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Original article

# Effects of pre-treatment with inulin on the kidney in lipopolysaccharide-induced endotoxemia in rats

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## Abstract

This study was aimed to evaluate the effects of inulin used as prebiotic on the kidney in lipopolysaccharide (LPS)-induced endotoxemia model.

Wistar Albino rats were divided into four groups: Control group, LPS (endotoxemia) group, Inulin + LPS group in which LPS (1.5 mg/kg, *E. coli*, Serotype 0111: B4) was treated after inulin (500 mg/kg) given by gavage for 21 days and Inulin group. The animals were sacrificed 24 h after the last LPS injection. Kidney samples were taken for biochemical and immunohistochemical analyses. Total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), malondialdehyde (MDA) and myeloperoxidase (MPO) values were determined. In addition, kidney sections were stained for inducible nitric oxide synthase (iNOS), tumor necrosis factor (TNF)- $\alpha$  and interleukine-6 (IL-6) expression, and leukocyte infiltration.

LPS caused oxidative stress and inflammation. Inulin administration could prevent oxidative stress and lipid peroxidation. Moreover, inulin decreased iNOS, TNF- $\alpha$  and IL-6 expression. However, it did not change the distribution of leukocytes in kidney tissues.

These results suggest to promising benefits of inulin as prebiotic in reducing the effects of endotoxemia. Further studies should be conducted to evaluate the capacity of prebiotics in endotoxemia.

**Key words:** acute kidney injury, endotoxemia, inulin, oxidative stress

## Introduction

Acute kidney injury (AKI) is one of the serious complications of sepsis and related to mortality and morbidity (Hoste et al. 2015). AKI is generally seen during serious infection due to Gram-negative bacteria (Suh et al. 2013) which have lipopolysaccharide (LPS). LPS causes to an immune/inflammatory host response and oxidative stress (Schrier and Wang 2004, Chvojka et al. 2010), and induces endotoxemia and sepsis

characterized by hemodynamic changes, hypoxia, microcirculatory dysfunction, organ injury, and production of reactive oxygen species and pro-inflammatory cytokines (Schrier and Wang 2004, Chvojka et al. 2010, Legrand et al. 2011). During sepsis and endotoxemia, systemic release of inflammatory cytokines and oxidants can lead to kidney injury and inflammation thereby affecting renal functions (Schrier and Wang 2004, Chvojka et al. 2010, Legrand et al. 2011).

The human gastrointestinal system contains micro-

organisms, which are bacteria, archaea, viruses, yeast, and fungi, called as the gut microbiota (Cani 2018). The microorganisms and their hosts have a symbiotic relationship that is important for physiological homeostasis. Cani (2017) has stated that the gut microbiota is likely to be at the intersection of physiological or pathological conditions according to the present literatures. The gut microbiota has a functional role in numerous physiological processes of the host including immunity, metabolic and nutritional homeostasis, and energy expenditure (Levy et al. 2017). The microbiota is linked with many diseases including diabetes, obesity, inflammation, neurodegenerative disorders, cardiovascular disease, and cancer (Cani 2017).

Many researchers have focused on effects of certain bacterial metabolites as short-chain fatty acids (SCFAs) or on effects of the prebiotic and probiotics on the gut microbiota in host health and disease (Gibson et al. 2017). The effects of potential novel therapeutic on the gut microbiota have been researched in many diseases. Treatment strategies are ranged from fecal microbiota transplantation, treatment with prebiotics, probiotics and synbiotics or selective decontamination of the digestive tract (Fay et al. 2017, Gibson et al. 2017, Tang et al. 2017).

Prebiotics are defined as non-digestible food components that stimulate the growth or activity of microorganisms conferring a benefit of the host (Fay et al. 2017, Gibson et al. 2017, Tang et al. 2017). Inulin is known as prebiotics (Gibson et al. 2017). The limited studies have evaluated the effect of inulin as prebiotic and its effect on LPS-induced AKI.

In this study, it was aimed to evaluate the effects of inulin on the kidney in LPS-induced kidney injury model.

## Materials and Methods

### Experimental design

This study was approved by Bezmiâlem Vakıf University Animal Experiments Local Ethics Committee (Date: 2021/Decision no: 54). Care and handling of animals was performed in accordance with the guidelines of the Institutional Animal Care and Use Committees. Experiments were performed on male 3 months old 27 Wistar albino rats (350-400 g) (Bezmiâlem Vakıf University, Istanbul, Turkey). The animals were fed commercial pelleted rodent diet and tap water *ad libitum*.

The animals were divided into four groups. Control group ( $n=8$ ) was given 0.9% physiological saline by gavage for 21 days and then 0.9% physiological saline was injected intraperitoneally (i.p.). LPS group ( $n=7$ ) was given 0.9% physiological saline by gavage for 21 days and then 1.5 mg/kg LPS (*E. coli*, Serotype

0111: B4, Sigma, Missouri, USA) was injected i.p. Inulin+LPS group ( $n=6$ ) was given 500 mg/kg inulin (Smart Kimya, Izmir, Turkey) by gavage for 21 days and then 1.5 mg/kg LPS was injected i.p. Inulin group ( $n=6$ ) was given 500 mg/kg inulin by gavage for 21 days and then 0.9% physiological saline given i.p. The animals were anesthetized with 50 mg/kg ketamine (İnterhas A.Ş. Ankara, Turkey) and 5 mg/kg xylazine (Bayer, Istanbul, Turkey) 24 h after the last injection. The kidney samples were taken for biochemical and immunohistochemical analyses.

### Measurement of oxidative stress parameters

The kidney samples were homogenized in a cold phosphate buffer (pH 7.4) using a homogenizer (IKA, Wilmington, NC, USA). The homogenates were centrifuged at 3000 rpm for 20 min at +4°C (Sorvall Super T21, Benchtop Centrifuge). The supernatants were collected for biochemical analysis. Protein levels in homogenates were determined using the bicinchoninic acid reaction (Smith et al. 1985).

Total antioxidant status (TAS) and total oxidant status (TOS) were determined in kidney samples according to the manufacturer's instructions (Rel Assay Diagnostics, Gaziantep, Turkey). It was measured TAS levels as mmol Trolox eq/l and TOS levels as  $\mu\text{mol H}_2\text{O}_2$  eq/l. The calculation of oxidative stress index (OSI) was performed using the formula (TOS/TAS) x 100 after determining the TAS and TOS values. The values were expressed as Arbitrary Unit.

### Lipid peroxidation analysis

The lipid peroxidation was measured in kidneys using the method by Ohkawa et al. (1979). Data were expressed in nmol/mg protein.

### Measurement of myeloperoxidase activity

Myeloperoxidase (MPO) activity was measured at 460 nm according to the method of Krawisz et al. (1984). The amount of enzyme required to degrade 1 mol of  $\text{H}_2\text{O}_2$ /min at 25°C was measured as MPO activity (1 unit), and the results were expressed in  $\mu\text{mol}/\text{min}/\text{ml}$ .

### Immunohistochemical analysis

The kidney tissues were fixed in 10% neutral buffered formaldehyde and embedded in paraffin as previously described by Legrand et al. (2011). The kidney sections (5  $\mu\text{m}$ ) were incubated with antibodies against inducible nitric oxide synthase (iNOS) (Thermo Fisher Scientific, B-1605-P), tumor necrosis factor (TNF)- $\alpha$  (Abcam ab66579), interleukine-6 (IL-6) (Abcam, 6672)

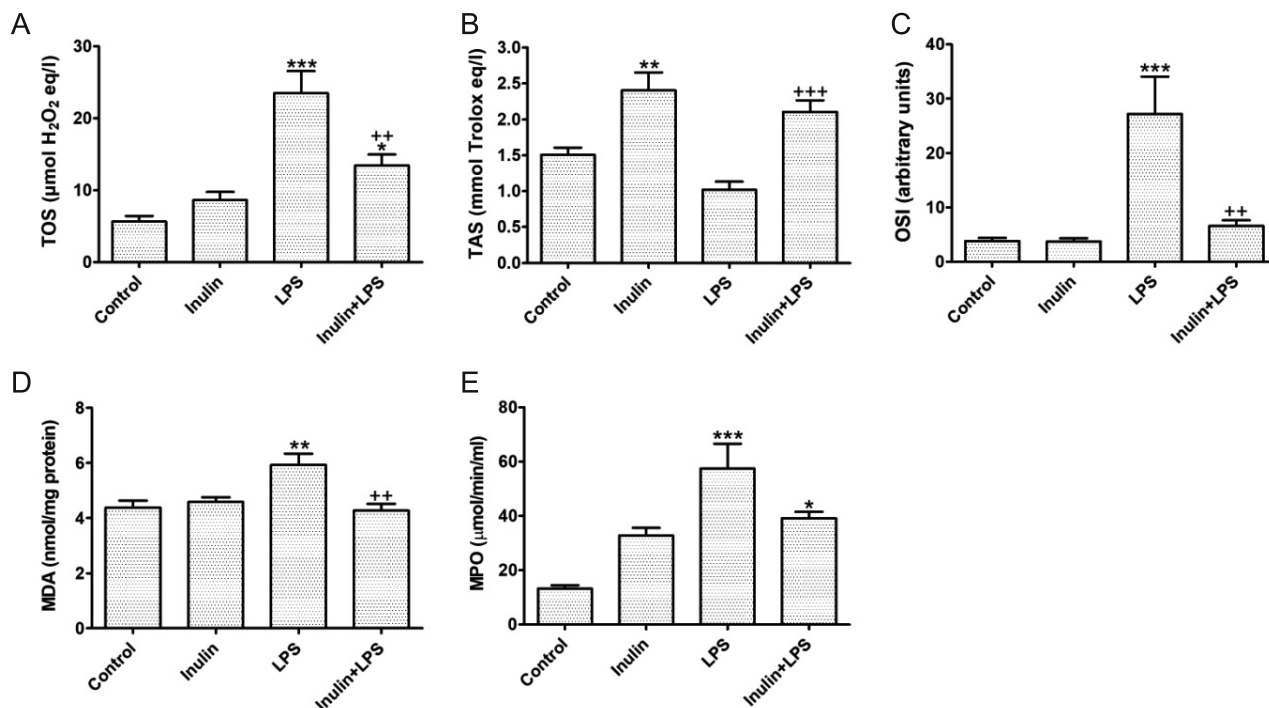


Fig. 1. Representative images for the TOS (A), TAS (B), OSI (C), MDA (D) and MPO (E) values in rat kidney tissues of Control, Inulin, LPS and Inulin+LPS groups. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs. Control group; ++ $p < 0.01$ , +++ $p < 0.001$  vs. LPS group. Data are shown as mean  $\pm$  SEM. Total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), malondialdehyde (MDA) and myeloperoxidase (MPO).

and myeloperoxidase (MPO) (Thermo Fisher Scientific, RB-373-A). Both intensity and distribution of the specific stainings were scored as previously described by Legrand et al. (2011). The MPO reaction was evaluated in selected glomeruli and peritubular areas in the kidney sections (Legrand et al. 2011). The photograph of reactions in the sections was taken with Image Pro-Plus Kameram 390CU.

### Statistical analysis

The results are expressed as the mean  $\pm$  SEM. The Shapiro–Wilk test was performed to determine the normality of the data. Data with non-normal distribution were established by the Kruskal Wallis test (non-parametric test). Data with normal distribution were established by one-way ANOVA. Post-hoc comparisons between the groups were performed with Tukey and Dunn tests. A value  $p < 0.05$  was considered statistically significant. The tests were performed with GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA).

## Results

### Oxidative stress results

LPS administration increased the TOS level in the kidney ( $p < 0.001$ ). The level of TAS in the kidney was

reduced in LPS-treated animals; however, this decrement was not statistically significant. Pre-treatment with inulin reduced the TOS level ( $p < 0.01$ ), while it increased TAS levels ( $p < 0.001$ ) in the kidney tissues of endotoxemic animals. Administration of inulin led to an increase in the TAS level compared with the control group ( $p < 0.01$ ) (Fig. 1A-B). Administration of LPS resulted in oxidative stress according to OSI values. OSI values were increased in LPS-treated group compared with the control group ( $p < 0.001$ ). Inulin could prevent an increase in OSI values compared with those found in the LPS group ( $p < 0.01$ ). OSI values in inulin-treated groups were similar to that found in the control group (Fig. 1C).

### Lipid peroxidation results

MDA level in the kidney was increased in LPS group ( $p < 0.01$ ). Inulin decreased the lipid peroxidation in kidneys of LPS-treated animals compared with LPS group ( $p < 0.01$ ). Inulin did not cause lipid peroxidation in the kidney compared with the control group (Fig. 1D).

### Myeloperoxidase activity results

MPO activity in kidneys was increased in LPS group ( $p < 0.001$ ). Inulin decreased MPO activity in kidneys; however, it was higher than that found con-



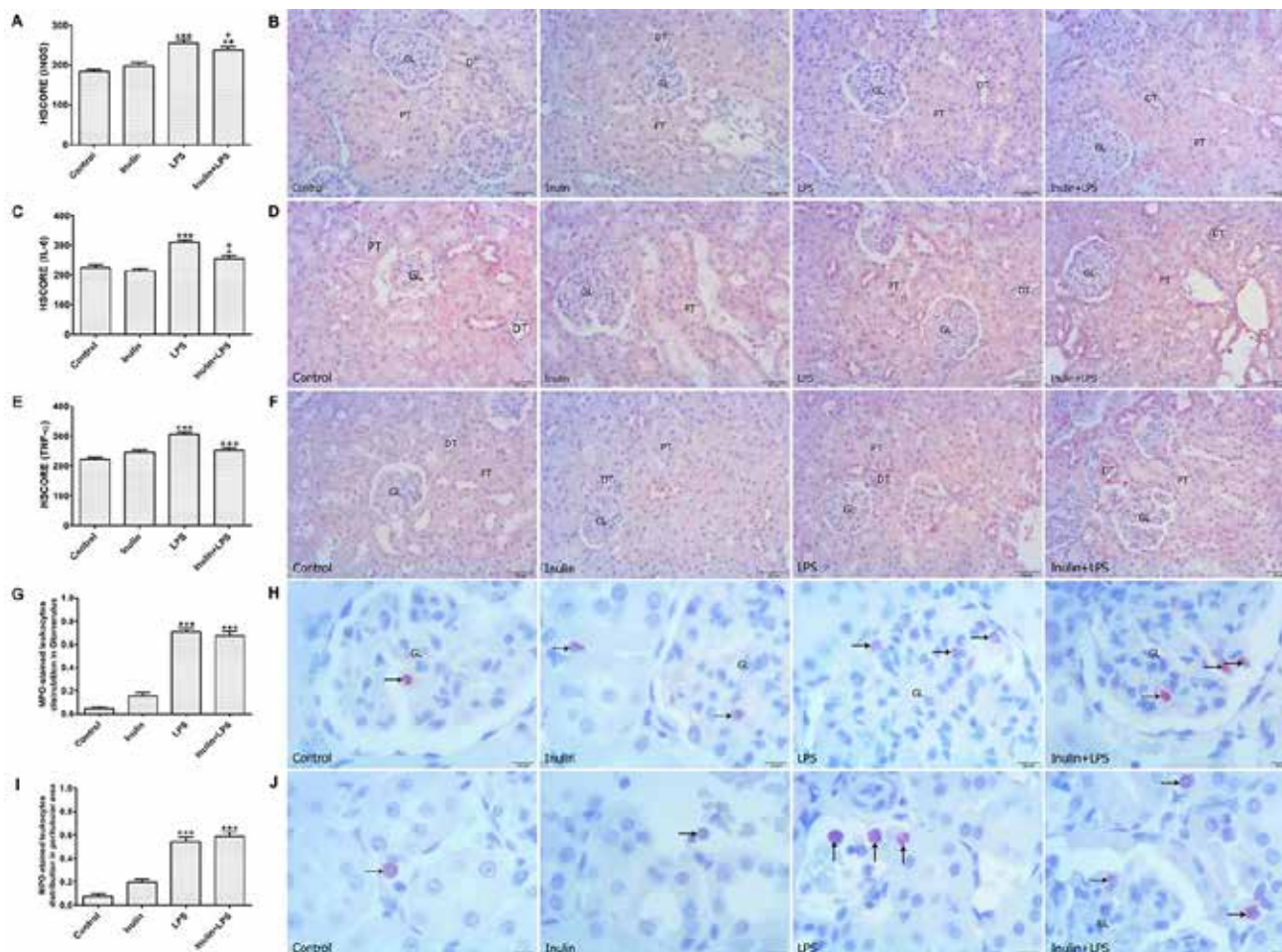


Fig. 2. Representative images for iNOS (A), IL-6 (C) and TNF- $\alpha$  (E) immunostaining intensity (HSCORE), the iNOS (B), IL-6 (D) and TNF- $\alpha$  (F) immunostaining, and the distribution of MPO-stained leukocytes ( $\rightarrow$ ) in glomerulus (G, H) and peritubular areas (I, J) in the kidney cortex of Control, Inulin, LPS and Inulin+LPS groups. Proximal tubule (PT), distal tubule (DT) and glomerulus (GL). Scale bar = 20  $\mu$ m, 50  $\mu$ m. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs. Control group; ++ $p < 0.05$ , +++ $p < 0.001$  vs. LPS group. Data are shown as mean  $\pm$  SEM. Nitric oxide synthase (iNOS), tumor necrosis factor (TNF)- $\alpha$ , interleukine-6 (IL-6) and myeloperoxidase (MPO).

control group ( $p < 0.05$ ). MPO activity in inulin groups was similar to that determined in the control group (Fig. 1E).

### Immunohistochemical results

LPS-induced endotoxemia caused an increase in iNOS, IL-6 and TNF- $\alpha$  reaction in the kidney tissues ( $p < 0.001$ ). The pre-treatment with inulin decreased iNOS ( $p < 0.05$ ), IL-6 ( $p < 0.05$ ) and TNF- $\alpha$  reaction ( $p < 0.001$ ) compared with the LPS, however, iNOS ( $p < 0.01$ ) and IL-6 ( $p < 0.05$ ) reactions were still higher than those in the control group (Fig. 2A-F). The number of MPO-stained leukocytes was increased both in glomeruli and in peritubular areas in the LPS groups ( $p < 0.001$ ) in comparison to that observed in the control group. The pre-treatment with inulin did not prevent the increase in MPO-stained leukocytes in the kidney tissue in LPS given animals (Fig. 2G-J).

### Discussion

It is suggested that infection is one of the most common diseases caused by microbiota dysbiosis (Wang et al. 2017). Understanding the importance of the gut microbiome in the intensive care unit (ICU) has led researchers to think about the role of the microbiome in critical illness such as sepsis (Akrami and Sweeney 2018). Patients with sepsis have a dysbiotic gut microbiota, however, the therapeutic potential and impact of the microbiota is not well-established in sepsis (Haak et al. 2018). Septic-caused AKI is the most common AKI syndrome in ICU (Suh et al. 2013, Hoste et al. 2015). This study involved the lipopolysaccharide (LPS)-induced AKI experimental animal model and the effects of inulin as prebiotic on oxidative stress in LPS-induced AKI were evaluated.

Current data have shown that the gut microbiota can modulate AKI. SCFAs (acetate, propionate, and butyr-

ate) had a protection role in renal dysfunction caused by injury in the AKI model induced by ischemia-reperfusion (Andrade-Oliveira et al. 2015). Al-Harbi et al. (2018) suggested that acetate improved sepsis-induced AKI based on results showing a decrease in renal myeloperoxidase activity, lipid peroxides and restoration of renal tissues. Jang et al. (2009) reported that renal functional and structural decline following ischemia-reperfusion injury was worse than that in the control germ free mice. In this study, it was thought that pre-treatment of inulin would be effective in LPS-induced AKI due to its effects on gut microbiota and permeability, and oxidative stress. Therefore, the effect of inulin as prebiotic on oxidative stress in LPS-induced AKI was evaluated. LPS caused an increment in oxidative stress and lipid peroxidation, and inflammation in the kidney tissue. In addition, the expression of pro-inflammatory cytokines such as TNF- $\alpha$  and IL-6, iNOS and leukocytes infiltration were enhanced in the kidney tissues.

Sepsis mediated an increase in the gut permeability and alteration of the gut-blood barrier, which may cause translocation of bacteria and toxins from the intestinal lumen to the mesenteric lymph and systemic circulation (Fay et al. 2017). Intestinal permeability was significantly enhanced 24 h after LPS treatment. Radical scavengers and cytokine inhibitors reduced LPS-induced intestinal barrier injury (Schulz et al. 2015). The increment of the intestinal permeability leads to an exacerbation of the systemic inflammatory response. The inflammation further aggravates kidney injury (Zhang et al. 2018). Inflammation and oxidative stress can be influenced by changes in the gut microbiota and/or their metabolites. The modulation of the gut microbiota may contribute to alteration and ameliorate the detrimental effects of kidney diseases (Andrade-Oliveira et al. 2019). Protection of the gut microbiota, mucosa and permeability is necessary and important not only to the host but also to the microbiota. The protection may be accomplished through intestinal barrier integrity, inflammatory and anti-inflammatory responses, and composition of microbiota (Fay et al. 2017). In this study, the pre-treatment with inulin prevented the oxidative stress, lipid peroxidation and inflammation, and expression of pro-inflammatory cytokines and iNOS in LPS-induced AKI. However, it did not change the distribution of leukocytes in the kidney tissues. The results indicated that inulin as a prebiotic has an antioxidative property in LPS-induced AKI. The antioxidative property of inulin is supported by increasing total antioxidant capacity level in tissues of inulin treated animals.

It was suggested that oligofructose-enriched inulin can modify some inflammatory markers in type 2 diabe-

tes and suboptimal daily dietary fiber intake (Dehghan et al. 2014). Heil et al. (2019) suggested that inulin-type fructans-rich vegetable can led to modification of the gut microbiota composition and function. Pre-treatment with inulin in methotrexate-administered mice decreased MDA level and increased glutathione levels, catalase, and superoxide dismutase activity in the liver (Kalantari et al. 2019). Ávila et al. (2020) evaluated the protective effects of probiotics, prebiotic and fecal microbiota transplantation on oxidative and inflammatory parameters in the intestine of two rat models of sepsis induced by LPS or zymosan, and determined that probiotics, prebiotics, and symbiotics exerted different effects on the parameters. Inulin could prevent LPS-induced changes in the expression of some proteins, which promote inflammation and intestinal motility and reduce the radical-mediated oxidative stress (Guarino et al. 2017). Pre-treatment with inulin prevented LPS-induced human colon mucosa damage, and this effect was associated with its protective effect against LPS-induced oxidative stress (Pasqualetti et al. 2014). Similar to the mentioned studies, the present results indicate that inulin has an antioxidant property in kidney injury.

In conclusion, the present study suggests promising benefits of using inulin as a prebiotic in reducing the effects of endotoxemia. These results indicate that targeting the effects of inulin on the microbiome and oxidative stress for therapeutic purposes is one of promising strategy to improve outcomes in LPS-induced acute kidney injury. Further studies should be conducted to evaluate the capacity of prebiotics in kidney disease.

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