

# The Effect of Ethanolic Extracts Mixture from Magnoliae cortex and Carthami semen on the Progression of Experimental Periodontitis in Beagle Dogs

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## I. Introduction

Periodontal disease is an inflammatory process caused by periodontopathic bacteria in dental plaque<sup>1-3</sup>), which results in gingival inflammation and periodontal destructions. So, many antiseptics and antibiotics have been used for the treatment and prevention of periodontitis<sup>4-9</sup>). From the general reluctance in using antibiotics for the treatment of chronic infections such as periodontitis<sup>10</sup>), interest has largely been directed toward antiseptics. Most commonly used antiseptics in clinical periodontics are bisbiguanides, phenolic compounds, and quarternary ammonium compounds and etc.,. While these agents have been used for many years, they exhibited many drawbacks such as tooth staining, development of resistant strains and desquamation of oral epithelium<sup>11-13</sup>). Therefore, several researches

have focused on developing new antimicrobial agents from natural products having no such drawbacks<sup>14-16</sup>).

The ethanolic extract of Magnoliae Cortex has been demonstrated to have antibacterial effects against periodontopathic microorganisms and ability to decrease collagenase production in periodontal ligament cells<sup>16</sup>), and also showed having a stimulatory effect on collagen and total protein production in periodontal ligament cells<sup>16-17</sup>). Moreover, Magnoliae Cortex could suppress IL-1 $\beta$  and PGE<sub>2</sub> production<sup>18-20</sup>). It has been also reported that Carthami Semen extracts increased the cellular activity (protein synthesis for example) and chemotactic activity of osteoblasts and periodontal ligament cells, and that it could increase new bone formation in surgically created rat calvarial defects<sup>20</sup>). Furthermore, in our pilot study, rats received the

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\* This study was supported by the grant of Seoul National University Hospital (02-1997-250-0).

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two extracts mixture orally, showed increased bone healing in calvarial defects. So, we mixed the two extracts and gaved it to beagle dogs which had experimentally induced periodontitis in order to test if the extracts had anti-periodontitis effects.

This study focused on the effect of oral administration of ethanolic extracts mixture from *Magnoliae Cortex* and *Carthami Semen* on the progression of experimental periodontitis in beagle dogs.

## II. Materials and Methods

*Magnoliae Cortex* and *Carthami Semen* were purchased from a crude drugs market in Taejon and voucher specimens have been deposited at the herbarium in College of Pharmacy, Chungnam National University. The crude drugs were crushed and then extracted with ethanol on water bath at 60 °C for 6 hours, separately. After filtration, the combined filtrates were concentrated under vacuum. The ethanol extracts of *Magnoliae Cortex* and *Carthami Semen* were mixed in the ratio of 1: 5 for the experiment. Five 18-month-old beagle dogs, weighing approximately 13 kg were used for this study. During the pretreatment phase, all beagle dogs were brought up to optimal periodontal health with supragingival scaling and daily tooth brushing. Fourteen teeth were selected in each beagle including maxillary second, third, fourth premolars, and mandibular second, third, fourth premolars, and first molar bilaterally. Wire and silk braided ligatures were then tied around the teeth in each animal to induce experimental periodontitis. After 8 weeks, ligatures were removed and supragingival scaling was done. From then on, three beagle dogs were received 1.5 g of the ethanolic extracts mixture of *Magnoliae Cortex* and *Carthami Semen* in a capsule every 12 hours for 12 weeks. Two beagles were not received the mixture and employed as the negative

control group. Gingival Index was measured on mesiobuccal, midbuccal, distobuccal sites of the teeth. For microbiological study, subgingival dental plaque was collected from midbuccal side of the maxillary third premolar and mandibular fourth premolar with paper points(#35). The supragingival area above the pocket to be sampled was cleaned with cotton pellet and dried prior to sample collections. Three sterile paper points were inserted into each site and left for 10 seconds. The tip of the paper points were cut off with sterile scissors and dropped into vials containing reduced transport fluid (VMGA III transfer media). Within 60 minutes, the samples were introduced into an aerobic chamber with an atmosphere of 85% N<sub>2</sub>, 10% H<sub>2</sub>, and 5% CO<sub>2</sub>. Samples were homogenized for 60 seconds by means of a vortex mixer. Suspensions were diluted by 10-fold serial dilution. From each dilution, 0.1ml was inoculated on different agar plate. Anaerobic bacteria were isolated by anaerobic culture on tryptic soy agar supplemented with 5% rabbit blood, 5.0 mg hemin per ml, and 0.5 mg menadione per ml in anaerobic chamber at 36°C. Aerobic bacteria were isolated by aerobic culture on tryptic soy agar supplemented with sheep blood in a CO<sub>2</sub> incubator containing 10% CO<sub>2</sub> at 37°C. Colony counts, expressed as colony forming units, of anaerobe were enumerated after 7 days and aerobe after 3 days incubation<sup>5)</sup>. Gingival index and microbiological assessments were repeated at week 4, 8, and 12. Paired t-test was used to compare the data between each time point and baseline, and independent t-test was used to analyze the difference between the groups. Statistical differences were determined at the values of p (0.05, 0.01 or 0.001).

## III. Result

Because this study was done under precise con-

trol, all variables including gingival indices, anaerobe counting, and aerobe counting were not significantly different between two groups at baseline.

Changes in the mean gingival index (GI) scores over the study period are shown in Table 1. AT baseline, mean GI scores were high in both groups due to ligatures. The scores were gradually decreased at both of the groups. At weeks 4 and 8, mean GI scores of test groups was slightly lower than that of control groups though not significantly different statistically. At week 12, GI scores of test groups were significantly lower than that of control

groups( $p < 0.05$ ). And at week 12, GI scores of test groups were significantly lower than that of baseline, but GI scores of control groups were not significantly lower than that of baseline.

Colony forming unit of anaerobic and aerobic bacteria are shown in Table 2 and 3 in logarithmic figures. In test groups, the numbers of cfu of anaerobe and aerobe were compared between baseline and each time points. In test groups, the numbers of cfu were gradually decreased over times. Three were significant differences between baseline and 4 weeks( $p < 0.01$ ), 8weeks( $p < 0.001$ ), and 12weeks

Table 1. Gingival Index in the Beagle dogs

Week	NC	MMC
0(baseline)	1.69±0.58	1.71±0.63
4	1.57±0.56	1.33±0.65
8	1.64±0.40	1.06±0.48 <sup>§</sup>
12	1.55±0.37	1.15±0.58 <sup>§†</sup>

The data represent the means with standard deviation.

NC: Negative control

MMC: Mixture of ethanolic extracts from Magnoliae Cortex and Carthami Semen.

§:  $p < 0.05$  as compared with week 0 in each group.

†:  $p < 0.05$  as compared with control groups at each time points.

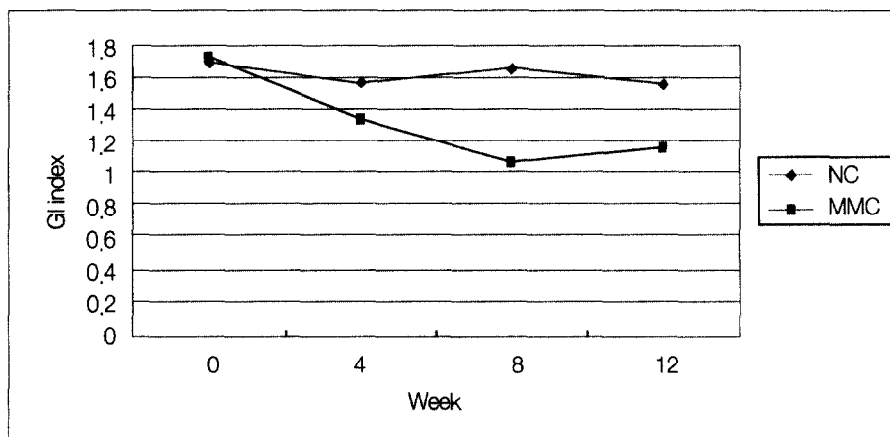


Figure 1. Gingival Index in the Beagle dogs

NC: Negative control,

MMC: Mixture of ethanolic extracts from Magnoliae Cortex and Carthami Semen.

**Table 2. Clinical attachment level of the Beagle dogs**

Week	NC	MMC
0	4,17±0,37	4,09±0,47
4	3,92±0,44	3,37±0,50 <sup>§†</sup>
8	4,06±0,56	2,67±0,41 <sup>§†</sup>
12	4,23±0,72	2,72±0,38 <sup>§†</sup>

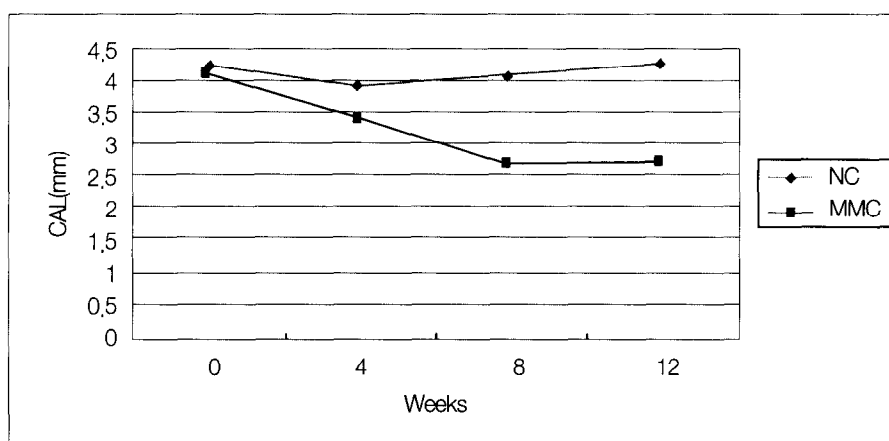
The data represent the means with standard deviation.

NC: Negative control

MMC: Mixture of ethanolic extracts from Magnoliae Cortex and Carthami Semen.

§: p<0,05 as compared with week 0 in each group.

†: p<0,05 as compared with control groups at each time points.



**Figure 2. Clinical attachment level of the Beagle dogs**

NC: Negative control,

MMC: Mixture of ethanolic extracts from Magnoliae Cortex and Carthami Semen.

**Table 3. Number of colony forming units of anaerobic bacteria in the Beagle dogs**

Week	NC	MMC
0	6,83 <sup>a</sup> ±0,16 <sup>b</sup>	7,04±0,29
4	7,12±0,10	5,44±0,21 <sup>†</sup>
8	7,03±0,10	5,95±0,44 <sup>**†</sup>
12	7,27±0,11	5,73±0,20 <sup>**†</sup>

a: Mean of cfu(in log<sub>10</sub> of counts)

b: S.D., of cfu(in log<sub>10</sub> of counts)

NC: Negative control

MMC: Mixture of ethanolic extracts from Magnoliae Cortex and Carthami Semen.

\*: p<0,01 as compared with week 0 in each group

\*\* : p<0,001 as compared with week 0 in each group

† : p<0,001 as compared with control groups at each time points.

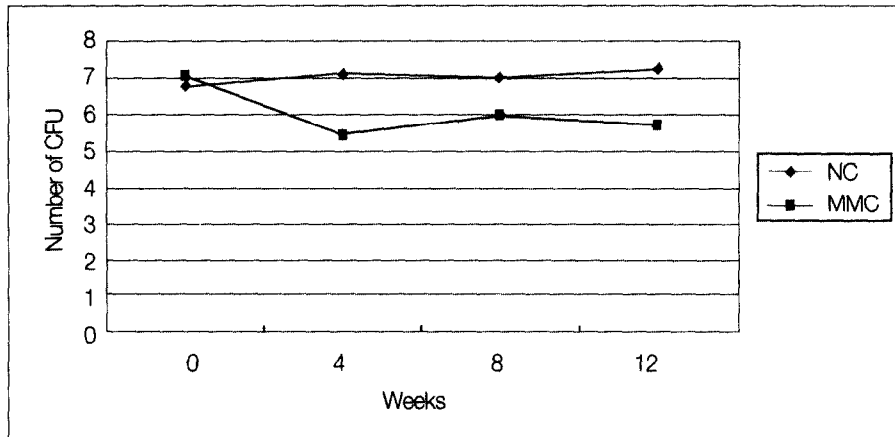


Figure 3. Number of colony forming units of anaerobic bacteria in the Beagle dogs. The number of the CFU is logarithmic scale

NC: Negative control,

MMC: Mixture of ethanolic extracts from Magnoliae Cortex and Carthami Semen,

( $p < 0.001$ ) at both control groups and test groups. Decreasing pattern was more prominent in anaerobic cfu than in aerobic cfu. But, in control groups, the numbers of cfu of anaerobe and aerobe were not decreased significantly over time. There were not any significant differences between baseline and at each time points at both groups. We also compared the numbers of cfu between the two groups at each time point. The numbers of cfu of anaerobe of test groups were significantly lower than that of control groups at 4, 8, and 12 weeks. In aerobic cfu, the similar results were obtained.

#### IV. Discussion

In a previous study, magnolol and honokiol, the main component of Magnoliae cortex, showed a significant antimicrobial activity against periodontopathic microorganisms and a relatively low cytotoxic effect on human gingival cells<sup>16</sup>. It was also reported that Carthami semem extract might have osteoblast activating effects<sup>20</sup>. The present study was designed to examine the effect of oral adminis-

tration of Magnoliae cortex and Carthami semen extract mixtures on the disease progression in beagle dogs with ligature-induced experimental periodontitis.

We measured Gingival index (GI) to monitor gingival inflammation. Gingival index has been employed to represent overall gingival inflammation<sup>22</sup>. After 12 weeks of medication, the GI scores of test groups were significantly lower than that of control groups and this might be due to the orally administered extracts mixtures. In the microbiological observation, the number of colony forming unit (CFU) of anaerobic bacteria of the control group did not show significant difference throughout the study period. Meanwhile, in the test group, the number of anaerobic bacteria colonies was significantly decreased at all time points (Table 2). In general, major putative pathogens of periodontal disease is almost anaerobic bacteria, for example, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Actinobacillus actinomycetemcomitans*<sup>1</sup>. So, this result suggests that the extract mixtures would be effective in decreasing the periodon-

Table 4. Number of colony forming units of aerobic bacteria in the Beagle dogs

Week	NC	MMC
0	5,72 <sup>a</sup> ±0,27 <sup>b</sup>	5,52±0,37
4	5,62±0,46	5,12±0,31 <sup>†</sup>
8	5,88±0,35	5,25±0,31 <sup>†</sup>
12	5,70±0,27	5,33±0,28 <sup>†</sup>

a: Mean of cfu(in log<sub>10</sub> of counts)

b: S.D., of cfu(in log<sub>10</sub> of counts)

NC: Negative control

MMC: Mixture of ethanolic extracts from Magnoliae Cortex and Carthami Semen.

\* : p < 0,05 as compared with week 0 in each group

† : p < 0,05 as compared with control groups at each time points.

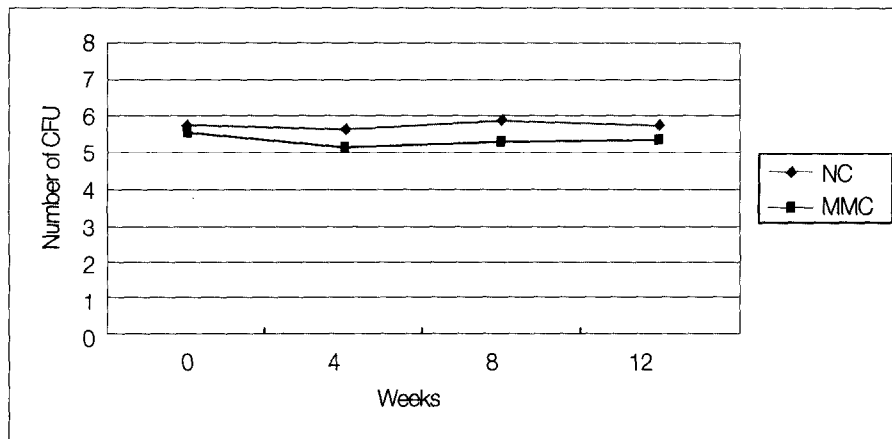


Figure 4. Number of colony forming units of aerobic bacteria in the Beagle dogs. The number of the CFU is logarithmic scale

NC: Negative control,

MMC: Mixture of ethanolic extracts from Magnoliae Cortex and Carthami Semen,

topathic microflora, and preventing progression of periodontal diseases. Likewise, in aerobic bacteria, CFU did not show significant changes in the control group, but the number of aerobic bacteria colonies decreased at all time points in the test group. The differences between the two groups were statistically significant at all time points (Table 3). Though both anaerobe and aerobe were decreased significantly over time in test groups, the decreasing pattern of anaerobe was much more prominent than

aerobe.

To inhibit alveolar bone loss resulting from periodontal disease progression in human, some kinds of NSAIDs have been introduced<sup>23-26</sup>. Waite et al<sup>23</sup> compared the periodontal status of a group of 22 subjects who had been taking a variety of NSAIDs for at least 1 year to that of an equal number of control subjects. Their observations showed that reduced gingival index scores and probing pocket depths were associated with NSAID therapy and

there was also a trend towards a reduced loss of attachment. But, the side effect of NSAID use such as gastric disturbances has deterred with its wide use for treatment of only periodontitis.

Many investigators have sought some new drug from natural extracts that would replace the previously used agents, such as antibiotics and anti-inflammatory drugs. Among them, sanguinaria-containing products have received extensive attention. Clinically meaningful and statistically lower gingival bleeding on probing scores were obtained with combined use of sanguinaria and zinc chloride containing tooth paste and oral rinse<sup>27-28</sup>. In this study, the mixture of Magnolia cortex and Carthami semen extracts showed significant effects on reducing gingival inflammation, anaerobic, and aerobic bacteria. These results might be due to the antimicrobial effects of Magnolia cortex<sup>16</sup> against periodontopathic microorganisms and PDL cell and osteoblasts activating effects of Carthami semen<sup>20</sup>.

Taken together, these results indicated that the oral administration of mixture of Magnolia cortex and Carthami semen extracts would be highly effective in slowing the destruction of periodontal tissue in beagles with periodontitis, which was presumed to be due to suppressive effects on production of inflammatory mediators, and that this mixture might be potentially useful as a drug for prevention and treatment of periodontitis.

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# 인위적으로 유발된 Beagle dog의 치주질환 진행에 후박과 홍화씨 추출물이 미치는 영향에 관한 연구

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후박추출물은 치주병인균에는 항균효과가 있고, 치은섬유아세포에는 세포독성이 적은 것으로 알려져 있으며 홍화씨 추출물은 골아세포를 활성화시키는 효과가 있어 골조직재생에 효과가 있는 것으로 알려져 있다. 이 실험의 목적은 비글견에 인위적으로 치주염을 유도하고 후박추출물과 홍화씨추출물의 혼합물을 투여함으로써, 이 두 가지 혼합물이 치주염 진행에 예방효과가 있는지, 또, 치료효과가 있는지 알아보는 것이다. 후박과 홍화씨를 구입하여 에탄올에서 추출하여 정제하고 진공상태에서 건조시켜 농축하였다. 후박추출물과 홍화씨추출물을 1:5의 질량비로 혼합하고 250mg씩 캡슐에 넣었다. 비글견의 상하악 소구치와 하악 제1대구치를 8주 동안 철사와 견사로 결찰하여 치주염을 유발하였다. 8주 후에, 실험군 비글견에게는 미리 후박추출물과 홍화씨추출물 혼합물을 매 12시간마다 하루에 1,500mg 씩 12주 동안 투약하였다. 대조군 비글견에는 투약을 하지 않았다. 투약을 시작한 후에 치은지수, 부착수준을 매 4주마다 측정하였다. 매 4주마다 치주낭에서 페이퍼포인트를 이용하여 혐기성세균과 호기성세균을 채취, 배양하여 각각의 집락 수를 세었다. 그 결과 실험군의 치은지수는 8주, 12주에서 대조군보다 유의하게 낮았고( $p < 0.05$ ), 임상적으로도 상당한 치은염의 개선을 보였다. 실험군의 부착수준 역시 4, 8, 및 12주에서 대조군보다 유의하게 낮았고( $p < 0.05$ ), 임상적으로 개선을 보였다. 실험군의 혐기성세균 집락 수는 감소하여 4, 8, 및 12 주에서 대조군에 비해 유의하게 낮았고( $p < 0.001$ ), 호기성 세균 집락 수는 4, 8, 및 12주에서 대조군에 비해 유의하게 낮았다. ( $p < 0.05$ ) 이 실험 결과, 후박 추출물과 홍화씨 추출물 혼합물이 치주질환의 예방 및 치료에 이용되어질 수 있을 것으로 생각된다.

주요어 : 후박 추출물, 홍화씨 추출물, 비글견, experimental periodontitis, 미생물검사