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: Briefly, 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, anti-inflammatory, 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 IO¹². 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 functions¹. Bacterial overgrowth and intestinal mucosal damage, as a result of mucosal disruption, motility dysfunction and increased intestinal volume, induce the development of BT after IO⁴. 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-
ment of BT. 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. This situation can results in systemic inflammatory response, infection, sepsis, and multiple organ failure. 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 in addition to antibacterial effect.

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); Ileocecal junction dissection was performed only, not treated with drug. Group 2 (IO); Ileocecal junction dissection and ileal ligation, 1 cm proximal to caecum with 3-0 silk suture, were performed, not treated with drug. Group 3 (IO + CAPE); Ileocecal junction dissection and ileal ligation (1 cm proximal to caecum with 3-0 silk suture) were performed and CAPE applied in appropriate doses (10 µmol/kg, intraperitoneal) used in the literature. 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. Ileocecal junction was dissected after a midline incision performed. The distal ileum (1 cm proximal to caecum) was ligated with 3-0 silk suture, 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 necro-
sis factor-alpha (TNFα) were analyzed.

Measurement of the PONX

Serum PONX levels were measured spectropho-
tometrically by modified Eckerson et al method14.
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 produced15. In the assay, ferrous ion so-
lution in Reagent 1, is mixed with hydrogen per-
oxide in Reagent 2. The sequential produced radic-
als 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 preci-
sion values, lower than 3%. The results are ex-
pressed as nmol Trolox Equiv./mg protein.

Measurement of TOA

TOA of supernatant fractions was determined
using a novel automated measurement method, de-
veloped by Erel16. 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 medi-
um. On the other hand, the ferric ion makes a col-
ored complex with xylenol orange in an acidic
medium. The color intensity measured spectropho-
tometrically is related to the total amount of oxida-
tant molecules in the sample. The assay is calibrat-
ed with hydrogen peroxide and the results are ex-
pressed in terms of nmol H2O2 Equiv./mg protein.

Measurement of the IL1β, IL6,
CRP and TNFα

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

Histopathological Assessment

Tissue specimens from the liver and ileal seg-
ment irrigated with isotonic saline solution, were
put into the 10% formalin solution for 24 hours.
After routine histological tissue processing, all tis-
sues were embedded in paraffin blocks, prepared by
slicing 5 µm thick sections, stained with hema-
toxylin-eosin (H&E) and standard protocols were
applied. In addition, the tissue sections from in-
testinal mucosa, stained with Giemsa for detection
of any bacteria infiltrated. The ileal mucosa and
liver tissue samples were examined for inflamma-
tory cell infiltration, and ileal mucosal injury and
bacterial infiltration of the ileal segments were al-
so examined by a pathologist blinded to the study
groups, using light microscopy (Nikon ECLIPSE
80i, Tokyo, Japan) on ×100, ×200 and ×400 mag-
nifications. The inflammatory changes and intesti-
nal 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 inflam-
mation; Score 3, severe mucosal changes with to-
tal villous atrophy and inflammation.

Statistical Analysis

“SPSS for Windows 11.5” (SPSS Inc., Chi-ca-
go, IL, USA) was used for statistical analysis.
Data was presented as mean (minimum, maxi-
num) 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 cat-
egorical 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. Tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) are the other important cytokines, and C-reactive protein (CRP) is a non-specific parameter in determining the inflammatory response. 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 atrophic 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 lengths and moderate to severe inflammation in IO group. Whereas 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. 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>
<thead>
<tr>
<th>SHAM (n = 10)</th>
<th>IO (n = 10)</th>
<th>IO + CAPE (n = 10)</th>
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<tbody>
<tr>
<td>TAS (mmol Trolox Eq./L)</td>
<td>0.72 ± 0.06</td>
<td>0.71 ± 0.09</td>
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<tr>
<td>PONX (U/L)</td>
<td>35.54 ± 8.52</td>
<td>18.68 ± 4.26&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>TOS (µmol H₂O₂ Equiv./L)</td>
<td>12.14 ± 1.21</td>
<td>33.52 ± 10.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>1.93 ± 0.86</td>
<td>7.59 ± 1.72&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>IL-6 (pg/mL)</td>
<td>31.25 ± 8.45</td>
<td>65.83 ± 20.44&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>IL-1β (pg/mL)</td>
<td>0.47 ± 0.11</td>
<td>1.62 ± 0.59&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>CRP (mg/L)</td>
<td>30.46 ± 4.64</td>
<td>165.26 ± 41.06&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

Data were given as Mean ± SD. <sup>a</sup>Significantly different when compared with S group, (p ≤ 0.001); <sup>b</sup>Significantly different when compared with IO group (p ≤ 0.001); <sup>c</sup>Significantly different when compared with IO group (p = 0.005).
testinal area disturbs the intestinal ecologic balance 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. Also some studies supporting this hypothesis demonstrated that intestinal obstruction causes intestinal epithelial injury with subsequent increased mucosal permeability and BT. As a result, bacterial overgrowth in disturbed indigenous flora may be translocated to the systemic circulation, MLNs and liver and it is possible that translocated bacteria in the systemic circulation, MLNs and liver can lead to sepsis causing multiple organ failure. 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. 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 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 in addition to antibacterial effect.
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 shows 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 shows only a few inflammatory cells and mild edema (H&E stain, ×200).

**Table II.** Microbiological culture results of the groups.

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

**Table III.** Histopathological grading of the groups.

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<th>SHAM (n = 10)</th>
<th>IO (n = 10)</th>
<th>IO + CAPE (n = 10)</th>
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</thead>
<tbody>
<tr>
<td>Liver inflammation score</td>
<td>0.10 ± 0.32</td>
<td>1.30 ± 0.7a</td>
<td>0.50 ± 0.53a</td>
</tr>
<tr>
<td>Ileal mucosal injury score</td>
<td>1.10 ± 0.32</td>
<td>2.50 ± 0.71a</td>
<td>0.60 ± 0.52b</td>
</tr>
</tbody>
</table>

Data were given as Mean ± SD. *Significantly different when compared with S group (p < 0.05); †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 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 inflammation also showed that the intestinal mucosal injury score and 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 and anti-inflammatory properties 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.

References


