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The effects of caffeic acid phenethyl ester (CAPE) on bacterial translocation and inflammatory response in an experimental intestinal obstruction model in rats

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Abstract. – OBJECTIVE: Intestinal obstruction (IO) is a disease which generates approximately 20% of emergency surgery and tends to with high mortality. Prevention of oxidative stress, bacterial translocation and tissue damage caused by IO is an important medical issue. Caffeic acid phenethyl ester (CAPE) is an anti-inflammatory, antioxidant, anti-bacterial and immunomodulatory agent. In this experimental study, we aimed to investigate the effects of CAPE on bacterial translocation, inflammatory response, oxidative stress and tissue injury caused by intestinal obstruction in a rat model.

MATERIALS AND METHODS: Breafly, thirty Wistar albino rats divided into three groups as Sham (n=10), IO (n=10) and IO + CAPE (10 μmol/kg day, intraperitoneal) (n=10). The tissues from the study groups were examined biochemically, microbiologically and histopathologically.

RESULTS: In CAPE treated group, decreased serum levels of proinflammatory cytokines (TNF-α, IL-6, IL-1β) and CRP ($p < 0.05$), additionally increased serum levels of antioxidant parameters (PONS, TAS) ($p < 0.05$), were observed after IO. Microbiologically, the rates of positive cultures of the lymph node, spleen, liver and blood were significantly decreased in CAPE treated group compared to the IO group. Also histopathological examination showed that the intestinal mucosal injury score and hepatic portal inflammation score were significantly decreased in the CAPE treated group ($p < 0.05$).

CONCLUSIONS: It is suggested that intraperitoneal administration of CAPE might has potential antibacterial, anti-inflammatory, antioxidant and immunomodulatory effects in IO. So, further studies on IO are needed to evaluate exact antibacterial, antiinflammatory, antioxidant and immunomodulatory effects of CAPE.

Key Words:
Intestinal obstruction, CAPE, Bacterial translocation, Inflammatory response.

Introduction

Intestinal obstruction (IO) is a disease which generates approximately 20% of emergency surgery and tends to with high mortality. High mortality rates are generally related with multiple organ failure caused by increased bacterial translocation (BT) together with septic peritonitis in IO[1,2]. The most important task of the small intestine is forming a functional and mechanical barrier to the antigens, toxins, and microorganisms, as well as, digestion and absorption functions[3]. Bacterial overgrowth and intestinal mucosal damage, as a result of mucosal distruption, motility dysfunction and increased intestinal volume, induce the development of BT after IO[4]. In addition, immundysfunction induced by oxidative stress, as a result of intestinal barrier dysfunction and the imbalance of inflammatory and anti-inflammatory cytokines, accelerates the develop-

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ment of BT[2]. As a result of bacterial translocation, bacteria and their products passes through the peritoneal area, mesenteric lymph nodes (MLNs), liver, spleen and systemic circulation, which are normally sterile[5]. This situation can results in systemic inflammatory response, infection, sepsis, and multiple organ failure[3]. Hence, prevention of oxidative stress, bacterial translocation and tissue damage caused by intestinal obstruction is an important medical issue. However, according to our literature research, there is no adequate study on this subject. Caffeic acid phenethyl ester (CAPE), an active component of *honeybee propolis* extracts, has immunomodulatory, anti-inflammatory and antioxidant properties[6-11] in addition to antibacterial effect[12,13].

In this study, we aimed to investigate the effects of CAPE on bacterial translocation, inflammatory response, oxidative stress and tissue injury caused by intestinal obstruction in a rat model.

Materials and Methods

Chemical

CAPE was purchased from Sigma, st Louis, MO, USA (cat#: C8221, cas#: 104594-70-9) and dissolved in ethyl acetate according to the information catalog.

Animals

Randomly selected thirty Wistar albino rats, each weighing 200-250 g, were included into the experimental study at Dicle University Health Sciences Application and Research Center. The study was approved by the Committee of Experimental Animals of Dicle University and complied with the Guide for the Care and Use of Laboratory Animals. The rats were housed in cages under standard conditions in an air-conditioned room with constant temperature (22 ± 2°C) through 12 h light and dark cycles, allowed free access to standard rat chow and water. Before surgery, the animals were fasted overnight the day, but had access to water *ad libitum*.

Thirty Wistar albino rats were randomly divided into three groups; Group 1 (Sham); Ileocaecal junction dissection was performed only, not treated with drug. Group 2 (IO); Ileocaecal junction dissection and ileal ligation, 1 cm proximal to caecum with 3-0 silk sutur, were performed, not treated with drug. Group 3 (IO + CAPE); Ileocaecal junction dissection and ileal ligation (1 cm proximal to caecum with 3-0 silk sutur) were performed and CAPE applied in appropriate doses (10 μmol/kg, intraperitoneal) used in the literatüre. The effect of CAPE) at the end of the experimental study.

Experimental Protocol

For anesthesia, the rats were given 50 mg/kg ketamine hydrochloride and 10 mg/kg xylazine via intramuscular injection. For shaved skin cleansing, 10% povidone iodine solution was used. Ileocaecal junction was dissected after a midline incision performed. The distal ileum (1 cm proximal to caecum) was ligated with 3-0 silk sutur, obstructed the passage but not inhibited the vascular circulation. Intraperitoneally 2 ml saline was given, and finally laparatomy was closed in one layer. After a 24 hour period, the rats were anaesthetized like before mentioned and sacrificed by taking blood from the heart for biochemical analyses. Immediately, a thoracoabdominal midline incision was performed under complete sterile conditions and peritoneal swabs were taken for culture. For microbiological analyses, a 1 ml blood from the inferior vena cava and, samples of the liver and spleen were collected. For histopathological examinations, tissue specimens from the liver and ileum were put into plastic containers including 10% formaldehyde solution, for 24 hours. The serum obtained from the centrifugated blood, rapidly transferred to plastic Eppendorf covered tubes, stored at -80°C in deep freezer, for biochemical analyses.

Microbiological Evaluation

The blood samples obtained from the heart, cultured aerobically and anaerobically using the peds battles. Identification was realized by the BD-Phoenix 100 TM system (Sparks, MD, USA). Peritoneal swabs and positive cultures were plated out on blood agar, eosin methylene blue (EMB) agar, chocolate agar or Sabouraud-dextrose agar. Liver, spleen and mesenteric limph modes (MLNs) were removed and placed in sterile glass bottles containing sterile brain-heart infusion media at the same time. The bottles were re-weighed and tissue homogenates were prepared in 2 ml brain-heart infusion using a sterile mortar and pestle. A portion (0.1 ml) of each homogenate was cultured on blood agar, EMB agar, chocolate agar and Sabouraud-dextrose agar. After 24 h and 48 h of incubation at 37°C, all agar plates were examined and the incidence of bacterial translocation was calculated by determining

the number of rats with positive bacterial culture divided by the total number of rats studied for each group.

Biochemical Analyses

In the blood samples, paraxonase (PONX), total antioxidant capacity (TAC), total oxidant activity (TOA), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), C-reactive protein (CRP) and tumor necrosis factor-alpha (TNFα) were analyzed.

Measurement of the PONX

Serum PONX levels were measured spectrophotometrically by modified Eckerson et al method[14]. Initial rates of hydrolysis of paraoxon (0.0-diethyl-0-p-nitrophenylphosphate) were determined by measuring liberated- p-nitrophenol at 405 nm at 37°C. The results are expressed as U/L.

Measurement of the TAC

TAC of supernatant fractions was determined using a novel automated measurement method in which hydroxyl radical, the most potent biological radical, is produced[15]. In the assay, ferrous ion solution in Reagent 1, is mixed with hydrogen peroxide in Reagent 2. The sequential produced radicals such as brown colored dianisidinyl radical cation, produced by the hydroxyl radical, are also potent radicals. Antioxidative effect of the sample against the potent-free radical reactions initiated by the produced hydroxyl radical, is measured by using this method. The assay has excellent precision values, lower than 3%. The results are expressed as nmol Trolox Equiv./mg protein.

Measurement of TOA

TOA of supernatant fractions was determined using a novel automated measurement method, developed by Erel[16]. Oxidants in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules abundantly present in the reaction medium. On the other hand, the ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity measured spectrophotometrically is related to the total amount of oxidant molecules in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of nmol H_2O_2 Equiv./mg protein.

Measurement of the IL1β, IL6, CRP and TNFα

IL1β, IL6 and TNFα (Diasource; Nivelles, Belgium) were determined using the enzyme amplified sensitivity immunassay method. The serum Hs-CRP levels (DRG International, Mountainside, NJ, USA) were determined using the enzyme-linked immunosorbent assay method.

Histopathological Assessment

Tissue specimens from the liver and ileal segment irrigated with isotonic saline solution, were put into the 10% formalin solution for 24 hours. After routine histological tissue processing, all tissues were embedded in paraffin blocks, prepared by slicing 5 μm thick sections, stained with hematoxylin-eosin (H&E) and standard protocols were applied. In addition, the tissue sections from intestinal mucosa, stained with Giemsa for detection of any bacteria infiltrated. The ileal mucosa and liver tissue samples were examined for inflammatory cell infiltration, and ileal mucosal injury and bacterial infiltration of the ileal segments were also examined by a pathologist blinded to the study groups, using light microscopy (Nikon ECLIPSE 80i, Tokyo, Japan) on ×100, ×200 and ×400 magnifications. The inflammatory changes and intestinal mucosal damage were scored as follows: For intestinal mucosal inflammation score; Score 0, normal intestinal mucosal architecture; Score 1, mild inflammation and edema; Score 2, moderate inflammation and edema with neutrophils and eosinophils; Score 3, severe inflammation with many neutrophils and eosinophils. And intestinal mucosal damage were scored as follows; Score 0, normal intestinal mucosal architecture; Score 1, mild mucosal changes with subepithelial edema and inflammation; Score 2, moderate mucosal changes with subtotal villous atrophy and inflammation; Score 3, severe mucosal changes with total villous atrophy and inflammation. For liver injury; Normal portal architecture with a few or no inflammatory cell (score 0); Mild portal inflammation and edema (score 1); Moderate portal inflammation and edema (score 2); Severe portal inflammation and edema (score 3).

Statistical Analysis

"SPSS for Windows 11.5" (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data was presented as mean (minimum, maximum) values for biochemical values. The groups were compared by using the nonparametric "Kruskal-Wallis test". For binary comparisons of continuous variables "Mann-Whitney U test" was used, and "Chi-square test" was used for categorical variables. p value of less than 0.05 was considered significant.

Results

In the Table I, the biochemical results of the study are summarized. Accordingly, levels of the inflammatory cytokines (IL-1β, IL-6, CRP and TNF-α) were increased in IO group and significantly decreased in IO+CAPE group compared to the IO group. In the literature, it has been demonstrated that interleukin-6 (IL-6) is one of the pro-inflammatory cytokines induced by oxidative and inflammatory stress, and the concentration of IL-6 is an important parameter in determining the severity of inflammatory damage[17]. Tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) are the other important cytokines[18], and C-reactive protein (CRP) is a non-specific parameter in determining the inflammatory response[19]. On the other hand, in our study, serum TOA levels were significantly decreased in IO+CAPE group compared to the IO group and serum PONX activity was significantly increased in IO+CAPE group compared to the IO group. These findings showed that oxidative stress associated with intestinal obstruction.

The biochemical and microbiological results were consistent with the results of histopathological examination; histopathologically, mucosa of the ileum and hepatic portal areas showed moderate inflammation, and some groups of bacteria were seen in the intestinal mucosal areas some of which showed subtotal atropfic changes in some villi in IO group (Figure 1 a,b,c,d) whereas those in IO+CAPE group showed a few blunted villi with mild inflammation and edema (Figure 2 a,b,c,d).

In the Table II, the histopathological scores of the ileum and liver were shown. Accordingly, the mucosal inflammation and mucosal damage scores of the ileum were higher in IO group ($p < 0.05$) and inflammation score of the liver were significantly higher in IO group than S group ($p < 0.05$). Liver inflammation scores of IO+CAPE group were also higher than S group ($p < 0.05$). The inflammation scores of the liver and ileum were significantly decreased in IO+CAPE group compared to the IO group ($p < 0.05$). Intestinal villi showed subtotal atrophic changes with shortening the lenghts and moderate to severe inflammation in IO group. Whereas the mucosal damage with atrophy and inflammatory changes were significantly decreased in IO+CAPE group compared to the IO group ($p < 0.05$).

Bacterial culture results were shown in the Table III. Accordingly; the bacterial culture results showed no differences between the groups in terms of peritoneal cultures. The MLN, spleen, liver and blood cultures were significantly positive in IO group compared to the S group (20%-90%; 10%-70%; 0%-70% and 0%-80% respectively). However, the rates of positive cultures of the MLN, spleen, liver and blood were significantly decreased in IO+CAPE group compared to the IO group (90%-20%; 70%-0%; 70%-0% and 80%-10% respectively). The bacteria isolated from the cultures, in order of frequency, were; *Escherichia coli* (25%), *Enterococcus faecalis* (20%), *Pseudomonas aeruginosa* (15%), and *Klebsiella* spp (15%), *Staphylococcus aureus* (15%) *Proteus mirabilis* (10%)

Discussion

Intestinal obstruction is known as one of the most important causes of abdominal emergencies[19]. Because intestinal mucosa can be injured with acute intestinal obstruction resulting in increased intestinal permeability. On the other hand, bacterial overgrowth in a damaged in-

Table I. Biochemical results of the study.

	SHAM (n = 10)	IO (n = 10)	IO + CAPE (n = 10)
TAS (mmol Trolox Eq./L)	0.72 ± 0.06	0.71 ± 0.09	1.16 ± 0.35[a,b]
PONX (U/L)	35.54 ± 8.52	18.68 ± 4.26[a]	36.91 ± 6.78[b]
TOS (μmol H_2O_2 Equiv./L)	12.14 ± 1.21	33.52 ± 10.58[a]	14.44 ± 2.13[b]
TNF-α (pg/mL)	1.93 ± 0.86	7.59 ± 1.72[a]	1.41 ± 0.47[b]
IL-6 (pg/mL)	31.25 ± 8.45	65.83 ± 20.44[a]	32.63 ± 7.90[b]
IL-1β (pg/mL)	0.47 ± 0.11	1.62 ± 0.59[a]	0.79 ± 0.64[c]
CRP (mg/L)	30.46 ± 4.64	165.26 ± 41.06[a]	85.50 ± 15.68[a,b]

Data were given as Mean ± SD. [a]Significantly different when compared with S group, ($p \leq 0.001$); [b]Significantly different when compared with IO group ($p \leq 0.001$); [c]Significantly different when compared with IO group ($p = 0.005$).

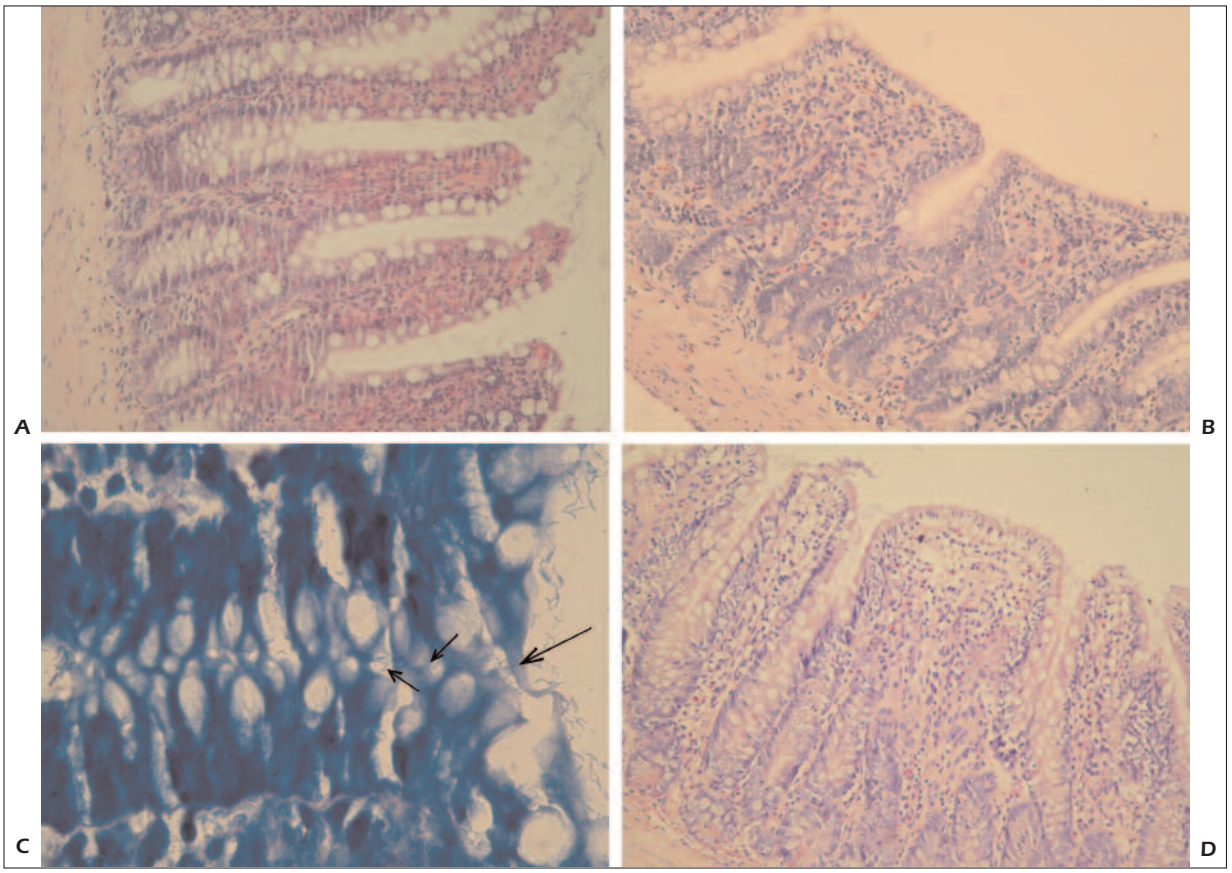

Figure 1. ***A,*** SHAM group. Normal appearing intestinal mucosa showing mild inflammation (H&E stain, ×100). ***B,*** IO group. Subtotal villous atrophic changes in the intestinal mucosa with moderate inflammation (H&E stain, ×100). ***C,*** IO group. Bacteria (*arrows*) infiltrating the intestinal mucosa with inflammatory cell infiltration (Giemsa stain, ×400). ***D,*** IO+CAPE group. A blunted villus with mild to moderate inflammation and nearly normal appearing villi in the intestinal mucosa (H&E stain, ×100).

testinal area disturbes the intestinal ecologic balance[20,21] and can precipitates bacterial translocation. It has been hypothesized that failure of the intestinal barrier function leading increased mucosal permeability is a major promoter of BT[22]. Also some studies supporting this hypothesis demonstrated that intestinal obstruction causes intestinal epithelial injury with subsequent increased mucosal permeability and BT[23-27]. As a result, bacterial overgrowth in disturbed indigenous flora may be translocated to the systemic circulation, MLNs and liver[28] and it is possible that translocated bacteria in the systemic circulation, MLNs and liver can lead to sepsis causing multiple organ failure[29,30]. So clinically it is very important to prevent oxidative stress, bacterial translocation and tissue damage caused by intestinal obstruction. But this might not be enough because in addition to injury of the intestinal mucosal barrier, deterioration of the balance of intestinal flora and immune disfunction can also stimulate bacterial translocation[31]. As a result, besides the prevention of bacterial translocation, supporting the host immune system can contribute to the reduction of complications arising from IO. In the literature, various studies indicate that selective use of antibiotics, prevention of intestinal mucosal damage and supporting immunologic mechanisms can prevent bacterial translocation[32] and also related complications. In accordance with the literature, the present study demonstrated that bacterial translocation was significantly increased in the IO group compared with the control. Also, histopathological examination showed increased inflammation with tissue injury in the intestinal mucosa and hepatic portal inflammation in the IO group. It has been demonstrated that caffeic acid phenethyl ester (CAPE), an active component of honeybee propolis extracts, has immunomodulatory, anti-inflammatory and antioxidant properties[8-11] in addition to antibacterial effect[12,13].

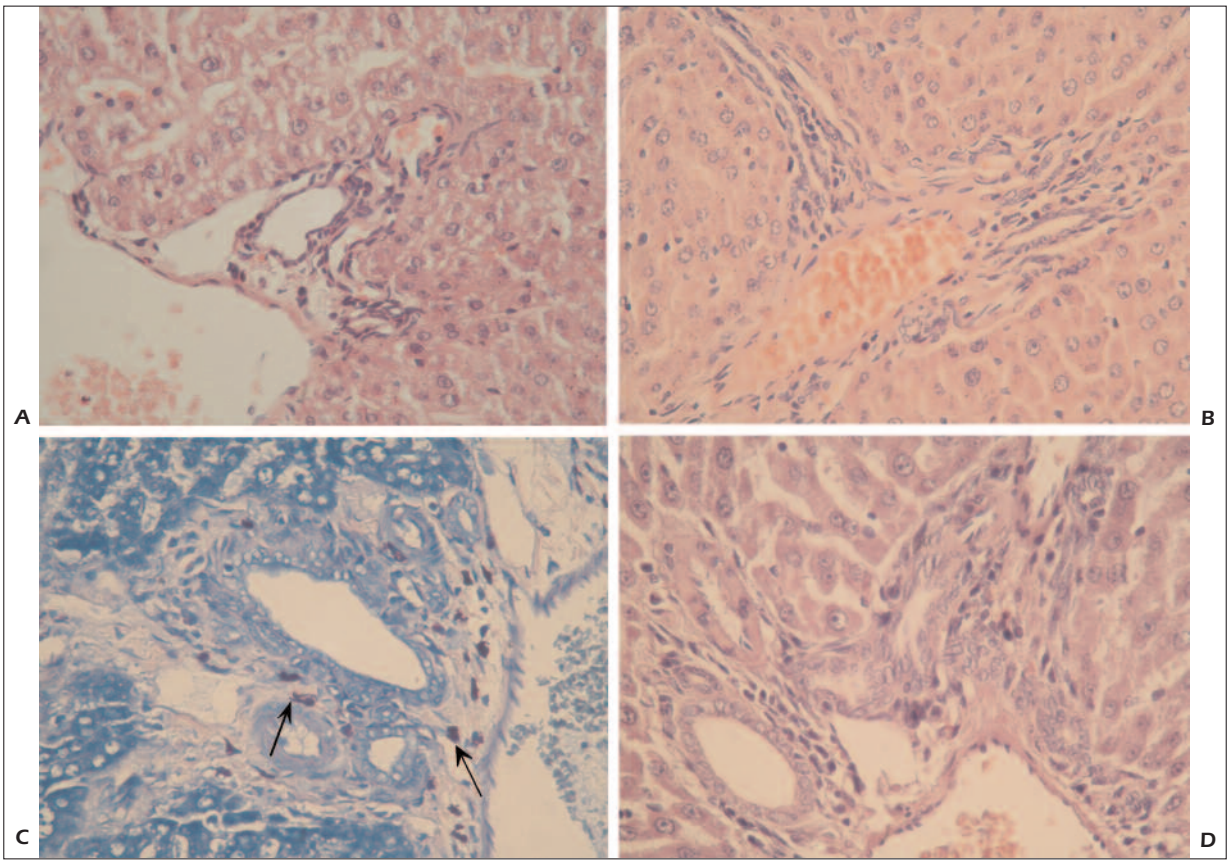

Figure 2. ***A,*** SHAM group. Portal area of the liver showing only mild edema with normal appearing periportal liver parenchyma (H&E stain, ×200). ***B,*** IO group. Portal area of the liver showes moderate lymphocytic infiltration and edema (H&E stain, ×200). ***C,*** IO group. Inflammatory cells (*arrows*) infiltrating a portal area of the liver (Giemsa stain, ×200). ***D,*** IO+CAPE group. Portal area of the liver showes only a few inflammatory cells and mild edema (H&E stain, ×200).

Table II. Microbiological culture results of the groups.

	SHAM (n = 10)	IO (n = 10)	IO + CAPE (n = 10)
Blood culture (c/d)	0/10 (0%)	8/10 (80%)	1/10 (10%)
Liver culture (c/d)	0/10 (0%)	7/10 (70%)	0/10 (0%)
Spleen culture (c/d)	1/10 (10%)	7/10 (70%)	0/10 (0%)
MLN culture (c/d)	2/10 (20%)	9/10 (90%)	2/10 (20%)
Peritoneal culture (c/d)	3/10 (30%)	3/10 (30%)	4/10 (40%)

Table III. Histopathological grading of the groups.

	SHAM (n = 10)	IO (n = 10)	IO + CAPE (n = 10)
Liver inflammation score	0.10 ± 0.32	1.30 ± 0.7[a]	0.50 ± 0.53[b]
Ileal mucosal injury score	1.10 ± 0.32	2.50 ± 0.71[a]	0.60 ± 0.52[b]

Data were given as Mean ± SD. [a]Significantly different when compared with S group ($p < 0.05$); [b]Significantly different when compared with IO group ($p < 0.05$).

Our study demonstrated that intraperitoneal administration of CAPE (10 μmol/kg day) decreased serum levels of proinflammatory cytokines (TNF-α, IL-6, IL-1β) and CRP suggesting antiinflammatory effect of CAPE, in addition, increased serum levels of antioxidant parameters (PONS, TAS) after IO. Also bacterial translocation rates in CAPE treated rats were significantly lower than those in the IO group. In accordance with these findings, histopathological examination showed that the intestinal mucosal injury score and hepatic portal inflammation score were significantly decreased in the CAPE treated group compared with the IO group. The increase in PONS and TAS levels in the CAPE treated group also suggested that antioxidant and free radical scavenging effects of CAPE. C-reactive protein (CRP), known as a marker of systemic inflammation, is the prototypical acute phase reactant. Cevikel et al[19] emphasized that serum CRP levels may be useful for predicting and determining the severity of bacterial translocation in cases of acute intestinal obstruction. In the present study, bacterial translocation rates were correlated with increased CRP levels in the serum whereas CAPE treated group showed significant decrease in CRP levels compared to the IO group.

In accordance with these findings, histopathological examination also showed that the intestinal mucosal injury score and the hepatic portal inflammation score were significantly decreased in the CAPE treated group. The protective effect of CAPE on the intestinal and hepatic tissue injuries in IO might be due to its immunomodulatory[8] and anti-inflammatory properties[33] in addition to inhibition of TNF-α. So these results suggest that CAPE might has potential anti-inflammatory, antibacterial, antioxidant and perhaps immunomodulatory beneficial effects in IO.

Conclusions

Intraperitoneal administration of CAPE might has potential antibacterial, antiinflammatory antioxidant and immunomodulatory effects in IO. Also these beneficial effects may be useful for patients with IO in the future. However, further studies are needed to evaluate exact antibacterial, antiinflammatory, antioxidant and immunomodulatory effects of CAPE.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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