Original Article

Effects of Borneol and Thymoquinone on TNBS-Induced Colitis in Mice

(inflammation / cytokines / colitis / borneol / thymoquinone)

Š. JUHÁS, Š. ČIKOŠ, S. CZIKKOVÁ, J. VESELÁ, G. IĽKOVÁ, T. HÁJEK, K. DOMARACKÁ, M. DOMARACKÝ, D. BUJŇÁKOVÁ, P. REHÁK, J. KOPPEL

Institute of Animal Physiology, Slovak Academy of Sciences, Košice, Slovak Republic

Abstract. Components of plant essential oils have been reported to have health benefit properties, including antioxidative, anti-tumour, antimicrobial, anti-stress, and immunomodulative activities. We examined the anti-inflammatory effects of thymoquinone, the active ingredient in the volatile oil of *Nigella sativa* seeds, and borneol, the active component of *Salvia officinalis* essential oil, on TNBS-induced colitis in mice. Thymoquinone was added to the commercial diet at a concentration of 0.05 % and borneol at two concentrations (0.09% and 0.18%) and fed to ICR mice 5 days before induction of TNBS colitis. Seven days after TNBS administration the mice were killed and macroscopic and histological scores were evaluated. Cytokine mRNA expression in colonic tissue was assessed using quantitative real-time RT-PCR. We did not detect any significant changes in macroscopic and histological scores between experimental and control groups, but we observed a significant decrease in proinflammatory cytokine (IL-1β and IL-6) mRNA expression in colon tissue in the 0.09% and 0.18% borneol-treated groups of mice in comparison to the control group. Surprisingly, we were not able to confirm anti-inflammatory effects of thymoquinone in TNBS colitis. In conclusion, our data show that borneol is able to significantly suppress proinflammatory cytokine mRNA expression in colonic inflammation, although no significant morphological changes are visible.

Introduction

A great number of plant species contain various chemical substances exhibiting health benefit properties, antioxidative, anti-inflammatory and mainly antimicrobial effects, and their preventive and therapeutic use is increasing. Recently, a number of natural products have been tested in animal models of colitis for the development of new therapeutics. The TNBS colitis model in mice and rats has been used to demonstrate the potential anti-inflammatory effects of curcumin, hange-shashin-to (HST) herbal formula, marine products (bolinaquinone and petrosaspongiolide M) and others (Sugimoto et al., 2002; Kawashima et al., 2004; Busserolles et al., 2005).

Thymoquinone is the main constituent of the volatile oil of *Nigella sativa* seeds (El-Dakhakhny, 1963). Thymoquinone has been shown to be a potent inhibitor of eicosanoid generation, namely thromboxane B_2 and leucotrienes B_4, by inhibiting both cyclooxygenase and lipooxygenase, respectively (Houghton et al., 1995). Both the volatile oil and thymoquinone have been found to produce a significant dose-dependent anti-inflammatory effect as evidenced by the significant inhibition of carrageenan-induced oedema formation in rat hind paws and reduction of cotton seed pellet granuloma weight in rats (Mutabagani and El-Mahdy, 1997). Thymoquinone administration leads to improvement in experimental allergic encephalomyelitis (EAE), presumably due to its inhibition of oxidative stress (Mohamed et al., 2003). Borneol, a rare variety of camphor, is a bi-cyclic monoterpene which is synthesized from turpentine oil or camphor, and is present in the essential oils of numerous medicinal plants, including sage (*Salvia officinalis*), valerian (*Valeriana officinalis*), chamomile (*Matricaria chamomilla*), rosemary (*Rosmarinus officinalis*), and lavender (*Lavandula officinalis*). Extracts of these plants are used traditionally to relieve anxiety, restlessness and insomnia. Borneol is a common ingredient in many traditional Chinese herbal formulas with current Chinese name *bingpian*. It is used more frequently for topical applications than for internal use. These applications are numerous, but especially apply to injuries, burns, rheumatic pains, haemorrhoids, skin diseases, and ulcerations of the mouth, ear, eye, and nose. Moreover, it stimulates the digestive system by increasing production of

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Corresponding author: Juraj Koppel, Institute of Animal Physiology, Slovak Academy of Sciences, Šoltésovej 4-6, 040 01 Košice, Slovak Republic. Fax: (+421) 55 7287842; e-mail: koppel@saske.sk

Abbreviations: PCR – polymerase chain reaction, TNBS – 2,4,6-trinitrobenzene sulphonic acid.

gastric juices; tones the heart and improves circulation; treats bronchitis, coughs and colds; reduces swelling; relieves stress; and can be used as a tonic to promote relaxation and reduce exhaustion (Buchbauer et al., 1992). So far, only a few studies have been published about the effects of plant monoterpenes and quinones on the gastrointestinal system. Our aim, therefore, was to examine whether dietary addition of borneol and thymoquinone could have positive effects in experimental intestinal inflammation induced by TNBS administration in mice.

Material and Methods

Animals and treatment

Male mice (ICR strain, Velaz, Prague, Czech Republic; 6 weeks old) were randomly divided into three experimental (N = 16) and two control (positive; N = 15, and sham; N = 12) groups. All animal experimentation was reviewed and approved by the Ethical Committee of the Institute of Animal Physiology.

Thymoquinone (purity 99 %) and [(1S)-endo]-(-)-borneol] (purity 98 %) from Sigma-Aldrich Chemie (Steinheim, Germany) were added to commercial rodent diet (Diet for laboratory mice and rats SPF, M1; Frantisek Machal, Ricmanice, Czech Republic) in 1% edible soya oil (Brölio, sidlo?, Germany) and fed to mice (for 5 days before colitis induction and 7 days during colitis) at the following concentrations: thymoquinone – 0.05% (corresponding to a daily intake of 75 mg/kg), borneol – 0.09% and 0.18% (corresponding to 135 mg/kg and 270 mg/kg/day). The thymoquinone dose (continuous) was 7.5 times higher than the effective dose (single administration) in acetic acid colitis in rats (Mahgoub, 2003), and the borneol doses were derived from our previous experiments (Domaracky et al., 2007) with sage essential oil (containing 18 % of borneol). The above doses were tested in preliminary experiments which showed that average food intake and body weight gains were similar to the control animals. Control animals were fed the diet with the vehicle only (1 % edible soya oil). Feed and water were available *ad libitum*.

TNBS colitis

The mice were anaesthetized with ketamine and xylazine, colitis was induced by intrarectal administration of 70 mg/kg of the hapten reagent TNBS (Fluka Chemie, Buchs, Switzerland) in 50% ethanol, and the animals were then kept in a vertical position for 30 seconds. The sham group received 50% ethanol alone using the same technique. The total injection volume was 40 µl. 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 seven days after TNBS administration. The colons were removed, opened longitudinally and cleared of faecal material with gentle spray of 0.9% saline solution. The extent of mucosal damage was assessed using the colon macroscopic scoring system adapted from Wallace et al. (1989). *Ulceration*: 1 – focal hyperaemia, no ulcer; 2 – ulceration, no hyperaemia/bowel wall thickening; 3 – ulceration, inflammation at one site; 4 – ulceration, inflammation at two 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 blotted dry and weighed. The colon weight/body weight ratio was calculated. Immediately after weighing, the macroscopically most intensively affected segment was cut longitudinally into strips for microscopic examination and assessment of tissue cytokine levels.

Histopathological assessment of colonic damage

The colons of seven mice from each group were used for microscopic examination. For histological evaluation, colon tissues (cca 5 mm x 5 mm) were fixed in 4% formalin in 0.1 M phosphate buffer, dehydrated with increasing concentrations of ethanol, embedded in paraffin, and sectioned. Sections (4–6 µm thick) were mounted on slides, cleared, hydrated and stained with haematoxylin and eosin. The slides were examined and photographed with an Olympus BX51 microscope (Olympus, Tokyo, Japan). Histological changes were classified according to Ameho's score (Ameho et al., 1997).

Real-time RT-PCR quantification of cytokine mRNA expression

We developed a quantitative real-time RT-PCR assay for cytokine mRNA quantification in the used model (TNBS-induced colitis in mice). Firstly, primers for selected cytokines and housekeeping genes were designed and PCR reactions were optimized (regarding specificity of obtained PCR products and efficiency of PCR amplifications). Afterwards, preparation of the RT-PCR template for construction of standard curves was optimized (to achieve similar amplification conditions in standards and samples, and correlation coefficients of standard curves in the range from 0.99 to 1.0). Finally, to normalize cytokine mRNAs quantity, housekeeping genes were tested to find those with stable expression in the used experimental model.

Informatics analysis, oligonucleotide primers

Gene sequences were obtained from GenBank (available at http://www.ncbi.nlm.nih.gov/). Primer design and optimization regarding primer dimer, self-priming formation, and primer melting temperature was done with Primer 3 software (Rozen and Skaletsky, 2000, available at http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). To check the primers specificity, the

Table 1. Oligonucleotide primers for real-time PCR

Gene name [a]	Primer sequence (5‘- 3‘) [b]	Position cDNA [c]	Accession # [d]
IL-1β	FP: AAGTGATATTCTCCATGAGCTTTGT	535	NM_008361
	RP: TTCTTCTTTGGGTATTGCTTGG	700	X04964
IL-6	FP: TGGGAAATCGTGGAAATGAG	209	NM_031168
	RP: CTCTGAAGGACTCTGGCTTTG	462	M20572
IL-10	FP: CAACATACTGCTAACCGACTCCT	261	NM_010548
	RP: TGAGGGTCTTCAGCTTCTCAC	433	M84340
TNF-α	FP: CGTCGTAGCAAACCACCAAG	438	NM_013693
	RP: TTGAAGAGAACCTGGGAGTAGACA	587	M38296
GM-CSF	FP: GCAATTTCACCAAACTCAAGG	277	NM_009969
	RP: CTCATTACGCAGGCACAAAAG	521	X03020
IFN-γ	FP: ATCAGGCCATCAGCAACAAC	360	NM_008337
	RP: ATCAGCAGCGACTCCTTTTC	578	NT_039501
β-act	FP: AAATCGTGCGTGACATCAAAG	700	NM_007393
	RP: AAGAAGGAAGGCTGGAAAAGAG	880	NT_081055
HPRT	FP: TGGATACAGGCCAGACTTTGTT	603	NM_013556
	RP: ACTTGCGCTCATCTTAGGCTTT	757	NT_039706
SDHA	FP: CATGCCAGGGAAGATTACAAAG	1806	NM_023281
	RP: AGTAGGAGCGGATAGCAGGAG	2025	NT_078713

Primers for β-actin are located on the same exon, other primers are located on different exons.
[a] IL, interleukin; TNF-α, tumour necrosis factor α; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon γ ; β-act, β actin; HPRT, hypoxanthine guanine phosphoribosyl transferase 1; SDHA, succinate dehydrogenase complex subunit A; B2M, β-2 microglobulin
[b] FP, forward primer; RP, reverse primer
[c] Position of the first (5‘) primer nucleotide within the cDNA sequence
[d] GenBank accession numbers of cDNA (upper lines) and genomic (lower lines) sequences

query of GenBank database with primer nucleotide sequences was carried out with the Blast program (Altschul et al., 1997). Analysis of PCR products for recognition sites of restriction enzymes was performed using the program Webcutter 2.0 (available at http://rna.lundberg.gu.se/cutter2/). PCR products were verified by agarose gel electrophoresis, by digestion with appropriate restriction enzymes and by melting curve analysis in real-time PCR system Mx 3000P (Stratagene, La Jolla, CA). Oligonucleotide primers (Table 1) were synthesized at Merck (Darmstadt, Germany).

Isolation of total RNA and cDNA synthesis

Total RNA was isolated from the mouse colon (about 15 mg of tissue for each sample) with TRIzol Reagent (Invitrogen Life Technologies, Karlsruhe, Germany) according to the manufacturer’s instructions. Total RNA preparations were then cleaned and DNase I treated with RNeasy Micro kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. In order to quantify total RNA extracted from each sample, optical density at 260 nm was measured. The integrity of the RNA was assessed by denaturing agarose gel electrophoresis.

The RNA (0.75 μg from each sample) was reverse transcribed at 42°C for 1 h in 30 μl containing 300 units of Superscript™ II Rnase H– Reverse Transcriptase (Invitrogen Life Technologies), 7 μM anchored oligo dT13VN, 50 mM Tris-HCl pH 8.3, 3 mM $MgCl_2$, 75 mM KCl, 10 mM DTT, 500 μM dNTPs (dATP, dTTP, dCTP, dGTP), 60 units RNase OUT™ (Recombinant Ribonuclease Inhibitor, Invitrogen Life Technologies) and 0.75 μg acetylated bovine serum albumin (BSA). The reaction was terminated by heating at 95°C for 5 min. To check for the presence of genomic DNA contamination in the RNA preparations (forward and reverse primers for β-actin are located on the same exon), reverse transcriptase negative control (no reverse transcriptase in the reaction) was carried out in parallel, using the RNA pool prepared from aliquots of all RNA samples.

Relative standard curve generation

The pool of murine colon RNA gained from aliquots of all samples served as standard RNA. The standard RNA preparation was serially diluted and cDNA was synthesized from each dilution as described above. To compensate for different RNA amounts in the reverse transcription reactions yeast, total RNA was added in appropriate amounts to the colon RNA dilutions (all oligonucleotide primers were checked so as not to create any PCR product on the yeast cDNA template). The standard curve was generated using Mx 3000P 2.0 soft-

ware (Stratagene) by plotting cycles at threshold fluorescence (Ct) against the logarithmic values of standard RNA amounts (standard RNA amounts were expressed as dilution factors of the standard RNA preparation).

Real-time PCR and data analysis

PCR reactions were carried out in a 20 μl final volume in duplicates using SYBRGreen I as a fluorescent detection dye. The reactions contained 0.8 μl of cDNA (corresponding to 20 ng of sample total RNA), 1 unit of Platinum Taq DNA polymerase (Invitrogen Life Technologies), SYBRGreen I in final dilution 1 : 25000 (Sigma-Aldrich, Munich, Germany), 30 nM ROX (passive reference dye for correction of non-PCR related fluctuations in fluorescence signal, Stratagene), 0.2 mM dNTPs (dATP, dTTP, dCTP, dGTP), 50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM $MgCl_2$ (except for the β-actin reaction, where 1.5 mM $MgCl_2$ was used), forward and reverse primer in final concentration 0.25 μM (for *IL-1β*, *IL-12β*, *TNF-α*, *IFN-γ*, *HPRT* and *SDHA*) or 0.5 μM (for *IL-6*, *IL-10*, *GM-CSF* and *β-act*; see Table 2 for full gene names). PCR amplification was performed in the real-time PCR system Mx 3000P (Stratagene). After an initial step at 95 °C for 2 min (DNA denaturation and hot start DNA polymerase activation), 40 cycles with the following thermocycling conditions were carried out: 94 °C for 30 s, specific annealing temperature for 30 s, 72 °C for 30 s, specific temperature at which the fluorescence was acquired ("acquiring temperature") for 30 s. Measurement of fluorescence at an elevated temperature ("acquiring temperature", a few degrees of Celsius below the melting temperature of the specific PCR product) enables elimination of the fluorescence signal produced by incidental short non-specific PCR products. Amplification specificity was then checked by generation of a melting curve using 41 cycles with temperature increments of 1°C (starting with 55 °C) and fluorescence measurement in each cycle. Specific annealing, acquiring, and melting temperatures are shown in Table 2.

For each gene, standard cDNAs were amplified along with sample cDNAs in the same PCR run. The relative amount of target mRNA in each sample was determined from the relative standard curve (using sample Ct value) and expressed in arbitrary units corresponding to dilution factors of the standard RNA preparation. To ensure correctness of the quantification we normalized cytokine expression to the expression of three housekeeping genes. Firstly, expression stability of several housekeeping genes was tested using geNorm software (Vandesompele et al., 2002). Subsequently, the normalization factor for each sample was calculated (by geNorm software) as the geometric mean of the relative amounts of the three most stable housekeeping genes – *HPRT*, *SDHA* and *β-act* (see Table 2 for full gene names). Finally, the relative amount of cytokine mRNA in each sample was divided by the normalization factor of the sample. Normalization of cytokine expression to the HPRT expression (the most stably expressed housekeeping gene) and to the input of total RNA was also performed.

Statistical analysis

The results are expressed as mean ± SEM. The Kruskal-Wallis test and the Mann-Whitney U test were used for macroscopic and microscopic damage scores. The χ^2 test was used to detect differences in mortality rate. The Student t-test was used for the body weight and the colon weight/body weight ratio. The Kruskal-Wallis test was used for the comparison of differences in cytokine expression between groups and the Mann-

Table 2. PCR product sizes, DNA fragments after digestion with restriction enzymes, annealing, melting and acquiring temperatures

Gene name [a]	PCR product [b]	RE [c]	Ta [d]	Tm [e]	Tacq [f]
IL-1β	166 bp	Hinf I: 80+86 bp	64°C	86°C	82°C
IL-6	254 bp	FokI: 128+126 bp	66°C	83°C	80°C
IL-10	173 bp	EcoRI: 142+31 bp	66°C	86°C	82°C
TNF-α	150 bp	HaeIII: 68+82 bp	64°C	90°C	80°C
GM-CSF	245 bp	Hinf I: 202+43 bp	64°C	86°C	82°C
IFN-γ	219 bp	HinfI: 33+147+26+13 bp	62°C	88°C	82°C
β-act	181 bp	FokI: 63+118 bp	70°C	89°C	82°C
HPRT	155 bp	HinfI: 91+64 bo	68°C	83°C	80°C
SDHA	220 bp	FokI: 44+176 bp	65°C	87°C	80°C

[a] as in Table 1

[b] Sizes of PCR products in base pairs (bp)

[c] Lengths of DNA fragments (in base pairs) after digestion of PCR products with indicated restriction enzymes

[d] Ta, annealing temperatures used for thermal cycling

[e] Tm, melting temperatures of the PCR products determined by melting curve analysis

[f] Tacq, temperature at which the fluorescence signal was acquired ("acquiring temperature")

Whitney U test was used to compare difference between the group of untreated colitic animals and other groups of animals. Values of $P < 0.05$ were considered as significant.

Results

Body weight changes, mortality, macroscopic score

TNBS administration caused a dramatic decrease in body weight (15% after four days), which recovered gradually from day 5 but not fully to the initial weight in 7 days (Table 3). Sham control (receiving 50% ethanol without TNBS) showed transient loss of body weight. Statistically significant differences were observed only between TNBS control and sham groups (50% ethanol) on days 1–7 ($P < 0.001$). The mortality rates (Table 3) of mice with TNBS-induced colitis ranged from 12.5% (borneol high dose) to 37.5% (thymoquinone); there were no significant differences in the mortality rate between experimental and control groups, however. The macroscopic damage scores and colon weights of mice (Table 3) in the sham controls were significantly lower than those of mice in the TNBS controls. Colon weight and macroscopic damage scores in mice treated with both concentrations of borneol or thymoquinone were not significantly different from those of TNBS controls.

Histological findings in colon

The histological scores (Table 3) of sham mice were significantly lower than those of TNBS mice with colitis ($P < 0.05$). In the sham mice, no signs of leukocyte infiltration in the colon were observed (data not shown). There were great variances of histological findings in TNBS-treated mice. In all groups with TNBS-induced colitis, the entire colonic wall became thick due to oedema. Major lesions due to colitis were observed in the distal parts of the colon, and focal ulcers were detected in approx. 33% of colon samples from TNBS-treated mice. Distortion of crypts, loss of goblet cells, and infiltration of mononuclear cells were also observed (data not shown). The changes in Ameho scores between TNBS-treated groups were not significant (Table 3).

Real-time RT-PCR quantification of cytokine mRNA expression

Primer design and confirmation of primer specificity

Intron spanning primers (except for primers for β-actin) giving PCR products with size 150–250 bp were designed. Sequences and locations of the primers are shown in Table 1. Specificity of PCR amplification for each gene was confirmed by melting curve analysis and resulted in a single melting peak (melting temperatures for each gene product are shown in Table 2). In addition, agarose gel electrophoresis of each PCR amplification revealed a single product with the desired length (Table 2). Digestion of the PCR products with appropriate restriction enzymes resulted in DNA fragments with expected lengths (Table 2).

Expression of cytokine mRNA

Relative amounts of IL-1β, IL-6, IL-10, GM-CSF, IFN-γ and TNF-α mRNAs (for full gene names see Table 2) were significantly higher in animals with TNBS-induced colitis compared to the sham controls (Table 4). In TNBS-treated mice fed with borneol (both doses), the amounts of IL-6 and IL-1β mRNA were significantly ($P < 0.05$) lower compared to TNBS controls (Table 4). In contrast to that, we found no significant changes in cytokine mRNA amounts between TNBS-treated mice fed with thymoquinone and control TNBS mice (Table 4). Normalization of cytokine expression to the HPRT expression (the most stably expressed housekeeping gene) and to the input of total RNA showed

Table 3. Mortality, body weight changes, macroscopic and microscopic score, colon weight /body weight ratio

	Sham	**TNBS**	**TNBS +B 0.09**	**TNBS +B 0.18**	**TNBS +T**	
Number of used mice	12	15	16	16	16	
Number of surviving mice	12	12	12	14	10	
Mortality (%)	0	20	25	12.5	37.5	
Relative weight on day 7 (%)	104.8±0.57***	93.3±2.827	96.15±2.883	95.54±3.259	91.05±3.416	
Macroscopic score	0±0***	3.92±1.003	3.75±0.962	2.5±0.478	4.5±0.847	KW: ***
Microscopic score	0±0*	2.29±0.97	3.43±0.81	2.79±0.93	4.36±0.5	KW: **
(Colon weight / Body weight) x 100	1.57±0.054**	2.17±0.197	2.09±0.147	1.93±0.0144	2.37±0.163	

Values are arithmetical means ± SEM

Statistical difference between untreated colitic animals and other groups of animals (weight t-test; score Mann-Whitney test) : * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$,

KW, Kruskal-Wallis test

Sham, control animals; TNBS, untreated colitic animals; TNBS+B 0.09, colitic animals fed with 0.09 % borneol; TNBS+B 0.18, colitic animals fed with 0.18 % borneol ; TNBS+T, colitic animals fed with thymoquinone

Table 4. Cytokine mRNA expression

	Sham	TNBS	TNBS +B 0.09	TNBS +B 0.18	TNBS +T	
IL-1β	4.78±0.86**	490.8±200.3	40.4±6.83*	32.8±10.2*	578.3±166.0	KW: ***
IL-6	11.86±2.75**	393.6±146.2	38.2±11.5*	49.9±16.9*	521.8±103.5	KW: ***
GM-CSF	54.8±3.6**	513.8±225.4	126.6±20.0	98.7±15.3	327.1±102.1	KW: **
IFN-γ	19.4±4.9**	393.2±139.5	98.9±22.6	141.7±36.6	191.9±43.7	KW: ***
TNF-α	36.3±7.37*	267.0±121.4	222.5±44.9	129.8±27.5	346.0±58.2	KW: **
IL-10	182.5±21.7*	339.4±48.0	237.8±40.5	313.6±48.9	401.6±82.3	KW: *

The relative amount of cytokine mRNA in each sample was divided by the normalization factor (geometric mean of HPRT, SDHA and β-act amount) of the sample.
Values are arithmetical means ± SEM; number of samples (animals) in each experimental group was 6–8. Statistical difference between untreated colitic animals and other groups of animals (Mann-Whitney test):
* $P \leq 0.05$, ** $P \leq 0.01$ KW, Kruskal-Wallis test: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$
Sham, control animals; TNBS, untreated colitic animals; TNBS+B 0.09, colitic animals fed with 0.09 % borneol; TNBS+B 0.18, colitic animals fed with 0.18 % borneol ; TNBS+T, colitic animals fed with thymoquinone

similar results as normalization to the normalization factor (data not shown).

Discussion

Natural plant products have been used since ancient times and their use is increasing nowadays. Some essential oils and their constituents are known to have various health benefit properties, especially antibacterial, anti-inflammatory and antioxidative activities. In our study we examined the effects of borneol and thymoquinone in TNBS-induced colitis in mice. We developed a quantitative real-time RT-PCR assay to measure mRNA levels of five cytokines with pro-inflammatory properties (IL-1β, IL-6, GM-CSF, IFN-γ and TNF-α) and mRNA concentration of IL-10, which is an immunoregulatory cytokine with anti-inflammatory properties (Howard and O'Garra, 1992; Tomoyose et al., 1998). We found significantly elevated mRNA levels of all measured cytokines in mice with TNBS-induced colitis. Our results are in accordance with findings of several authors demonstrating an increased expression of pro-inflammatory cytokines in experimentally induced colitis (Hong et al., 2002a; Sugimoto et al., 2002; Kwon et al., 2005). Concurrently increased expression of proinflamatory cytokines and IL-10 found in our experiment has also been shown by other authors in different experimental models, including colitis (Segal et al., 1995; Halford et al., 1996; Tomoyose et al., 1998) and it can indicate later stages of colonic inflammation reflecting a regulatory circuit in which IL-10 counteracts the inflammatory process maintained by ongoing production of cytokines (Tomoyose et al., 1998). Our results show that prophylactic dietary administration of borneol at 0.09 and 0.18% concentrations is able to affect TNBS-induced inflammation in mice, as indicated by cytokine mRNA analysis. We observed significant differences only for IL-6 and IL-1β mRNA levels in correlation with their highest increase (~ 40 fold and more) in TNBS control animals compared to the sham group. We were unable to detect significant differences in other analysed parameters (macroscopic/microscopic scores, other cytokine mRNA levels), probably due to their relatively lower TNBS-induced increase and observed biological variability. Other plant extracts have also been shown to alter cytokine expression in mice with experimentally induced colitis. Administration of Polygalae root to mice with TNBS-induced colitis decreased production of IFN-γ and increased production of IL-4 (Hong et al., 2002b), while plant flavonoid rutin suppressed production of IL-1β and IL-6 in mice with dextran sodium sulphate-induced colitis (Kwon et al., 2005), and curcumin treatment in mice with TNBS-induced colitis decreased the expression of IFN-γ, TNF-α, IL-6 and IL-12 (Sugimoto et al., 2002).

The anti-inflammatory activity of black cumin (*Nigella sativa)* and its main component thymoquinone is well known (for review see Salem, 2005). Very recently, Tekeoglu et al. (2006) detected thymoquinone anti-inflammatory effects on experimentally-induced arthritis in rats at the same level as methotrexate, and decreased levels of TNF-α and IL-1β in circulation were observed. Similarly, Mohamed et al. (2005) showed that treatment of rats with thymoquinone at 1 mg/kg/day, concomitant to myelin basic protein but after the appearance of clinical signs, resulted in preventing and ameliorating experimental autoimmune encephalomyelitis. On the other hand, Hajhashemi et al. (2004) were unable to detect any anti-inflammatory effect of *per os* administered black cumin essential oil in carrageenan-induced paw oedema.

Interestingly, we were unable to detect any positive influence of thymoquinone on our experimental (TNBS) colitis, in contrast to thymoquinone beneficial effects against acetic acid-induced colitis in rats (Mahgoub,

2003). The observed discrepancy could be explained by differences in the experimental design, e.g. inducing agent – TNBS vs. acetic acid, animals – mice vs. rats, thymoquinone continuous vs. once daily application, duration of colitis 7 days vs. 24 hours.

In conclusion, our results indicate that dietary supplementation with plant compounds could have immunomodulatory effects during TNBS colitis in mice. The thymoquinone component of *Nigella sativa* probably stimulates the immune system, and does not counteract TNBS-induced colon inflammation. On the other hand, borneol dietary supplementation significantly decreases the concentrations of pro-inflammatory cytokines IL-1β and IL-6 compared with untreated TNBS mice.

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