Effect of the essential oil of thyme on intestinal morphology in Rat

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1. Introduction

*Thymus vulgaris* L. (thyme) is an aromatic plant belonging to the Lamiaceae family that has been used traditionally as condiments and flavoring agents. Thyme 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 anesthesiology as a preservative and stabilizer of halothane (Boudry & 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 oil 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 oil, has been noted to have a positive effect on intestinal morphology and secretion of mucin in the intestine (Jamroz et al., 2006). Non-steroidal 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. Non-steroidal 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 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 trophic 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.

2. Materials and Methods

2.1. Animals and design

In this study, 16 rats were chosen that treatment groups were comprised of eight groups of animals treated with thyme volatile oil 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 oil in male rat and Group D: received intramuscular injection of indomethacin and thyme volatile oil 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.

2.2. 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 2 h 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.

2.3. 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).

2.4. 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 & 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.

### 2.5. Statistical analysis

All data was checked for normality before analysis. The data was 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.

### 3. Results and discussion

Results of the essential oil analysis indicated that thyme oil 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).

Effects of feeding diets containing thyme volatile oils on small intestinal morphology (duodenum, jejunum and ilea) are shown in Table 1, 2 and 3. The thyme volatile oil significantly improved small intestinal morphology in indomethacin-treated with thyme rats when compared to the indomethacin rat groups. Results of duodenum and jejunum in rats were affected by inclusion of thyme volatile oil into the diets. VH, VW, CD and CW significantly improved in rats fed thyme volatile oil in duodenum and jejunum. However, the only significant difference in the ilea was in VH and CD. Intestinal villi are the main site of nutrient absorption and their better development could be the reason for higher nutrient absorption (Hanczakowska & 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 digestibility (Hanczakowska & Swiatkiewicz, 2012).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of thyme volatile oil on duodenum morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>VH</td>
</tr>
<tr>
<td>A</td>
<td>129.21$^a$</td>
</tr>
<tr>
<td>B</td>
<td>118.59$^a$</td>
</tr>
<tr>
<td>C</td>
<td>198.41$^a$</td>
</tr>
<tr>
<td>D</td>
<td>142.94$^b$</td>
</tr>
<tr>
<td>$P$ value**</td>
<td>0.001</td>
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<tr>
<td>SEM</td>
<td>0.80</td>
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</tbody>
</table>

*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

** Means in each column with different superscripts are significantly different ($p < 0.05$).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Effect of thyme volatile oil on jejunum morphology</th>
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<tbody>
<tr>
<td>Treatment</td>
<td>VH</td>
</tr>
<tr>
<td>A</td>
<td>111.87$^b$</td>
</tr>
<tr>
<td>B</td>
<td>104.26$^b$</td>
</tr>
<tr>
<td>C</td>
<td>179.09$^a$</td>
</tr>
<tr>
<td>D</td>
<td>161.87$^a$</td>
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<tr>
<td>$P$ value**</td>
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<tr>
<td>SEM</td>
<td>15.03</td>
</tr>
</tbody>
</table>

*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

** Means in each column with different superscripts are significantly different ($p < 0.05$).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Effect of thyme volatile oil on ilea morphology</th>
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<tr>
<td>Treatment</td>
<td>VH</td>
</tr>
<tr>
<td>A</td>
<td>158.31$^c$</td>
</tr>
<tr>
<td>B</td>
<td>151.63$^b$</td>
</tr>
<tr>
<td>C</td>
<td>208.98$^a$</td>
</tr>
<tr>
<td>D</td>
<td>196.87$^a$</td>
</tr>
<tr>
<td>$P$ value**</td>
<td>0.018</td>
</tr>
<tr>
<td>SEM</td>
<td>1.32</td>
</tr>
</tbody>
</table>

*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

** Means in each column with different superscripts are significantly different ($p < 0.05$).

Pappenheimer & 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. In addition, 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, hydro-alcoholic 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 hydro-alcoholic 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 phytogenic 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 mucus secretion at the surface of the jejunum villi when feeding broilers a mixture of 5 mg/kg of carvacrol. 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 & Swiatkiewicz, 2012).

Perhaps an increased villus height is paralleled by an increased expression of brush border enzymes and improved nutrient transport system (Hanczakowska & 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.

4. 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.

5. References

Abimosleh, S.M., Tran, C.D. and Howarth, G.S. 2013. Emu oil reduces small intestinal inflammation in the absence of clinical improvement in a rat model of indomethacin-induced enteropathy. Evidence-Based Complementary and Alternative Medicine, Article ID 429706, 10 pages.


