

Effect of Using Thyme on Intestinal Morphology in Rat Model

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Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in clinical medicine. Their utility is, however, often limited by the adverse effects they produce in the gastrointestinal tract. Oxidative stress has been shown to occur in the small intestine in response to the oral administration of indomethacin. In view of this, the effect of thyme, an agent with anti-oxidant properties, was evaluated on indomethacin-induced small intestinal damage in a rat model. The major components of thyme essential oils were determined by gas chromatography mass spectrometry. Tissues morphology affected by indomethacin, with and without treatment with thyme, was measured. The small intestine morphology (villus height, villus width, crypt depth and crypt width of duodenum, jejunum and ilea) from indomethacin-treated animals with thyme was significantly improved than those from indomethacin animals. Treatment with thyme was found to ameliorate these drug-induced changes. Thus, thyme appears to hold promise as an agent that can potentially reduce non-steroidal anti-inflammatory drugs induced small intestinal damage.

Key words: Indomethacin, Small intestine, Thyme.

Thymus Vulgaris L. (thyme) is an aromatic plant belonging to the lamiaceae family that has been used traditionally as condiments and flavoring agents. It is also used in folk medicine as remedies for different ailments of the digestive tract. Most of its bioactive effects come from essential oils, thymol and carvacrol. In addition to its flavouring role, thymol is commonly used in food and cosmetic industry as preservative and antioxidant. It is also used in dentistry as a protective agent against caries and plaques and in anaesthesiology as a preservative and stabilizer of halothane (Boudry and Perrier, 2008). Thymol is also a potent scavenger of reactive oxygen species (Kruk *et al.*, 2000) and has anti-inflammatory properties by inhibiting COX1 activity or release by neutrophils (Braga *et al.*, 2006). Thyme essential oils could be used as a growth promoter due to its

mode of action, including: improving feed flavor and intake, stimulating the secretion of digestive enzymes, increasing gastric and intestinal motility, gut development and antimicrobial activity (Jamroz *et al.* 2006; Tipu *et al.* 2006). Carvacrol, an active component of thyme essential oils, has been noted to have a positive effect on intestinal morphology and secretion of mucin in the intestine (Jamroz *et al.* 2006). Nonsteroidal anti-inflammatory drugs (NSAID) are a class of drugs that usually used for the treatment of acute and chronic conditions where pain and inflammation are present. Nonsteroidal anti-inflammatory drug inhibit the activity of both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) and thereby, the synthesis of prostaglandins and thromboxans. It is thought that inhibiting COX-2 leads to the anti-inflammatory analgesic and antipyretic effects and that these NSAID also inhibiting COX-1 may cause gastrointestinal bleeding and ulcers. The main adverse drug reactions associated with NSAID uses related to direct and indirect irritation of the

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gastrointestinal tract. Nonsteroidal anti-inflammatory drug cause a dual assault on the gastrointestinal tract: the acidic molecules directly irritate the gastric mucosa and inhibition of COX-1 and COX-2 reduces the levels of protective prostaglandins. Inhibition of prostaglandin synthesis in the gastrointestinal tract causes increased gastric acid secretion, diminished bicarbonate secretion, diminished mucus secretion and diminished tropic effects on epithelial mucosa. Nonsteroidal anti-inflammatory drug induced injury on gastrointestinal tract is well documented, and jejunal inflammation caused by indomethacin in rats is a broadly used experimental model of enteritis (Menozzi *et al.*, 2009). The pathogenesis of non-steroidal anti-inflammatory drugs induced small intestinal lesions remains unclear, although it is considered to be quite different from that of upper gastrointestinal tract ulcers due to the absence of acid and the presence of bacteria and bile in the small intestine (Yamada *et al.*, 2012). The aim of this study was to show the effect of indomethacin on small intestinal morphology, and to assess the role of thyme in the indomethacin induced changes.

MATERIALS AND METHODS

Animals and design

16 rat, were chosen for the present study. Treatment groups were comprised of 8 groups of animals treated with thyme volatile oils dissolved in distilled water to the desired concentration. Thyme volatile oil dissolved in distilled water was administered once daily (1 ml). Rats were divided randomly into four groups: Group A; received intramuscular injection of indomethacin in male rat, Group B; was received intramuscular injection of indomethacin in female rat, Group C; received intramuscular injection of indomethacin and thyme volatile oils in male rat and Group D; received intramuscular injection of indomethacin and thyme volatile oils in female rat. Animals were housed in individual cages and kept in a ventilated room with temperature regulated at 23°C, with a 12 h light and 12 h dark cycle. They were fed with commercial rat pellets and water was supplied *ad libitum* and were acclimatized for 2 days prior to experimentation.

Extraction of thyme volatile oils

Fresh plants were collected at the flowering stage and processed immediately after

harvest. Volatile oil was distilled from the ground plant material using Clevenger distillation apparatus (Herbal Exir Co., Mashhad, Iran). The samples were distilled for two hours and the oils obtained, dried with anhydrous sodium sulphate, and stored in dark sealed glass vials at +4°C until required. The main active compounds of the thyme were determined by GC/MS and contained thymol and its isomer, carvacrol at the rate of 21.9 and 31.9% respectively. The concentrations of two predominate components of thyme volatile oils; thymol and carvacrol have been reported to range from as low as 3% to as high as 60% of total essential oils (Khaksar *et al.*, 2013).

Anesthetic protocol

The animals were anesthetized using 0.05 ml inspiration chloroform and after those animals injected with intramuscular of indomethacin. The dose of Indomethacin (8 mg/kg) was determined from a previous study (Abimosleh *et al.*, 2013).

Data collection

At end of experiment, rats were sacrificed by CO₂ asphyxiation followed by cervical dislocation and then the mid part of duodenum, jejunum and ileum were excised for histomorphometric analysis. Briefly, the small intestine was divided into three segments: the duodenum, the jejunum and the ileum. The duodenum is recognizable as the first stretch of the intestine leading from the stomach, it is mostly straight. The jejunum and ileum are both curly parts of the intestine, with the ileum being the last section before the small intestine becomes the large intestine. Samples of duodenum, jejunum and ileum (0.5 cm×0.5 cm segments) were obtained at its midpoint and immersed in a 10% buffered formalin solution for 72 h. Then they were excised and washed by physiological saline. The samples were treated in tissue processor apparatus and embedded in paraffin wax (Bancroft and Gamble, 2002). Transverse sections were cut (6 µm) using a rotary microtome (LEICA RM 2145), placed on a glass slide and stained with hematoxylin and eosin, and analyzed under a light microscope to determine morphometric indices. Morphological parameters were measured using the Image Pro Plus v 4.5 software package. The measured morphometric variables (Sakamoto *et al.* 2000; Aptekmann *et al.* 2001) included: villus height (VH) measured from the villus-crypt junction; villus width (VW)

measured at midvillus height; crypt depth (CD) and crypt width (CW). The mean from 10 villus per sample was used as the average value for further analysis.

Statistical analysis

All data were checked for normality before analysis. The data were subjected to ANOVA using SAS (SAS, 2000). Differences between treatment means were evaluated by Duncan's multiple range tests. A value of $P < 0.05$ was considered significant.

RESULT AND DISCUSSION

Effects of feeding diets containing thyme volatile oils on small intestinal morphology (duodenum, jejunum and ilea) are shown in *Tab. 1, 2 and 3*. The thyme volatile oils significantly improved small intestinal morphology in indomethacin-treated with thyme rats when compared to the indomethacin rat groups. The results of duodenum and jejunum in rats were affected by inclusion of thyme volatile oils into the diets. VH, VW, CD and CW significantly improved in rats fed thyme volatile oils in duodenum and jejunum. However, the only significant difference in the ilea was in VH and CD. On the other hand, the results in the present study indicated that bacterial populations (data was not presented) in the small intestinal digesta were not affected by supplementation with thyme volatile oil. But In the small intestinal digesta, the viable counts of *Biüdobacterium* and *Lactobacillus* were

Table 2. Effect of thyme volatile oil on jejunum morphology

Treatment*	VH	VW	CD	CW
A	111.87 ^b	33.25 ^c	58.89 ^c	25.73 ^b
B	104.26 ^b	33.53 ^c	53.47 ^c	36.17 ^a
C	179.09 ^a	44.08 ^a	87.88 ^a	38.37 ^a
D	161.87 ^a	39.66 ^b	70.46 ^b	23.42 ^b
P value**	0.001	0.003	0.001	0.001
SEM	15.03	0.43	0.57	2.71

*A: injection of indomethacin in male rat
 B: injection of indomethacin in female rat
 C: injection of indomethacin and thyme volatile oil in male rat
 D: injection of indomethacin and thyme volatile oil in female rat
 **a,b,c Means in each column with different superscripts are significantly different ($P < 0.05$)

increased, whereas those of *Escherichia coli* were reduced for the broilers fed diets with thyme volatile oils versus control. The reason of the lack of effect of thyme volatile oils supplementation on the small intestinal bacterial populations in their study may be because the concentration of thyme volatile oils was not adequate to alter microbial populations.

Intestinal villi are the main site of nutrient absorption and their better development could be the reason for higher nutrient absorption (Hanczakowska and Swiatkiewicz, 2012). Velazques et al (2005) reported positive effects of herb extract on the development of the intestinal tract. As mentioned above, there is a relationship between villi height and nutrient absorption and

Table 1. Effect of thyme volatile oil on duodenum morphology

Treatment*	VH	VW	CD	CW
A	129.21 ^c	27.72 ^b	68.24 ^a	42.29 ^a
B	118.59 ^c	20.52 ^b	59.46 ^b	34.74 ^a
C	198.41 ^a	46.53 ^a	46.93 ^b	14.62 ^b
D	142.94 ^b	35.62 ^a	37.57 ^b	16.00 ^b
P value**	0.001	0.020	0.002	0.001
SEM	0.80	0.82	1.12	0.60

*A: injection of indomethacin in male rat
 B: injection of indomethacin in female rat
 C: injection of indomethacin and thyme volatile oil in male rat
 D: injection of indomethacin and thyme volatile oil in female rat
 **a,b,c Means in each column with different superscripts are significantly different ($P < 0.05$)

Table 3. Effect of thyme volatile oil on ilea morphology

Treatment*	VH	VW	CD	CW
A	158.31 ^b	35.42	74.27 ^b	36.14
B	151.63 ^b	35.14	68.12 ^b	34.87
C	208.98 ^a	39.95	109.83 ^a	41.00
D	196.87 ^a	39.56	94.55 ^a	36.14
P value**	0.018	0.520	0.011	0.309
SEM	1.32	0.38	1.14	0.58

*A: injection of indomethacin in male rat
 B: injection of indomethacin in female rat
 C: injection of indomethacin and thyme volatile oil in male rat
 D: injection of indomethacin and thyme volatile oil in female rat
 **a,b,c Means in each column with different superscripts are significantly different ($P < 0.05$)

digestibility (Hanczakowska and Swiatkiewicz, 2012).

Also Pappenheimer and Michel (2003) proved a decisive role of villi in the intestinal absorption of nutrients. Increasing villi height suggests an increased surface area for greater absorption of available nutrients. Also increase in villi to crypt ratio is related to an increase in digestion and absorption (Montagne *et al.*, 2003). Antimicrobial agents such as essential oils or their active components are known to reduce the intestinal microbial load, which in turn reduces the presence of toxins that are associated with changes in intestinal histomorphology, such as shorter villi and deeper crypts (Xu *et al.*, 2003). As regards that productive potential of the cell cycle in small intestine is resultant of cell proliferation, cell migration in pivot crypt-villi, death and cell ablation, then, digestive material with high viscosity in lumen can be caused loss of cell's villi, decrease villi high and increase production of cell crypt and finally increase in crypt death and produce deeper crypts. However, hydroalcoholic plant extracts from sage, thyme, and rosemary leaves did not exert any influence on intestinal villus height, villus surface area and crypt depth. Longer villi increase the absorptive surface of intestine, while smaller crypt indicates a decrease of enterocyte replacement and tissue turnover, and lower demand for tissue development as well. It can be stated that, increments in villus height and crypt death as directly correlated with enhanced epithelial cell turnover (Fang *et al.*, 1997). In contrary, some studies reported that there was no significant effect in the villus height, crypt death and villus surface area due to feeding 200 ppm of plant extract, based on a blend of oregano, cinnamon and pepper essential oils and 5000 ppm of hydroalcoholic extract from sage, thyme and rosemary leaves. Guo *et al* (2004) found no significant differences in intestinal morphology among a medicinal plant group and control group. Garcia *et al* (2007) also reported no significant differences in villus height and crypt death between birds in the control group and a group receiving a blend of plant extracts. However, little information is available on how phytochemical compounds may affect gastrointestinal histomorphology and functionality. Observation were reported by Jamroz *et al* (2006) who observed qualitative increase in

the number of goblet cells and in mucin secretion at the surface of the jejunum villi when feeding broilers a mixture of 5 mg/kg of carvacol. The positive effect of extracts from medicine plants on piglet villi height was reported also by Fang *et al* (2009). Now we do not have a satisfactory explanation of the beneficial effect of herbal extracts and their antioxidative activity on ileal structure. It is possible that it is due to free radical-scavenging activities of polyphenolic compounds (Asfar *et al.*, 2003; Hanczakowska and Swiatkiewicz, 2012).

Perhaps an increased villus height is paralleled by an increased expression of brush border enzymes and improved nutrient transport system (Hanczakowska and Swiatkiewicz, 2012). Demir *et al* (2003) showed that crypt death in the ileum was significantly reduced by including garlic and thyme in the diet compared with including antibiotics and oregano.

CONCLUSION

Summing up the results obtained, it can be stated that thyme volatile oils can have positive changes in small intestine morphology in rats due to NSAID-induced small intestinal damage.

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