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The anti-translocation and anti-inflammatory effect of cinnamon oil in mice with TNBS induced colitis

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Abstract: The bacterial translocation induced by colitis may cause the organ failure and sepsis. Therefore, it is necessary to find new possibilities for prevention and therapy of this problem. The purpose of this study was to examine *Escherichia coli* anti-translocation activity of cinnamon oil and its ability to reduce colonic damage in mice with TNBS (2,4,6-trinitrobenzenesulfonic acid) induced colitis. Mice received cinnamon essential oil in four various concentrations (0.5%, 0.25%, 0.125% and 0.063%) in the powdery commercial rodent diet, starting 21 days before induction of TNBS colitis. The colonic damage was assessed using the colon macroscopic scoring system (Wallace score). *E. coli* translocation to the mesenteric lymphatic nodules was evaluated by serial dilutions method for counting bacteria. Bacterial translocation was significantly reduced in first and third group (15.2% or 42.8% in cinnamon oil groups versus 100% in TNBS group). Cinnamon oil was effective also against the colonic damage in all cinnamon oil groups (macroscopically scores of grade 9 in TNBS group versus 5.25, 5.63, 5.13 and 3.25 in cinnamon oil groups). Our results confirmed that dietary administration of cinnamon oil could possess potential therapeutic effects on bacterial translocation and intestinal wall injury in colitis.

Key words: bacterial translocation; *E. coli;* colitis; cinnamon oil

Introduction

The gastrointestinal epithelium forms a barrier that separates the finely regulated homeostasis of the body interstitium from the harsh environment of the intestinal lumen (Clayburgh et al. 2004). After TNBS administration, the intestinal mucosal barrier is disrupted by inflammation and ulceration. In these circumstances, translocation of enteric bacteria and their products through the bowel wall to extra-intestinal sterile sites may occur (Akcan et al. 2008). Bacterial translocation may cause secondary infection of intra-abdominal inflammatory processes, such as intra-abdominal abscesses, or peritonitis. With regard to endotoxemia and its relationship with severity of disease, increased intestinal epithelial permeability precedes clinical relapse (Weber et al. 2007), showing that a permeability alteration may be an early event in disease reactivation (Clayburgh et al. 2004). Intestinal inflammatory diseases are a serious problem in human as well as veterinary medicine. The current medicinal therapies for inflammatory gut diseases involve treatment with nonsteroidal anti-inflammatory drugs, antibiotics, corticosteroids, and immunosuppressant, but the application of these drugs is limited due to their toxicity and side effects (Lichtenstein et al. 2006). Therefore, there is an increased interest in finding a new alternative products for treatment, that enhance gut-barrier function to diminish, avoid, or prevent bacterial translocation and have fewer side effects. One possibility is using plant extract exhibiting various health-beneficial properties, such as anti-oxidative, anti-inflammatory and mainly antimicrobial effects (Srinivasan 2005).

Consequently, this research article discusses recent evidence of potential therapeutic effects of cinnamon essential oils on bacterial translocation and intestinal wall injury in colitis. Our observed results indicate that dietary administration of cinnamon oil in adequate concentrations can reduce the degree of colonic tissue damage, and bacterial translocation and hereby to improve TNBS induced colitis in mice.

Material and methods

Animals

Female mice of the ICR (imprinting control regions mice), weighing 23–28 g were obtained from Velaz (Prague, Czech Republic). The mice were maintained under standard conditions of temperature (21 ± 1°C), relative humidity (55 ± 10%), and 12 hours/12 hours light/dark cycle. All animal work was in compliance with the Animal Ethics Committee of the Institute of Animal Physiology SAS, Košice.

After a period of adaptation, animals were randomized into six groups: group a: colitic animals fed with the 0.5% cinnamon oil (5,000 ppm), group b: colitic animals fed with the 0.25% cinnamon oil (2,500 ppm), group c: colitic animals fed with the 0.125% cinnamon oil (1,250 ppm), group d: colitic animals fed with the 0.063% cinnamon oil (630 ppm), group e: animals with TNBS (2,4,6-trinitrobenzenesulfonic acid) induced colitis and group f: control sham animals

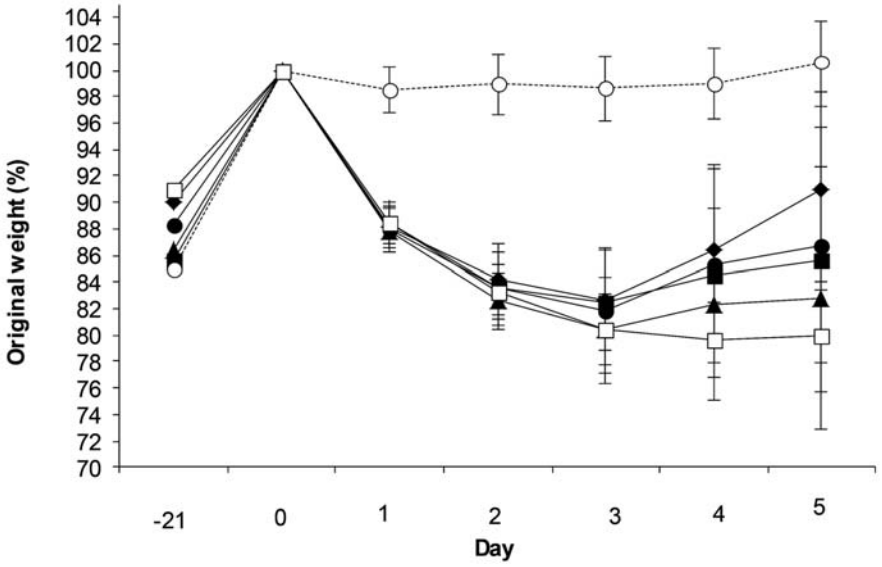

Fig. 1. Body weight changes. Changes in body weight are expressed as a percentage of the original weight on day 0. Values are arithmetical means ± SEM. Statistical significance of the differences between the group of untreated colitic animals (TNBS group) and other groups of animals was assessed using the Student *t*-test; $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$. a (●): colitic animals fed with the 0.5% cinnamon oil; b (■): colitic animals fed with the 0.25% cinnamon oil; c (▲): colitic animals fed with the 0.125 % cinnamon oil; d (◆): colitic animals fed with the 0.063 % cinnamon oil; e (□): animals with TNBS induced colitis; f (○): control sham animals. A: statistical difference between the e and a groups, (4, 5 day: $^{++}P < 0.05$); B: statistical difference between the e and b groups, (4 day: $^{++}P < 0.01$, 5 day: $^{+}P < 0.05$); C: statistical difference between the e and c groups; D: statistical difference between the e and d groups, (4, 5 day: $^{+++}P < 0.001$); E: statistical difference between the e and f groups, (1, 2, 3, 4, 5 day: $^{+++}P < 0.001$).

(treated only with ethanol). Number of animals for each group of treatment was 17 and experiment was repeated twice.

Essential plant extract

Cinnamon oil *(Cinnamomum zeylanicum)* obtained from Calendula (Nová Lubovna, Slovakia) (lot 5-029-002-10-04), in four various concentrations (0.5%, 0.25%, 0.125% and 0.063%) was served in experimental groups over all-time of experiment.

Cinnamon oil (500, 250, 125 resp. 63 µl) was dissolved in 1000 µl edible soya oil (Brölio, Germany) and added to 99 g powdery commercial rodent diet (Diet for laboratory mice and rats SPF, M1, Czech Republic). For dietary administration in positive control group TNBS and group sham without TNBS, edible soya oil was mixed with the powdery rodent diet to concentration of 1% (w/w). Diets were fed *ad libitum* during all experiment with starting 21 days before administration of TNBS enema (preventive model).

Induction of TNBS colitis

The mice were anesthetized with ketamine and xylazine, and colitis was induced by rectal instillation of TNBS as modified from Neurath et al. (1975). A 1.2 mm diameter catheter was advanced 4 cm into the colon, and of 30 µl/mouse of 2,4,6-trinitrobenzene sulfonic acid (TNBS-Fluka, Steinheim, Germany) dissolved in 0.5 ml of 50% ethanol in PBS (Phosphate Buffer saline) (vol/vol) was applied. The animal was held by the tail for 30 s to ensure uniform contact with colon mucosa. The control sham group received 50% ethanol alone using the same technique. Development of colitis was assessed daily by measurement of body weight. The mortality rate was observed during this study. The mice were killed by cervical dislocation 5 days after TNBS administration.

The colons were removed, cut longitudinally, and cleared of fecal material with gentle spray of 0.9% saline solution. The extent of mucosal damage was assessed using the colon macroscopic scoring system according Wallace et al. (1989). *Ulceration*: (1) focal hyperemia, no ulcer; (2) ulceration, no hyperemia/bowel wall thickening; (3) ulceration, inflammation at one site; (4) ulceration, inflammation at 2 or more sites; (5) major injury > 1 cm; 6–10 major damage > 2 cm. Adhesion: (1) minor (colon easily separated from other tissue); (2) major. Diarrhea: (1); Bowel wall thickening: (1).

E. coli translocation

The mesenteric lymphatic nodules were removed and the tissue were weighed separately and placed in a sterile grinding tube. The samples were homogenized with sterile sea sand in 1000 µl of PBS. After mechanical grinding, 500 µl of homogenate was transferred into a tube containing 4.5 ml of phosphate buffered saline and used to perform serial dilutions method for counting bacteria. From this dilution, 100 µl aliquots were plated onto McConkey No 3 CM 0115 agar (Oxoid) plates. All agar plates were incubated aerobically for 24 h at 37°C. Quantitative culture results were determined as the logarithm of the number of colony-forming units (cfu) per 10 mg of tissue, calculated from the formula: number of cfu × reciprocal of dilution × 10/weight of tissue.

Statistical analysis

Bacterial concentrations were represented as the logarithm cfu ± SEM per 0.01 g of mesenteric lymphatic nodules. The Mann-Whitney test was used to compare the rate of bacterial translocation and also macroscopic scoring system between TNBS group and cinnamon oil groups. Values of $P < 0.05$ were considered as statistically significant.

Results

As shown in Fig. 1, administration of TNBS caused a dramatic decrease in body weight (almost 20% after 3 days) (80.42 ± 2.64; percentage of the original weight ± SEM). Mice receiving 50% ethanol without

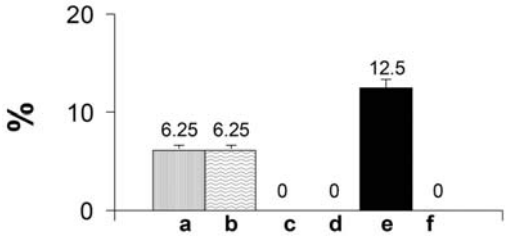

Fig. 2. Mortality rate. Results are shown as the percentage of dead animals in each experimental group. a: colitic animals fed with the 0.5% cinnamon oil; b: colitic animals fed with the 0.25% cinnamon oil; c: colitic animals fed with the 0.125% cinnamon oil; d: colitic animals fed with the 0.063% cinnamon oil; e: animals with TNBS induced colitis; f: control sham animals.

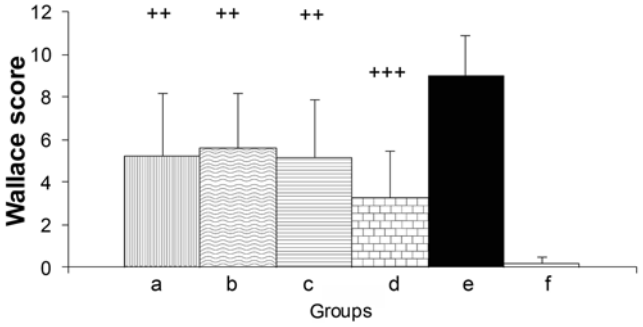

Fig. 3. Macroscopic scoring system are expressed as Wallace score ± SEM. a: colitic animals fed with the 0.5% cinnamon oil; b: colitic animals fed with the 0.25% cinnamon oil; c: colitic animals fed with the 0.125% cinnamon oil; d: colitic animals fed with the 0.063% cinnamon oil; e: animals with TNBS induced colitis; f: control sham animals; Statistical analysis: Mann-Whitney test $^{++}P < 0.01$; $^{+++}P < 0.001$.

TNBS (control sham group) showed only slight and transient loss of body weight (98.63 ± 2.43). In mice of cinnamon oil groups body weight was recovered gradually from day 3 when it became higher than the body weight of TNBS colitic animals (a: 81.82 ± 4.75; b: 82.44 ± 3.20; c: 80.35 ± 3.98; d: 82.65 ± 3.78). The mortality rate of mice with TNBS-induced colitis (e) was 12.5 ± 0.8 (% ± SEM), while that of the control sham group (f) was 0 ± 0. The mortality rate in cinnamon groups (a and b) was 6.25 ± 0.45 or 6.25 ± 0.47 and in cinnamon groups (c and d) was 0 ± 0 (Fig. 2). Macroscopic examinations showed severe injury and bowel wall thickening in colon in the TNBS group and slight oedema, in colon in the cinnamon oil treated groups. Ulceration in the intestines was severe in the TNBS group and slight in the cinnamon oil treated groups. In the positive control group, all mice developed diarrhoea and significant weight loss. Transmural colonic inflammation, with thickening, hyperaemia and macroscopic ulceration were seen in the TNBS group, yielding Wallace scores of grade 9.00 ± 1.875 (e). The comparable cinnamon oil treated groups yielded scores of 5.25 ± 2.938 (a), 5.63 ± 2.547 (b), 5.13 ± 2.75 (c) and 3.25 ± 2.188 (d). The macroscopic score of the sham group was 0.18 ± 0.298 (f). The results of macroscopic scoring system are summarized in Fig. 3. The decrease

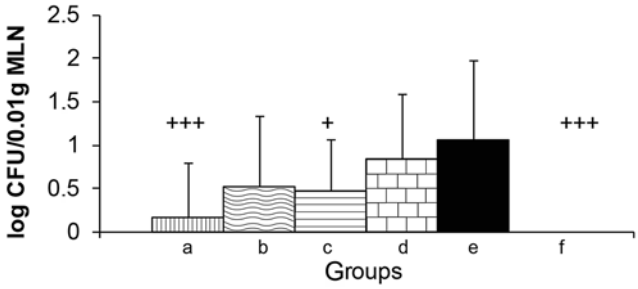

Fig. 4. *E. coli* translocation into mesenteric lymphatic nodules (MLN). The data are reported as log CFU/0.01 g ± SEM. a: colitic animals fed with the 0.5% cinnamon oil; b: colitic animals fed with the 0.25% cinnamon oil; c: colitic animals fed with the 0.125% cinnamon oil; d: colitic animals fed with the 0.063% cinnamon oil; e: animals with TNBS induced colitis; f: control sham animals; Statistical analysis: Mann-Whitney test $^{+}P < 0.05$; $^{+++}P < 0.001$

of bacterial translocation was recorded after application of all oil cinnamon concentrations, with statistical significance in two concentrations: 0.5% and 0.125%. The results are summarized in Fig. 4. The bacterial translocation in TNBS positive control group was 1.05 ± 0.92 (log CFU/0.01 g ± SEM), in presence of various cinnamon oils concentration bacterial translocation were 0.840 ± 0.74 in 0.063% cinnamon oil, 0.450 ± 0.59 in 0.125% cinnamon oil, 0.518 ± 0.80 in 0.25% cinnamon oil or 0.165 ± 0.616 in 0.5% cinnamon oil. Sham mice receiving only 50% ethanol intrarectally did not show any bacterial translocation to mesenteric lymph nodes.

Discussion

Various aromatic plants and their products have been reported to have health benefit properties (Baser et al. 2010). Cinnamon oil is involved in therapy of various diseases of the gastrointestinal tract, such as endotoxin shock, ischaemic bowel necrosis, infectious diarrhoea, ulcerative colitis and Crohn's disease (Capasso et al. 2000). The research work done in China has found that some herbs and their extracts, including cinnamon oil appear to have anti-inflammatory and soothing effects on the intestinal walls (Mau et al. 2001; Chao et al. 2005). These herbs help relieve spasm, ulceration, and inflammation. It has also been used to treat diarrhea and other problems of the digestive system. In present study, we examined whether dietary supplementation with cinnamon essential oil could have a protective effect on intestinal wall injury in colitis. We applied four concentrations (0.5%, 0.25%, 0.125% and 0.063%) of cinnamon essential oil to mice with TNBS-induced colitis, and found that administration of the two doses (0.125 and 0.063%), decreased the mortality rate from 12.5% (in animals with TNBS induced colitis) to 0 %, when others two higher concentration (0.5 and 0.25%) depressed mortality rate only to 6.25%. Furthermore, application of all cinnamon essential oil doses significantly accelerated the body weight gain recovery. Our results indicate that dietary administration of cinnamon essential oil significantly reduce the colonic dam-

age after TNBS administration. All tested concentrations of cinnamon oil (0.5%, 0.25%, 0.125%, 0.063%) produced significant amelioration of the colon inflammation in comparison with untreated mice. However the best results were achieved after application of the lowest concentration of cinnamon oil. The lower effectiveness was found after administration of higher doses of cinnamon oil that could be connected with possible cytotoxic effects of higher concentrations of this oil. In our preliminary experiments, we found negative cytotoxic effects of higher concentration of cinnamon essential oil on intestinal cells (Fabián et al. 2006). TNBS administration causes extensive colonic damage associated with marked defects in epithelial barrier and changes of intestinal permeability (Stein et al. 1998). Huang et al. 1993 reported that mucosal permeability increases because of mucosal atrophy in burns and this causes bacterial translocation. Seeing that, integrity of mucosal barrier is the major determinant of translocation, improvement of the colon damage and decreasing of mucosal permeability by application of cinnamon oil could help to reduce bacterial translocation.

In our study, bacterial translocation was lower in the cinnamon oil groups than in the TNBS group (76.2% in 0.063% cinnamon oil, 42.8% in 0.125% cinnamon oil, 49.52% in 0.25% cinnamon oil or 15.2% in 0.5% cinnamon oil versus 100% in TNBS group), that would be evoke moreover by antimicrobial properties of cinnamon essential oil (Lopez et al. 2005). The antimicrobial activities of various plant extract on the Gram (+) *Staphylococcus epidermidis* and the Gram (–) *Escherichia coli* F'lac K12 LE140, and on two yeast *Saccharomyces cerevisiae* 0425 δ/1 and 0425 52C strains were determined by Schelz et al. 2006. Our preliminary studies (Horosová et al. 2004) demonstrated dose depended antimicrobial activity of cinnamon essential oil. The preferable antimicrobial activity showed essential oil at higher doses, than lower ones. Unfortunately, effective high doses of essential oils had simultaneously strong cytotoxic effect on Caco-2 cells (Fabián et al. 2006). In accordance with this, we may assume that the presence of the evaluated oils at high concentrations in the digestive system could cause injury to intestinal cells. On the other hand, too low doses of essential oils can be insufficient to reduce the intensity of inflammation, such as also bacterial translocation. Thus, the use of optimal doses is essential for good efficacy of essential oils in attenuating inflammation.

The present data indicate that dietary administration of cinnamon oil in appropriate concentrations can reduce the degree of colonic tissue injury, and bacterial translocation and thereby ameliorate TNBS induced colitis in mice, however rather narrow range of proper concentrations is limiting for its therapeutic/preventive potential.

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References

Akcan A., Kucuk C., Sozuer E., Esel D., Akyildiz H., Akgun H., Muhtaroglu S. & Aritas Y. 2008. Melatonin reduces bacterial translocation and apoptosis in trinitrobenzene sulphonic acid-induced colitis of rats. World J. Gastroenterol. **14 (6):** 918–924. PMID: 18240350

Baser K.H.C. & Buchbauer G. 2010. Handbook of Essential Oils: Science, Technology and Applications. CRC Press, Boca Raton, London, New York, 994 pp. ISBN: 978-1-4200-6315-8.

Capasso R., Pinto L., Vuotto M. L. & Di Carlo G. 2000. Preventive effect of eugenol on PAF and ethanol-induced gastric mucosal damage. Fitoterapia **71 (1):** 131–137. DOI: 10.1016/S0367-326X(00)00188-X

Clayburgh D.R., Shen L. & Turner J.R. 2004. A porous defense: the leaky epithelial barrier in intestinal disease. Lab. Invest. **84:** 282–291. PMID: 14767487

Fabián D., Sabol M., Domaracká K. & Bujňáková D. 2006. Essential oils—their antimicrobial activity against *Escherichia coli* and effect on intestinal cell viability. Toxicology in Vitro **20 (8):** 1435–1445. DOI: 10.1016/j.tiv.2006.06.012

Horosová K., Bujňáková D. & Kmeť V. 2004. Antimikrobiálne účinky rastlinných silíc na *E. coli* z poodstavovej hnačky [Antimicrobial effects of essential oils on *E. coli* from post-weaning diarrhoea]. Slov. Vet. Čas. **29 (5):** 30–31.

Huang K.F., Chung D.H. & Herndon D.N. 1993. Insulin-like growth factor 1 (IGF-1) reduces gut atrophy and bacterial translocation after severe burn 2 injury. Arch. Surg. **128:** 47–54. DOI: 10.1001/archsurg.1993.01420130051009

Chao L.K., Hua K., Hsu H., Cheng S., Liu J. & Chang S. 2005. Study on the Antiinflammatory activity of essential oil from leaves of *Cinnamomum osmophloeum*. J. Agric. Food Chem. **53:** 7274–7278. DOI: 10.1021/jf051151u

Lichtenstein G.R., Abreu M.T., Cohen R. & Tremaine W. 2006. American Gastroenterological Association. American Gastroenterological Association Institute technical review on corticosteroids, immunomodulators, and infl iximab in inflammatory bowel disease. Gastroenterology **130:** 940–987. PMID: 16530532

Lopez P., Sanchez C., Batlle R. & Nerin C. 2005. Solid and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. J. Agric. Food Chem. **53 (17):** 6939–6946. DOI: 10.1021/jf050709v

Mau J.L., Chen C.P. & Hsieh P.C. 2001. Antimicrobial effect of extracts from Chinese Chive, Cinnamon, and Corni Fructus J. Agric. Food Chem**. 49 (1):** 183–188. DOI: 10.1021/jf000263c

Neurath M.E., Fuss I., Kelsall B. L., Stüber E. & Strober W. 1995. Antibodies to interleukin-12 abrogate established experimental colitis in mice. J. Exp. Med. **182:** 1281–1290. DOI: 10.1084/jem.182.5.1281

Schelz Z., Joseph M. & Judit. H. 2006. Antimicrobial and antiplasmid activities of essential oils. Fitoterapia **77 (4):** 279–285. DOI: 10.1016/j.fitote.2006.03.013

Srinivasan K. 2005. Role of spices beyond food flavoring: Nutraceuticals with multiple health effects. Food Rev. Int.**21:** 167–188. DOI: 10.1081/FRI-200051872

Stein J., Ries J. & Barrett K.E. 1998. Disruption of intestinal barrier function associated with experimental colitis: possible role of mast cells. Am. J. Physiol. **274 (Pt. 1):** G203–G209.

Wallace J.L., MacNaughton W.K., Morris G.P. & Beck P.L. 1989. Inhibition of leukotriene synthesis markedly enhances healing in an animal model of inflammatory bowel disease. Gastroenterology **96:** 29–36.

Weber C.R. & Turner J.R. 2007. Inflammatory bowel disease: is it really just another break in the wall? Gut **56:** 6–8. PMID: 17172583

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