Anti-Inflammatory Effects of Rosmarinus officinalis Essential Oil in Mice

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Abstract

Essential oils are plant secondary metabolites possessing various pharmacological properties. primarily anti-oxidative, antimicrobial or immunomodulative, but they can exhibit toxic and allergic effects as well. The aim of this study was to compare the effects of Rosmarinus officinalis essential oil dietary administration in carrageenan paw oedema and trinitrobenzene sulfonic acid (TNBS) colitis. ICR mice received rosemary essential oil at three concentrations (1 250, 2 500 and 5 000 ppm) in the standard laboratory diet starting two weeks before the experiments. The inflammation of paws induced by carrageenan application was evaluated by measurement of paw swelling, paw weight and myeloperoxidase activity. In the TNBS model the mice were killed by cervical dislocation 3 days after colitis induction and the mortality, changes in the body weight of mice, colon weight : body weight ratio, macroscopical scores and myeloperoxidase activity were analysed. Furthermore, IL-1B and IL-6 cytokine levels in colonic tissue were quantified using ELISA assay. Dietary supplementation with 5 000 ppm of rosemary essential oil initially after 2 h increased but after 24 h suppressed the extent of paw oedema. The same dose in the TNBS model exhibited protective effects on colonic mucosa and significantly decreased macroscopic scores for colonic inflammation. On the other hand, in colon samples from mice fed the diet with 1 250 ppm of rosemary essential oil we detected decreased myeloperoxidase activity and significantly lower levels of IL-6 compared to TNBS control animals.

Our results indicate that rosemary essential oil is able to influence several variables of murine experimental inflammatory models depending on the concentration used. We conclude that the anti-inflammatory effects of rosemary essential oil should be interpreted carefully due to its time-and dose-related effects.

Inflammation, TNBS colitis, carrageenan paw oedema, rosemary essential oil, cytokines

It is known that a large number of plant species contain various bioactive compounds that may have health-beneficial properties, anti-oxidative, anti-inflammatory and antimicrobial effects, and their preventive and therapeutic use is increasing. Various plant products have been already tested in different animal models of inflammation for the development of new preventive and curative remedies.

Carrageenan-induced mouse paw oedema has been widely used for analysis of the antiinflammatory effects of plant extracts and essential oils (Maruyama et al. 2006; Fernandes et al. 2007; Juhás et al. 2008a). Experimental colitis in mice and rats induced by the administration of various chemical agents is frequently used to study various compounds as possible therapeutic agents for inflammatory bowel disease (Ikeda et al. 2008). The intrarectal application of trinitrobenzene sulfonic acid (TNBS) produces severe necrotic lesions that heal after several weeks and leave scars and fibrosis as sequelae. Moreover, this model of colitis significantly increased IFN- γ levels in colonic samples only (Pérez-Navarro et al. 2005). Currently used therapies of inflammatory bowel disease seem to be suboptimal and consequently different complementary and alternative medicines are used for symptom relief and improved quality of life. Accordingly, many of these alternative therapies are able to modulate the immune system and disrupt the proinflammatory cascade through a variety of mechanisms, including antioxidant effects, alterations in

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Phone: +421 55 7287841 Fax: +421 55 7287842 E-mail: koppel@saske.sk http://www.vfu.cz/acta-vet/actavet.htm cell signalling (in particular the NF-kB pathway), cytokines, proinflammatory mediators, and disruption of bacterial flora (Clarke and Mullin 2008). These alternative treatments include herbal therapy consisting of anti-oxidant, anti-inflammatory and antimicrobial plants administration, too. We have described very recently that thyme essential oil in the diet was able to attenuate murine TNBS-induced colitis depending on the concentration used (Juhás et al. 2008a).

Rosmarinus officinalis is known as a common herb and household plant broadly used all around the world for different medicinal purposes, being a component of various established anti-inflammatory plant drug preparations, and having a long tradition of use for treating headaches, colds and colic, as well as other diseases (Darshan and Doreswamy 2004). Anti-oxidant properties of rosemary and their compounds have been confirmed recently (Cheung and Tai 2007; Atsumi and Tonosaki 2007); furthermore, rosemary extracts and their triterpenes (ursolic acid, oleanolic acid and micromeric acid) have been shown to exert anti-inflammatory activity *in vivo* (Altinier et al. 2007).

Up to now only a few studies have analysed the effects of rosemary or its essential oil on the gastrointestinal apparatus (Galisteo et al. 2000). Our aim therefore was to examine whether the dietary addition of rosemary essential oil could have positive effects in experimental intestinal inflammation induced by TNBS administration in mice.

Materials and Methods

Animals and treatment

Weight-matched ICR mice (5–6-week-old males from the Institute's breeding facility, weighing 28–32 g) were randomized into groups. The organization of the experiment, the investigations conducted, and the related documentation complied fully with legislative regulations governing the protection of experimental animals in the Slovak Republic. All animal experimentation was reviewed and approved by the Ethics Committee of the Institute of Animal Physiology.

Rosmarinus officinalis essential oil (Ph. Eur. 4) purchased from Calendula, a.s. (Nová Ľubovňa, Slovakia, lot 5-014-009-12-06, containing approx. 25% cineole, 19% α -pinene, 19% camphor, 17% *p*-cymene, 9% camphene, 5% β-pinene and 2% borneol) was added to powdery commercial rodent diet (Diet for laboratory mice and rats SPF, M1; František Machal, Ricmanice, Czech Republic) in 1% edible soy oil (Brölio, Germany) at the following concentrations: 5 000 ppm (wt/wt) - group A; 2 500 ppm (wt/wt) - group B; 1 250 ppm (wt/wt) - group C. The diet for control and sham groups was prepared similarly using only 1% edible soy oil. The diets were fed *ad libitum* during the experimental period starting 14 days before administration of TNBS enemas. The mice of experimental group D were treated with dexamethasone (DEXAMED, Medochemie Ltd., Limassol, Cyprus) at a dose of 3 mg/kg b.w. s.c. 2 h before carrageenan application (paw oedema model) or 0.25 mg/kg b.w. s.c. starting 2 h before TNBS instillation, and then every 24 h until the sacrifice of the animals. Dexamethasone has anti-inflammatory effects and attenuates the enhanced neutrophil infiltration in many animal inflammatory models including carrageenan paw oedema and TNBS colitis.

Carrageenan paw oedema

Male ICR were anaesthetized with the mixture [ketamine 5% (42.5%) / xylasine 2% (7.5%) / NaCl 0.9% (50%)] at 60 μ l/20 g body weight i.p. Each group of animals received subplantar administration of 50 μ l of saline to the left paw or 50 μ l of carrageenan 1% (w/v) (SIGMA, Steinheim, Germany) in saline to the right paw. The volume was measured using a Mitutoyo thickness gauge (Mitutoyo, No. 7313, Japan) immediately before subplantar injection, and 2, 4, 24 h thereafter. The increase in paw volume was calculated as the difference between the right paw volume (carrageenan) and the left paw volume (saline) measured at each time point. The mice were killed by cervical dislocation after the last time point (24 h) and the carrageenan paws were rapidly amputated at the tarsocrural joint and weighed on an analytical balance.

Induction of TNBS colitis

Mice were anaesthetized with the mixture [ketamine 5% (42.5%) / xylasine 2% (7.5%) / NaCl 0.9% (50%)] at 60 μ l/20 g body weight i.p. and colitis was induced by intrarectal administration of 120 mg/kg of the hapten reagent TNBS (Fluka, Steinheim, Germany) in 50% ethanol, and they were then kept in a vertical head-down position for 30 s. The sham group received 50% ethanol alone through the same technique. The total injection volume was 40 μ l. Development of colitis was assessed daily by the measurement of body weight. The mortality rate was observed during this study. Mice were killed by cervical dislocation 3 days after TNBS administration. Afterwards the colons were removed, opened longitudinally and cleared of faecal material with a gentle spray of 0.9% saline solution. The extent of mucosal damage was assessed using the colon macroscopic scoring system (Bukovská et al. 2007, adapted from Wallace et al. 1989).

		Table 1. Effects of the rose	mary essential oil dietary adm	inistration on carrageenan	-induced paw oedema in mice	
	u		Swelling thickness (10 ⁻² mm)		Right paw weight (10 ⁻² g)	MPO activity (AU/g)
		2 h	4 h	24 h		
Control	13	42.3 ± 9.3	73.0 ± 8.3	120.5 ± 34.1	26.6 ± 2.6	71.86 ± 28.35
DEX	12	46.2 ± 6.7	$37.7 \pm 7.0^{***}$	$47.0 \pm 9.3^{***}$	$21.6 \pm 1.1^{***}$	$37.75 \pm 13.36^{***}$
RoEO 1250	12	48.1 ± 7.6	71.8 ± 9.7	120.7 ± 33.3	26.5 ± 2.1	57.48 ± 17.29
RoEO 2500	12	39.8 ± 11.2	$60.3 \pm 12.2^{**}$	$94.6\pm18.8^*$	25.4 ± 1.9	60.51 ± 20.29
RoEO 5000	12	$56.2 \pm 10.7^{**}$	78.2 ± 13.5	$82.5 \pm 31.7^{**}$	25.4 ± 2.2	60.62 ± 19.52
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values are arithmetical means \pm DU. DEA – animals treated with 5 mg/kg dexamethasone; KOEU 129U - animals fed with 2500 ppm rosemary essential oil; ROEO 5000 - animals fed with 5000 ppm rosemary essential oil; ROEO 5000 - animals fed with 5000 ppm rosesmary essential oil; RoEO 5 $P \le 0.001$ $P \le 0.01$, 490 nm \times 100). Significant differences between control and experimental groups (Student's *t*-test): * $P \leq 0.05$, Ulceration: 1 - focal hyperaemia, no ulcer; 2 - ulceration, no hyperaemia/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.

Diarrhoea: 1.

Bowel wall thickening: 1.

After scoring, the detached colon was dried and weighed. The colon weight/body weight ratio was calculated as a marker of colonic inflammation. Immediately after weighing, the macroscopically most intensively affected segment was cut for assessment of IL-1 β and IL-6 protein expression and myeloperoxidase activity.

Myeloperoxidase (MPO) analysis

Myeloperoxidase activity correlates directly to the degree of neutrophil infiltration in the tissues. Samples (40-60 mg) of paws (carrageenan) or colonic samples (TNBS colitis) were homogenized on ice in 50 mM of phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide. After freezing and thawing for three cycles, samples were sonicated (90 s) and then centrifuged at 12 000 g at 4 °C for 30 min. The supernatant was diluted with distilled water (1 : 10). The MPO activity of the diluted supernatant (50 µl) was assessed in 96-well plates (Gama group a.s., Trhové Sviny, Czech Republic). Supernatant aliquots were added to 50 µl of the reaction mixture, which contained citrate buffer (1.8405 g Na, HPO₄, 0.511 g citric acid, 0.01 g menthiolate in 100 ml of distilled water), 4.62 mM of 1,2phenylenediamine, and 4.76 mM of H₂O₂ solution. MPO activities are presented as arbitrary units per g of paws or mg of colonic tissue. The enzyme activity was determined spectrophotometrically at 490 nm on a Microplate Spectrophotometer µQuant (BioTek Instruments, Winooski, VT, ÛSA).

Quantification of cytokine protein using ELISA

Colon tissue samples (20–30 mg) were snap frozen in liquid nitrogen and stored at -70 °C. They were homogenized in 800 µl of ice-cold PBS containing protease inhibitor P8340 (Sigma-Aldrich, Munich, Germany) and the homogenates were centrifuged at 12 000 × g at 4 °C for 15 min. Total protein in the tissue supernatants was determined using Bradford protein assay (Bradford 1976) with BSA as the standard. IL-1β and IL-6 concentrations were determined using ELISA assay, according to the manufacturer's instructions (Endogen, Pierce Biotechnology, Inc., Rockford, USA) on a Microplate Spectrophotometer µQuant (BioTek Instruments, Winooski, VT, USA).

Statistical analysis

The results are expressed as means \pm SD. Kruskal-Wallis and Mann-Whitney U tests were used for macroscopic damage scores. ANOVA and Duncan tests were applied for IL-1 β and IL-6 concentrations using ELISA assay. The Chi-square test was used to analyse differences in mortality rate. Student's *t*-test was used for differences in paw width and weight, the colon weight: body weight ratio and MPO enzyme activities. Values of P < 0.05 were considered significant.

Results

Mouse paw oedema

As early as the first measurements, 2 h after induction of paw oedema, we detected a significant increase in paw swelling in mice fed the diet with 5 000 ppm of rosemary essential oil (RoEO), compared to control mice (P <0.01, Table 1). In contrast to this, 24 h after carrageenan administration we observed a significant reduction in paw

	/body	weight ratio, bacterial	translocation, MPC) activity, IL-1 β and	IĽ-6 protein level	, ,))
	Sham	TNBS	DEX	RoEO 1250	RoEO 2500	RoEO 5000	
nber of treated mice	10	12	12	12	12	12	
nber of surviving mice	10	11	11	11	11	12	
tality (%)	0	8.33	8.33	8.33	8.33	0	
tive weight on day 3 (%)	$98.24 \pm 4.13^{***}$	81.92 ± 2.57	81.31 ± 2.61	82.85 ± 3.95	81.41 ± 1.75	82.76 ± 2.13	
on weight (% of b.w.)	$1.08 \pm 0.19^{***}$	1.77 ± 0.19	$1.58 \pm 0.31^{*}$	1.63 ± 0.35	1.92 ± 0.22	1.77 ± 0.38	
roscopic score	$1.0 \pm 2.16^{***}$	8.82 ± 1.66	7.09 ± 2.63	7.09 ± 2.26	7.73 ± 1.49	$6.75 \pm 1.49^{**}$	KW: ***
31	$33.4 \pm 5.6^{***}$	1059.4 ± 704.5	682.9 ± 476.6	670.9 ± 435.2	1338.7 ± 748.1	950.9 ± 499.0	ANOVA: ***
	$215.9 \pm 66.4^{***}$	1331.6 ± 460.7	$886.1 \pm 523.5^*$	$574.0 \pm 272.4^{***}$	1130.5 ± 447.0	1380.7 ± 543.1	ANOVA: ***
) activity ²	$13.83 \pm 10.1^{*}$	23.03 ± 6.4	19.11 ± 9.2	$17.59 \pm 5.2^{*}$	25.51 ± 6.8	20.57 ± 5.1	
			-	-	-		

Table 2. Effects of the rosemary essential oil dietary administration on TNBS induced colitis in mice - mortality, body weight changes, macroscopic score, colon weight

Kruskal-Wallis test; ¹(pg IL/mg total protein); ²(absorbance 490mm/mg × 10000); Sham - control animals (50% ethanol); TNBS - untreated colitic animals (TNBS + ethanol); RoEO 1250 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 5000 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 - colitic animals fed with 250 ppm rosemary essential oil; RoEO 2500 /alues are arithmetical means ± SD. Significant differences between untreated colitic animals and other groups of animals (weight and MPO activity t-test; bacterial translocation and macroscopic score Mann-Whitney test; mortality Chi-square test; IL-1 β and IL-6 protein Tevel Duncan test): $P \leq 0.05$, " $P \leq 0.01$, " $P \leq 0.01$, " $P \geq 0.$ colitic animals fed with 5 000 ppm rosemary essential oil oedema (P < 0.01). Mice fed with a lower (2 500 ppm) concentration of RoEO in the diet demonstrated a significant decrease in paw oedemas at later measurement points (4h P < 0.01, 24h P < 0.05), similarly as in the dexamethasone-treated (3 mg/kg)group where paw swelling was significantly reduced compared to the control group (4 h P < 0.001, 24 h P < 0.001). Paw weight and MPO activity of mice on diets with all three RoEO concentrations were decreased in comparison with control mice, but not significantly. We detected a significant decrease in paw weight (P < 0.001) and MPO activity (P < 0.001) in the dexamethasonetreated mice compared to controls.

TNBS induced colitis

The macroscopic damage scores and colon weight: body weight ratio of intact mice and mice in the sham group were significantly lower than that of mice in the TNBS group (P < 0.001, Table 2). Colon weight : body weight ratios in mice fed all concentrations of rosemary essential oil in diet were comparable with mice in the TNBS group. Treatment with dexamethasone caused a significant decrease in the colon weight : body weight ratio compared to the control TNBS group (P < 0.05). In contrast to this, the dexamethasone medication apparently did not protect the colon mucosa and we observed only a moderate non-significant decrease of macroscopic scores. RoEO addition to the diet induced a decrease in macroscopic scores, but the changes were not significant in groups on 1 250 ppm and 2 500 ppm RoEO-enriched diet compared to the control TNBS group. However, the macroscopic scores of the group on 5 000 ppm RoEO diet were significantly lower than those of the control mice (TNBS group) (P < 0.01).

Sham mice receiving only 50% ethanol intrarectally showed a significant reduction in MPO activity (P < 0.05) as well as lower concentrations of IL-1 β and IL-6 (P < 0.001) in colon samples compared to the TNBS-treated mice (Table 2). In the group treated with dexamethasone (0.25 mg/kg/day), the colon MPO activity and IL-1 β concentration

decreased in comparison with the control mice (TNBS) group, but the changes were not significant. In contrast, the levels of IL-6 protein in the colon were markedly reduced by dexamethasone treatment (P < 0.05). In the TNBS group and groups on the diets with 5 000 and 2 500 ppm of RoEO, the MPO activities and concentrations of IL-1 β and IL-6 protein in colon samples were comparable. However, we observed a significant reduction in colon MPO activity (P < 0.05) and IL-6 protein (P < 0.001) in mice on the 1 250 ppm RoEO-enriched diet compared to the untreated mice in the TNBS group.

Discussion

We analysed the anti-inflammatory effects of dietary administration of Rosmarinus officinalis essential oil in mice using carrageenan-induced mouse paw oedema and TNBSinduced colitis. A single dose of TNBS administered at the start of the experiment resulted in an acute local inflammatory response. Our results show that rosemary essential oil at a high concentration (5 000 ppm) in the diet was able to attenuate the colon inflammation as proved by the decreased macroscopic score. These facts could be hypothetically related to a synergy of biologically active compounds of rosemary essential oil, in particular their antiinflammatory as well as antimicrobial, anti-oxidative effects (Bozin et al. 2007), because luminal bacterial microflora plays an important role in the pathogenesis of TNBS-induced colitis in mice. In the paw oedema model we observed that higher concentrations of RoEO (5 000 and 2 500 ppm) in the diet decreased mice paw oedema 24 h after carrageenan application. However, we detected the augmentation of paw oedema in mice treated with 5 000 ppm RoEO in the diet 2 h after carrageenan application. It could be speculated that the highest concentration of RoEO (containing eucalyptol, α -pinene and borneol) inhibited acetylcholinesterase (Savelev et al. 2003) and produced vascular relaxation and plasma leakage during the first hours after carrageenan application (Ghisdal et al. 2005).

Rosmarinus officinalis essential oil contains a rich mixture of terpenes; in our preparation the most represented compound was 1.8-cineole (eucalyptol), which is known to have anti-inflammatory and antinociceptive effects (Santos and Rao 2000; Juergens et al. 2003). Therapeutic concentrations of cineole significantly inhibited cytokine production in lymphocytes (TNF- α , IL-1 β , IL-4 and IL-5) and in monocytes (TNF- α , IL-1 β , IL-6 and IL-8) with smaller effects on chemotactic cytokines (Juergens et al. 2004). Interestingly IL-6(-/-) mice compared with the wild-type mice, had significantly reduced intestinal inflammation as evidenced by epithelial damage, neutrophil infiltration, colon thickness and proinflammatory cytokine expression, following treatment with TNBS (Gay et al. 2006). Moreover, Santos et al. (2004) demonstrated that cineole administered by intrarectal instillation has therapeutic effects on TNBS-induced colitis in rats. In animals pre-treated (but not treated after TNBS administration), cineole significantly reduced the gross damage scores, wet weights (mg/cm) and myeloperoxidase activity of colonic segments. These data support our results indicating the protective effects of preventative rosemary essential oil in diet in TNBS colitis. At least two other components of this essential oil, α -pinene and borneol, could also be involved in the biological effects of rosemary essential oil. In a dose-related fashion, α -pinene inhibits the nuclear translocation of NF-kappa B induced by LPS in THP-1 cells (Zhou et al. 2004). TNBS-induced colitis in mice could be attenuated by antisense oligonucleotides inhibiting the p65 subunit of NF- kappa B, which suggests a critical role for NF- kappa B in mediating inflammatory responses (Kawada et al. 2007). We have shown very recently (Juhás et al. 2008b) that borneol (900 and 1800 ppm in diet) was able to suppress significantly proinflammatory cytokines (IL-1 β and IL-6) mRNA expression in TNBS colitis, although no significant morphological changes were detected. Similarly to the above experiments, in this study we have demonstrated reduced levels of IL-1 β (non-significant) and IL-6 (significant changes) protein in the colon tissue in the group fed with 1 250 ppm of rosemary essential oil in the diet compared to the TNBS

control group. Anti-inflammatory effects of 1 250 ppm of rosemary essential oil were confirmed also by the apparent decrease of neutrophil infiltration at the site of colonic inflammation reflected in the lower MPO activity.

Our study indicates that rosemary essential oil dietary application is able to affect murine experimental inflammatory models depending on the concentration used. Obviously, it is necessary to study in greater detail the immunomodulatory properties of rosemary extracts. We conclude, however, that the anti-inflammatory effects of rosemary essential oil should be interpreted with caution, due to its contradictory dose-related effects.

Protizápalové účinky éterického oleja z Rosmarinus officinalis u myší

Éterické oleje sú sekundárne metabolity rastlín, ktoré majú rozličné farmakologické vlastnosti, v prvom rade antioxidačné, antimikrobiálne alebo imunomodulačné, ale môžu tiež spôsobovať toxické a alergické účinky. Cieľom teito štúdie bolo porovnať účinky éterického oleja z *Rosmarinus officinalis* podávaného v krmiye na edém labky vyvolaný karagenanom a kolitídu indukovanú podaním kyseliny trinitrobenzénsulfónovej (TNBS). Myši kmeňa ICR boli kŕmené štandardnou laboratórnou diétou s prídavkom rozmarínového éterického oleja v troch koncentráciách (5000, 2500 a 1250 ppm) dva týždne pred začiatkom experimentu. Zápal labiek indukovaný aplikáciou karagenanu bol hodnotený meraním opuchu labiek, hmotnosťou labiek a aktivitou myeloperoxidázy. V TNBS modeli boli myši usmrtené cervikálnou dislokáciou 3 dni po indukcii kolitídy, pričom boli analyzované mortalita, zmeny telesných hmotností myší, pomer hmotnosti hrubého čreva a telesnej hmotnosti myší, makroskopické skóre a aktivita myeloperoxidázy. Navyše boli kvantifikované koncentrácie cytokínov IL-1 β a IL-6 v tkanive hrubých čriev pomocu ELISA kitu. Diéta s 5000 ppm rozmarínového éterického oleja na začiatku po 2h vyvolala zväčšenie ale po 24h zmenšenie rozsahu opuchu labky. Rovnaká dávka v modeli TNBS kolitídy mala ochranné účinky na mukózu kolónu a signifikantne znížila makroskopické skóre zápalu hrubého čreva indukovaného TNBS. Na druhej strane sme v porovnaní s TNBS kontrolnými zvieratmi zistili zníženie aktivity enzýmu myeloperoxidázy a signifikantne nižšiu koncentráciu IL-6 vo vzorkách hrubého čreva myší kŕmených s 1250 ppm koncentráciou rozmarínového éterického oleja v krmive. Naše výsledky svedčia o tom, že rozmarínový éterický olej v krmive môže ovplyvniť niektoré parametre experimentálnych modelov zápalu u myší v závislosti na dávke. Protizápalové účinky rozmarínového etérického oleja je potrebné interpretovať opatrne vzhľadom na ich závislosť na čase a použitej dávke.

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