Fibrinolytic effects of *Matricaria chamomila* in preventing peritoneal adhesions

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**ABSTRACT**

Abdominal wall surgeries usually result in a common consequence which is peritoneal adhesion. *Matricaria chamomila* (MC) is a remedial herb with anti-inflammatory biologically active compounds which are effective in prevention of post-surgical adhesions. In this prospective study, 50 female rats were divided into 5 groups which received normal saline, chamomile extracts of 100mg/kg 200mg/kg 400 mg/kg and a control group. Intra-peritoneal ketamine 30 mg/kg was administered for anesthesia. After surgical preparation of the abdomen, several scratches were made in the peritoneum and the abdominal wall was closed. Repeated laparotomy was performed 2 weeks later, and the peritoneum was biopsied for histo-pathological examination and grading of the adhesions. Data were analyzed with SPSS 13. Analysis was done using student’s t-test, chi square, Kruskal-Wallis and Mann-Whitney U tests. If the value of *p* ≤ 0.05 the results were considered significant. The results show that adhesion, fibrosis, inflammation, and post-surgical vascularization was significantly less in chamomile group compared to rats receiving normal saline or the control group (*p* ≤ 0.001). Chamomile has protective anti-inflammatory effects proved by significant restraining of the post-surgical adhesions. (Abstract truncated at 185 words)

**Keywords:** peritoneum adhesions; *Matricaria Chamomila*; Rat; fibrinolytic agents

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**INTRODUCTION**

Peritoneal adhesions are fibrous bands of tissue joining intra-abdominal organs to each other or to the abdominal wall [1, 2]. Adhesions are formed when the parietal or visceral peritoneum is damaged and the mesothelial layer basal membrane is exposed to surrounding tissues. The sequence of adhesion formation can be described as: 1- Tissue ischemia, 2- Inflammation, 3- Fibrin deposition, 4- Fibrin organization, 4- Collagen formation, and finally 5- maturation with the formation of adhesions [1,4]. Inflammatory mediators also may play an important role in adhesion formation [5]. Almost concurrently, migration of inflammatory cells, release of cytokines, and activation of the coagulation cascade results in an inflammatory response [6,7]. The long-lasting approval of herbal medicines may be explained by the affinity of herbs to work gradually, usually with minimal toxic side effects. One of the most common herbs used for medicinal purposes is chamomile [8]. The common variation used for remedial purposes is Matricaria (MC). One of the medicinal plants that have been widely used is Matricaria Chamomile (MC) [9]. The flowering parts of chamomile can be used principally for an inflammatory condition [10]. Use of MC has been sustained by the segregation and documentation of several biologically active chemicals including polyphenols (flavonoids) and essential oil extracted from chamomile flowers [11]. MC containsterpenoids and azulenes (including chamazulene and enyedecolo ether) [12]. Anti-inflammatory properties ofterpenoids, bisabolol and chamazulene have been well known [10]. Chamazulene strongly...
suppresses the formation of leukotriene B4 in the neutrophil granulocytes [13], and also inhibits lipid peroxidation through antioxidant activity [14]. Furthermore, chamazulene inhibits peroxidation of arachidonic acid resulting reduced inflammatory mediators derived from arachidonic acid. A number of papers have been published on the identification of the phenolic compounds of MC including apigenin, quercetin, and luteolin [15,16]. Some reports suggest that flavonoids have a significant part in inflammatory processes and immune functions by inhibiting several enzymes which are activated during certain inflammatory conditions [15]. For instance, luteolin, belonging to flavone (a subclass of flavonoids) has specific anti-inflammatory activities such as antioxidative enzymes activation, nuclear factor KappaB (NF-κB) pathway suppression, inhibition of pro-inflammatory substances, and reduction of the enhanced vascular permeability [17]. Apigenin, another flavone in the MC, that is non-toxic, non-mutagenic, and a potent antioxidant, effectively blocks intercellular adhesion molecule-1 up-regulation, leukocyte adhesion in response to cytokines, subdues cyclooxygenase-2 expression, and is a potential apoptosis inducer [18]. Therefore, the effect of chamomile extract on the prevention of intra Peritoneal adhesions among rats was evaluated in this study.

MATERIALS AND METHODS

Animals and Anesthesia
Female Wistar rats (weighing 200-250g) taken from the central Animal House of Hormozgan University of Bandar Abbas, were used in this study. They were housed in environmentally controlled conditions (22±2°C, 12-hour light-dark cycle), with free access to standard pellet diet and water. Animals were kept in cages with raised floor to prevent coprophagy. After adaptation; the animals were randomly assigned to five different groups of equal numbers. The rats were prepared for surgery with an injection of ketamine (30mg/kg ip) for anesthesia. The surgical procedures were performed under sterile conditions.

Chemicals
The following chemicals were used: Extract of MC flowers Kashan (Iran), 0.9% NaCl (Serum Samen, Mashhad, Iran).

Induction of Adhesions
All of the animals were fasted for at least 12 hours immediately before surgery. After hair removal, the abdomen was cleaned with 1% antiseptic povidone-iodine solution and a 3 cm midline laparotomy was made. The peritoneal wall of the control group was completely stunned using sharp forceps and then the abdomen was sutured using nylon string 0.4. As for the normal group, 2 ml of Normal saline 0.9% was used for five minutes with IP after the manipulation then the abdomen was sutured. The other groups’ abdomen was sutured after administering 100-200 mg of chamomile extract (400 mg/kg). All the surgeries were carried out by the same person under one single procedure. The animals’ nutrition was controlled for two weeks.

Experimental Groups
Group 1 (Control group)
Group 2 (Normal Salin group)
Group 3 (Matricaria chamomilla 100 mg/kg group)
Group 4 (Matricaria chamomilla 200 mg/kg group)
Group 5 (Matricaria chamomilla 400 mg/kg group)

Assessment of Adhesions
2 week after surgery, the animals were sacrificed. The abdominal cavity was shaved and opened via a 8 cm incision based in the lower abdomen for complete exploration. Blind evaluations were carried out; adhesions were graded from 0 (absent) to 4 (severe) according to the Mazuji classification (19) (Table 1). In general, Grades 0 and 1 adhesions have no clinical significance, whereas Grades 3 and 4 adhesions can be a cause of intestinal obstruction. Adhesion rate is used to describe the presence of an adhesion of any grade. After biopsy; the samples were fixed in formalin and sent to pathology lab for histology evaluations. The findings were compared with Adhesion Grading Classification.

Table 1 - Adhesion grading according to Mazuji classification

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description of Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No adhesion</td>
</tr>
<tr>
<td>1</td>
<td>Very small, irregular adhesion</td>
</tr>
<tr>
<td>2</td>
<td>Easily separable medium intensity adhesion</td>
</tr>
<tr>
<td>3</td>
<td>Intense, not easily separable regular adhesion</td>
</tr>
<tr>
<td>4</td>
<td>Very intense, not easily separable, homogeneous adhesion</td>
</tr>
</tbody>
</table>

Histology
Histopathology examination was performed by two investigators. Adhesion-carrying tissues were excised en-bloc and fixed in a 10% buffered formaldehyde solution. Histological study was performed after
staining with hematoxylin and eosin. The evaluated parameters were fibrosis, inflammation, and vascular proliferation, rated on a modified semi-quantitative scale of 0-3 (20-22). The amount of fibrosis was scored as follows: 0, no fibrosis; 1, minimal, loose fibrosis; 2, moderate fibrosis; and 3, florid dense fibrosis. Inflammation was scored as follows: 0: no inflammation; 1: presence of giant cell, occasional lymphocytes and plasma cell; 2: presence of giant cell, plasma cells, eosinophils and neutrophils; and, presence of many inflammatory cells and micro-abscesses. Vascular proliferation was scored as: 0: no vascular proliferation; 1: mild vascular proliferation; 2: moderate vascular proliferation; and 3: intense vascular proliferation.

**Statistical Analysis**

Statistical analysis was performed using the Statistical Package for Social Science (SPSS 13). Differences in the numbers of animals without adhesions in the different treatment groups were evaluated using a Chi-square test. A t-test was used to determine differences between two independent populations. Kruskal-Wallis and Mann-Whitney U test were used if required. A value of p ≤ 0.05 was considered significant.

**RESULTS**

### Adhesion Grade and Rate

Throughout the investigation, several animals died during or after surgery. Adhesion rates and grades of the groups are presented in table 2. In comparing adhesion grades, a significant difference was found between the control group and the prevention groups (p=0.004). The more the dose of chamomile extract, the lesser the abdominal adhesion; administering chamomile extract with a dose of 200 mg/kg, adhesion was absent in 90% of the cases and only 10% had (minimal) grade 1 adhesion.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>adhesion rate (%)</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Control Group)</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>10/10(100)</td>
<td>Local hemaoma(n=3)</td>
</tr>
<tr>
<td>(Normal Saline Group)</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>6/10(60)</td>
<td>-</td>
</tr>
<tr>
<td>(MC100mg/kg Group)</td>
<td>10</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>6/10(60)</td>
<td>-</td>
</tr>
<tr>
<td>(MC200mg/kg Group)</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4/10(40)</td>
<td>-</td>
</tr>
<tr>
<td>(MC400mg/kg Group)</td>
<td>10</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1/10(10)</td>
<td>Local hemaoma(n=4)</td>
</tr>
</tbody>
</table>

* p ≤ 0.05 as compared to the control group and to the prevent groups (Group 3, 4 and 5) and with 2 group.

Adhesion rate is used to describe the presence of an adhesion in any grade. On the other hand, in Group 5 (MC400mg/kg Group) the adhesion grade differed significantly from that of the other groups (p≤0.05) (Table2). There were no adhesions in Grades 2 to 4 in the MC400mg/kg Group. Furthermore, most adhesion in the control, Normal Saline Group (60%) was Grade 2 or 3 and MC400mg/kg Group were Grade 1 (10%). Based on these findings, it was concluded that MC significantly reduced peritoneal adhesion development rate and adhesion grades. The adhesions in Group 5 (MC400mg/kg group) developed in the uncovered areas in the abdomen. But in groups 1 and 2 (Control and Normal Saline) showed the highest incidence of postsurgical adhesion in comparison of 5 groups (p≤0.05) (Table 4).

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Control Group)</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>(Normal Saline Group)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>(MC100mg/kg Group)</td>
<td>10</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>(MC200mg/kg Group)</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>(MC400mg/kg Group)</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* p ≤ 0.05 as compared to the control group and to the prevent groups (Group 3, 4 and 5) and with 2 group.
specifically present this difference more completely compared with the findings of this study detect that chamomile extract could prevent adhesion. The results of group 5 show that chamomile extract plays an important role in reducing inflammation resulting from abdominal surgeries. Several drugs and substances are used locally or systematically for this purpose, including mechanical barriers and physical, chemical, and pharmacological agents [20,21, 23-31]. However, despite positive reports, none of these have been approved for a standard therapy of adhesions [20,21,23]. The findings suggest that adhesions following abdominal surgeries are affected by a complex of factors including inflammation. The inflammatory cells finally migrate to the affected region and leading to cytokine release and activation of clotting factors. It is suggested that inflammatory mediators may play an important role in adhesion formation. There is experimental evidence that certain mediators, such as transforming growth factor-B (TGF-B) and interleukins, decrease the fibrinolytic capability of the peritoneum and increase the adhesion formation [5, 32-34]. Chamomile has a extensive usage and is highly reputed among herbal medicines during the history due to its anti-inflammatory, analgesic, antimicrobial, antispasmodic and sedative properties [10, 11].

Based on previous studies and our study, the main anti-inflammatory mechanism of MC is summarized in Table 1. Probable mechanisms describing the role of MC compounds in prevention postsurgical adhesion include prevention of NF-κB activation and suppression of iNOS and COX-2 gene expression via apigenin and luteolin. The next mechanism is the antioxidant activity and prevention of lipid peroxidation of arachidonic acid, which blocks production of inflammatory substances via apigenin, luteolin, and chamazulene. Moreover, mechanisms such as inhibition of XO, vasodilatation inhibitory, and induction of apoptosis might also be important in the expression of anti-inflammatory effect of MC in prevention postsurgical adhesion. This study suggests that chamomile extract plays an important role in reducing inflammation resulting from abdominal surgeries (p<0.05). Besides, no grade 3 or 4 adhesion was observed among groups 4 and 5 compared with the control group, specifically when chamomile extract was used with a dose of 200 and 400m/kg (p<0.05). Most of the adhesion, fibrosis, inflammation and increased vascular proliferation was observed in the control group. (Table 3). Nicholas et al, have shown that apigenin has anti-inflammatory effects by inactivation of NF-κB pathway through suppression of p65 phosphorylation which in turn blocks the expression of pro-inflammatory cytokines, such as interleukin 1 and 6 (IL-1, 6) and tumor necrosis factor-alpha (TNF-α) in human monocytes [35]. This effect regulates prostaglandin (PG) and NO production and suppresses inflammation [36]. Luteolin has anti-inflammatory effects including activation of antioxidative enzymes, suppression of the NF-κB pathway, and inhibition of pro-inflammatory substances. Luteolin, ineffective in decreasing inflammation through lessening of enhanced vascular permeability [17]. It also subsides production of NO and Prostaglandins (PGE2) as well as the expression of iNOS, cyclooxygenase-2 (COX-2), TNF-α and IL-6 viablocking NF-κB activation pathway [37]. Histological findings of this study detect that chamomile extract could prevent adhesion. The results of group 5 specifically present this difference more completely compared with the control group.

Histology
The histologic findings of adhesion differed significantly among the Matricaria chamomilla (MC) group and other groups according to fibrosis (p= 0.000), inflammation (p= 0.002), and vascular proliferation (p= 0.014). The (MC) group had a moderate score for fibrosis and the lowest scores for inflammation and vascular proliferation (Table 3). Histologic examination showed Groups 1 and 2 had the highest scores for fibrosis, inflammation and vascular proliferation. (Table 3).

DISCUSSION
Post-surgical adhesion is a significant clinical problem for every surgical specialty. Evaluation of the causes and resources of inhibition of adhesion formation has been a major aim in many investigations. Based on previous studies and our study, the main anti-inflammatory mechanism of MC is summarized in Table 1. The findings of this study suggest that chamomile extract could prevent adhesion. The results of group 5 specifically present this difference more completely compared with the control group.
CONCLUSION

It is noteworthy to state that this is a pioneer study and that the previous studies didn’t consider this feature of chamomile. Therefore, further studies are required on different animal groups for reassurance.

Table 1. Main anti-inflammatory mechanisms of MC.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Compound</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modulation of NF-Kb</td>
<td>Apigenin</td>
<td>Nicholas et al. [35]</td>
</tr>
<tr>
<td></td>
<td>Luteolin</td>
<td>Chen et al. [37]</td>
</tr>
<tr>
<td>Vascular dilation inhibitor</td>
<td>Luteolin</td>
<td>Seelinger et al. [17]</td>
</tr>
<tr>
<td>LTB4 blocker</td>
<td>Chamazulene</td>
<td>Safayhi et al. [13]</td>
</tr>
<tr>
<td>Antioxidant activity</td>
<td>Apigenin</td>
<td>Lee et al. [18]</td>
</tr>
<tr>
<td></td>
<td>Luteolin</td>
<td>Seelinger et al. [17]</td>
</tr>
<tr>
<td></td>
<td>Chamazulene</td>
<td>Rekka et al. [14]</td>
</tr>
<tr>
<td>Suppresses gene expression of iNOS and COX-2</td>
<td>Apigenin</td>
<td>Nicholas et al. [35]</td>
</tr>
<tr>
<td></td>
<td>Luteolin</td>
<td>Chen et al. [37]</td>
</tr>
</tbody>
</table>

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CONFLICT OF INTEREST

NONE

REFERENCES


**Citation of this article**