA, TAC, CRP, WBC and Creatinine

| | Normal Group | Sham operated Group | CLP Group | CLP and saline Group | CLP and 0.4 mg/kg oxytocin Group |
|---|---|---|---|---|---|
| **MDA (nM)** | 75.7 ± 9.5 | 94.5 ± 12.3 | 168.1 ± 8.9 * | 161.3 ± 10.2 * | 132.1 ± 4.3 # |
| **TAC (μM)** | 62.1 ± 5.8 | 60.4 ± 4.5 | 22.7 ± 5.3 * | 25.6 ± 3.8 * | 108.5 ± 7.6 ## |
| **TNF-α (pg/ml)** | 24.2 ± 6.2 | 28.6 ± 9.5 | 239.2 ± 18.6 ** | 235.5 ± 15.3 ** | 118.6 ± 21.5 ## |
| **WBC /μL** | 4.28 ± 0.69 | 4.65 ± 0.43 | 10.43 ± 1.15 * | 11.24 ± 2.06 * | 6.13 ± 0.21 # |
| **CRP (mg/dl)** | 0.36 ± 0.08 | 0.42 ± 0.12 | 0.75 ± 0.25 * | 0.78 ± 0.39 * | 0.52 ± 0.14 # |
| **Creatinine (mg/dl)** | 0.31 ± 0.04 | 0.33 ± 0.05 * | 1.23 ± 0.15 | 1.06 ± 0.05 * | 0.68 ± 0.06 # |

Results were presented as mean ± SEM.
* $p < 0.001$,
** $p < 0.00001$ different from normal and sham-operated groups;
# $p < 0.01$,
## $p< 0.0001$ different from CLP and CLP + saline group.

**Table 2.** Comparison between the groups according to the level of Plasma TNF- α, MDA, TAC, CRP, WBC and Creatinine

| | Normal Group | Sham operated Group | CLP Group | CLP and saline Group | CLP and 0.4 mg/kg oxytocin Group |
|---|---|---|---|---|---|
| **Loss of brush-border** | 0.1 ± 0.1 | 0.1 ± 0.1 | 2.8 ± 0.1 ** | 2.6 ± 0.2 ** | 1.6 ± 0.2 # |
| **Tubular dilatation** | 0 | 0.3 ± 0.3 | 2.6 ± 0.3 ** | 2.8 ± 0.1 ** | 1.8 ± 0.3 # |
| **Mononuclear histological index(MNHI)** | 0.1 ± 0.1 | 0.6 ± 0.2 * | 2.5 ± 0.5 ** | 2.8 ± 0.1 ** | 1.1 ± 0.1 ## |
| **Erytrocyte extravasation** | 0 | 0.1 ± 0.1 | 0.8 ± 0.1 * | 1.1 ± 0.1 * | 1.0 ± 0.2 |
| **Cast Formation** | 0 | 0.1 ± 0.1 | 0.6 ± 0.2 * | 0.8 ± 0.1 * | 1.1 ± 0.1 |

Results were presented as mean ± SEM.
* p< 0.05,
** p< 0.00001, different from normal and sham-operated groups;
# p<0.05,
# p< 0.001, different from CLP and CLP + saline group.

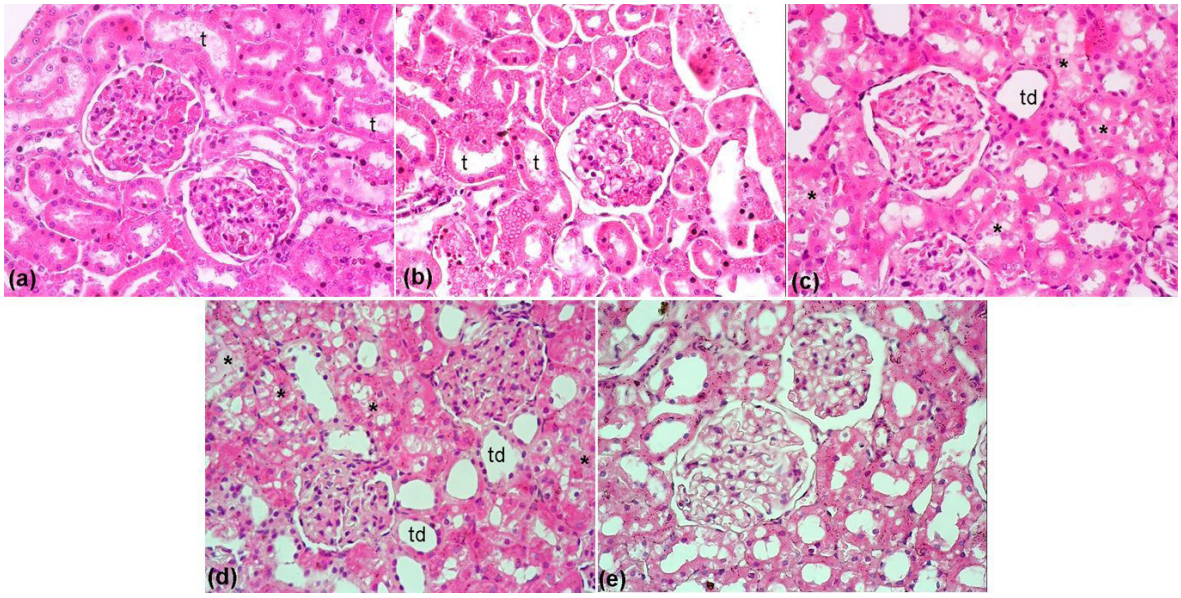


**Figure 1.** Histopathological images of all groups. H&E staining. Original magnification X40. Scale bar = 125 µm, a) Normal group, b) Sham-Operated group, c) CLP group, d) CLP + saline group, e) CLP + OT group. * Tubular cast, td tubular dilatation, t normal renal tubul, Dt Distal tubul, PT Proksimal tubul

## Discussion

The results of our study indicate that OT reduces sepsis induced lipid peroxidation as evidenced by decreased MDA levels, serum CRP levels and ameliorates histopathologically confirmed renal damage. The increase in serum levels of TNF-α, which is the key cytokine in the inflammatory cascade in sepsis is also reversed by OT treatment. The mechanism of action of OT responsible of all these curative effects is probably due to its significant role on the elevation of total antioxidant capacity. The incidence and mortality rate of sepsis remains high, and sepsis is still the most common cause of death in the intensive care units. The disease differs widely in severity, but for those patients who become critically ill, poor outcome correlates very closely with the severity of the bodys own inflammatory responses to infection. A sufficient inflammatory response is a prerequisite for successfully combating bacterial infection. However, self-inflicted damage to host tissues, such as those of lung, liver, circulatory and renal systems, as a consequence of an overtly aggressive inflammatory response appears to be the key component for a poor patient outcome rather than an inability to fight infection (14). Macrophages and neutrophils are two innate immune cell types intimately associated with the excessive inflammatory response characterizing severe sepsis. These cells both produce and respond to cytokines, chemokines, and other pro-inflammatory mediators released from endothelial, epithelial, and other cell populations activated by microbial products. Host tissues, as well as infiltrating cells, such as neutrophils, are injured by production of tissue damaging reactive oxygen intermediates, proteases, and general inflammatory environment brought about by these

cells. Stimulated in situ macrophages and neutrophils also exacerbate the disease by releasing chemokines and cytokines which set up a positive feedback loop causing even more severe inflammation. If the disease progresses, organ failure develops in severe cases, with a concomitant high mortality rate. Once bacterial products including peptidoglycans and lipotechoic acid, interact with toll like receptors (TLR) on cell surfaces, kinases that lead to enhanced transcription of cytokines and other pro-inflammatory rnediators are synthesized (15). This initiates further cycles of cytokine release from various cell types further promoting the inflammatory response. Both TNF-α and IL-1β have important roles in enhancing inflammatory responses involved in sepsis. For example, mice in which TNF receptors have been knocked out demonstrate improved survival from sepsis (16). TNF-α and IL-1β activate inflammatory cells which leads to the release of reactive oxygen metabolites. In turn, these metabolites cause lipid peroxidation of cell membranes and oxidative degeneration of cellular components ending in cell lysis (17). In the present study, the level of lipid peroxidation in plasma samples measured as MDA levels were significantly elevated in all groups except the control and sham-operated group, but was decreased in the group treated with OT. This result was compatible with Iseri et al.'s study in which tissue MDA levels were also significantly decreased in OT treated group in a septic rat model (11). OT, normally is a neurohypophyseal hormone acting to facilitate uterine contractions and milk ejection in postpartum women (6,11). But recent studies gave rise to its antiinflammatory and immunumodulatory effects. On various tissues under inflammation it was shown to display antiinflammatory and antifibrotic effects (11). In one of these studies OT was documented to increase the survival of ischemic skin flaps in rats (9). In another study in patients with diabetic foot lesions OT was shown to have therapeutic effects. In various studies OT was shown to decrease IL-6 synthesis (18) and increase prostacyclin release [19]. This prostacycline discharge aids in inhibiting platelet aggregation. OT was also shown to decrease TNF-α release in response to oxidative tissue injury (11).

Oxidant injury has been implicated in the pathogenesis of renal inflammatory processes and is characterized as an imbalance between the amount of free radicals and antioxidants in favor of free radicals (20). Oxidant injury in kidneys causes mainly structural alterations, loss in energy status and alterations in the aminoacid transport in renal brush border. These changes lead to damage in cellular organelles including membrane lipids, proteins and nucleic acids and ultimately lead to apoptotic cell lysis and cell death (21). In a previous study OT was shown to decrease renal paranchymal damage and preserve renal function in pyelonephritic rats through the inhibition of myeloperoxidase activity (22). In an ischemia/reperfusion model of rat kidneys it was reported that OT treatment prevents the development of acute renal failure and preserves renal tissue morphology along with the alleviation of oxidant tissue responses (23). Our results reveal significant decrease in loss of brush-border, tubular dilatation and mononuclear cell infiltration in kidney specimens of rats that were treated with OT. Along with the beneficial effects on MDA this is mainly attributable to its effect in reducing lipid peroxidation.

## Conclusion

Finally, results of the current study clearly indicate that OT treatment has antiinflammatory and antioxidant actions and ameliorates sepsis-induced renal damage. On the basis of alike studies in the literature we strongly recommend that large-scaled prospective human studies should be performed to better understand its therapeutic effects and use this hormone in favor of septic patients.

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