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EFFECTS OF *CURCUMA LONGA L., CURCUMA AROMATICA SALISB., ZINGIBER OFFICINALE ROSC.* EXTRACT ON INFLAMMATORY TEMPOROMANDIBULAR JOINT PAIN IN RAT'S

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ABSTRACT

Objective: Zingiberaceae plants(*Curcuma longa L.* (C.L), *Curcuma aromatica Salisb.* (C.A) and *Zingiber officinale Rosc.* (Z.O)) which are well known as effects of anti-inflammation or anti-nociception. We investigated that the Zingiberaceae plants are involved in control of inflammatory temporomandibular joint(TMJ) pain in rats. **Methods:** Male Sprague-Dawley rats weighing 240-280 g were used in this study. Experiments were carried out using inflammatiry pain model that was caused by the injection of 5% formalin into the TMJ(30 $\mu\ell$). The number of scratching or rubbing to the injection site was recorded for 9 consecutive 5-minute intervals following injection of formalin. The experimental groups were separated into temporomandibular joint pain; control group (formalin, 5%), vehicle group (5% formalin after sodium carboxymethyl cellulose), single administration group, single mixed administration group, repeated administration group. The experiments were carried out various concentrations of Zingiberaceae plants extract. **Results**: Consequently, oral administration of C.L, C.A, and Z.O (p.o., concentrations of 12.5, 25 mg/ml) in inflammatory pain model significantly decrease the nociceptive behavior in a concentration dependent manner. And it tended to decrease at low concentration (6.25 mg/ml) of single mixed and repeated administration more than single administration. **Conclusions**: These results suggest that C.L, C.A, and Z.O extract administration regulate the inflammatory TMJ pain. Therefore, C.L, C.A, and Z.O extract may be a potential therapeutic treatment for inflammatory TMJ pain.

KEYWORD: *Curcuma longa L., Curcuma aromatica Salisb., Zingiber officinale Rosc.., TMJ, Inflammation*.

INTRODUCTION

Plants which belong to the ginger family (Zingiberaceae) are perennial herbaceous and ginger, turmeric, and turmeric root are representative. First of all, ginger (*Zingiber officinale Roscoe*) is a plant with dark yellow, yellow, or red color, and it is widely used as a spice for food. The unique stimulating flavor of ginger is due to gingerol, shogaol, zingerone, and others, and previous studies have reported that it contains diverse physiologically active compounds such as flavonoids and terpenoids.[7] Especially, shogaol and gingerol among the major components of ginger are known to be effective inhibitors of prostaglandin (PG) and leukotriene synthase. Many studies have reported that ginger has anti-cancer effects, anti-inflammatory effects, stomach protection, anti-ulcer, and antiemetic effects.[9] Moreover, 6-gingerol, one of the ginger extract, is known to effectively inhibit the activity of cyclooxygenase-2 (COX-2).[8] Turmeric root (*Curcuma longa L., turmeric*) has traditionally been used for herbal medicines, spices, and an edible plant.[10] Moreover, the known major efficacies of turmeric root are to promote the detoxification of the liver, help the secretion of bile. Due to these efficacies, it is used for aromatic stomachic medicine, cathartic jaundice medicine, gallbladder disease, acute hepatitis medicine and, consequently, the demand of turmeric root is high for various prescriptions and pharmaceutical raw materials in South Korea and other countries. Moreover, as the pharmacological effects of curcuminoids, which are the biologically active substances originated from turmeric root, is getting known, many studies have been actively performed especially in the medical field.[11] Curcuminoids are mainly composed of three compounds: curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BMC). Among them, many studies have reported diverse efficacies of curcumin, which is a yellow pigment and has shown various pharmacological activities, in medicine, pharmacy, and food science for a long time.[12] Lastly, curcumin is the main component of turmeric, which belongs to genus Curcuma, Zingiberaceae, and many studies have revealed the arteriosclerosis inhibition, anti-inflammatory, anticancer, antioxidant, and antimicrobial effects of it.[14] These studies have proved the effective pharmacological activity of turmeric root, turmeric, and ginger, which belong to Zingiberaceae. Many studies

have been performed actively to prove the efficacy of many natural products in addition to plants in Zingiberaceae. However, there is no study evaluating the effects of natural products on inflammatory disease or pain control occurring in the orofacial region. Therefore, this study aimed to examine the effects of turmeric root, turmeric, and ginger extract on the occurrence and control of pain in the inflammatory temporomandibular joint (TMJ).

Orofacial pain that is associated with the hard and soft tissues in the head, face and all structures within the oral cavity express in a similar form of pain (e.g., tooth diseases, temporomandibular disorders (TMD), and neuropathic pain). Moreover, Orofacial pain has complex and unique signaling pathways through the trigeminal nerve system, so it is difficult to distinguish lesions clearly and treat them.[15] Especially, TMJ pain can be caused by various reasons including bad habits, malocclusion and trauma. Moreover, vasocontraction and muscle tension, which are due to the stress hormone around the TMJ where many blood vessels and nerves are distributed, can be the cause of the pain. The major symptom of TMJ pain is the pain occurring around the masticatory muscle, the frontal part of the ear, and TMJ and can be associated with facial pain, headache, migraine, and cervical pain. It is mainly caused or exacerbated by mastication or mandibular functions and accompanied by symptoms such as condylar lock, crackling jaw, constraints of mandibular movement, asymmetric exercise.[23,24] There are many data reporting that pain reliever, muscle relaxants, and tricyclic antidepressant are effective for treating TMD pain as medication treatment. Although these medications are useful for treating the pain, problems such as drug addiction, side effects, and conflicts with previously used medications.[25] Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used and they are chemical drugs that commonly have analgesics, anti-inflammatory, and antipyretic actions. However, NSAIDs is known to cause various side effects and, especially, they are known to cause various complications in the gastrointestinal tract in high-frequency. Although new medicines or medical supplies have been made from diverse natural materials and have been applied to clinical treatments, there are not many studies on medications for oral and dental diseases yet.[27]

Therefore, this study aimed to evaluate the control effects of pain behavior response by injecting turmeric root, turmeric, and ginger extract based on the application of inflammatory TMJ pain model, which was induced by applying formalin to experimental animals.

MATERIALS AND METHODS

1. Materials

1) Animal

Male Sprague-Dawley albino rats (240-280 g) were supplied from Hyochang Science (Daegu, Republic of Korea) and they were between 7 and 8 weeks old. The feed and water supplies were unlimited. Room temperature was maintained between 23 and 25℃, and a 12-hour day and night cycle was provided. Stress was minimized as much as possible before the experiment including behavioral suppression. This study complies with the ethical regulations on the conscious animal experiment of the Korean Pain Society and it was conducted after obtaining the approval of the Animal Ethics Committee of Dong-Eui University (R2018-012).

2) Reagents

Turmeric root powder (product of the Republic of Korea) and turmeric powder (product of India) were purchased from Cheongwoon Distribution Co., Ltd., and ginger powder (product of the Republic of Korea) was purchased from Hyundai Food Co., Ltd. The experiment groups were a vehicle (5% sodium carboxymethyl cellulose [CMC-Na]), a 5% formalin-injection group, a turmeric root extract injection group (C.L group), a turmeric extract injection group (C.A group), and a ginger extract injection group (Z.O group). Reagents were used after diluting with 5% CMC-Na. The administration of each drug was divided into single dose (once), repeated dose (once a day for three days) and single mixed dose (turmeric root, turmeric, and ginger) groups.

2. Methods

1) Expression of inflammatory temporomandibular joint pain and evaluation of the effects of turmeric root, turmeric, and ginger effects.

Turmeric root, turmeric, and ginger extract were orally administered using oral zonde (ø1.8(15 gauge)*80mm) for rats at a dose of 1 ml each. Drug administration was performed 30minutes before pain induction. A preliminary experiment was carried out to check the location of the articular cavity by injecting 1% Evans blue dye in the target volume of the formalin. The drug injection region was confirmed again after observing the pain behavior response of experimental animals. The position of the articular cavity was estimated by touching the head of condylar and the posteroinferior border of the zygomatic arch. When the tip of a needle penetrated into the cavity and touched the mandibular fossa, it was considered as the inside of the articular cavity. The cannula for injecting formalin was prepared by connecting 30gauge needle at one end of a polyethylene tube and an insulin syringe (0.25×8 mm) is connected to the opposite end of it. After anesthetizing a rat using ether inhalation, 30 $\mu\ell$ of 5% formalin was injected by touching the articular cavity, it was observed to recover consciousness within a few seconds after injecting formalin. Rubbing or scratching the TMJ was considered as the pain indication after the formalin injection. The response was recorded every five minutes for nine consecutive times (45 minutes observation time). It was divided into the first phase pain behavior response (0-10minutes) and the second phase pain behavior response (11-45minutes) and the responses were evaluated

accordingly.

2) Statistical Analysis

The experimental results were analyzed statistically using IBM SPSS statistic ver. 22 (IBM Co., Armonk, NY, USA). One-way ANOVA with repeated measurements was used and LSD test was conducted for post-hoc analysis. Statistical significance was determined at $p<0.05$. The results were expressed in mean ± standard error (SEM).

RESULTS

1. Effects of turmeric root extract on regulating inflammatory temporomandibular joint's pain behavior response

The effects of turmeric root on regulating pain behavior response in the inflammatory TMJ were examined after injecting 5% formalin (30 $\mu\ell$) into the right articular cavity of experimental animals (Fig. 1). The first phase pain behavior response in the TMJ was 21.17 ± 3.20 and 10.50 ± 6.18 times for the formalin group and the vehicle group, respectively. Moreover, it was 9.17 ± 6.92, 16.17 ± 6.79, and 10.83 ± 7.28 times for the C.L group according to the concentration 6.25, 12.5, and 25 mg/ml, respectively. There was no significant difference between the vehicle group and the experimental groups. The results of the second phase pain behavior response showed that pain behavior response was 176.67 ± 10.12 and 154.00 ± 18.16 times for the formalin group and the vehicle group, respectively, while that of the 6.25 mg/ml C.L group and that of the 12.5 mg/ml C.L group were 120.17 ± 7.67 and 90.00 ± 14.85 times, respectively. It was found that pain behavior response was significantly lower in these groups than the formalin group and the vehicle group. Moreover, the pain behavior response of the 25 mg/ml C.L group was 71.67 ± 11.51 times, which was significantly lower than the control group, the 6.25 mg/ml C.L group, and the 12.5 mg/ml C.L group ($p<0.05$).

The temporal dynamics of pain behavior response was also examined (Fig. 2). It was observed that the pain behavior response induced by formalin injection increased from 20 minutes after administration, peaked at 30 minutes, began to decrease from 35 minutes, and slightly increased from 40 minutes. The 6.25 and 12.5 mg/ml C.L group peaked at 25 minutes after administration and began to decrease from 30 minutes. The 25 mg/ml C.L group significantly reduced the pain behavior response from 20 minutes, when it began to increase due to formalin injection.

2. Effects of turmeric extract on regulating inflammatory temporomandibular joint's pain behavior response

The inflammatory TMJ pain was induced by administrating 5% formalin (30 $\mu\ell$) into the right articular cavity of experimental animals, and the pain behavior response control effects of turmeric were shown as below (Fig. 3). It was found that the first phase pain behavior response in the TMJ was 17.33 ± 4.36 and 6.25 ± 4.25 times for the formalin group and the vehicle group, respectively. It was 9.67 ± 3.02, 4.50 ± 3.76, and 2.50 ± 1.63 times in the C.A group according to the concentration (6.25, 12.5, and 25.0 mg/ml), respectively. There was no significant difference between the vehicle group and the experimental groups. The results of the second phase pain behavior were 176.50 ± 10.17 and 161.00 ± 14.20 times for the formalin group and the vehicle group, respectively, which were high. However, those of the 6.25 and 12.5 mg/ml C.A groups were 123.83 ± 2.23 and 98.33 ± 13.31 times, which were significantly lower than those of the formalin group and those of the vehicle group. Moreover, it was 73.00 ± 8.94 times in the 25 mg/ml C.A group, which indicated that it was the significantly lowest among all treatments ($p<0.05$).

The temporal dynamics of pain behavior response were evaluated (Fig. 4). Pain behavior response induced by formalin injection increased after 20 minutes, peaked at 30, began to decrease at 35 minutes, and slightly increased at 45 minutes. The oral administration of 6.25 mg/ml C.A group peaked at 20 minutes after formalin injection and began to decrease from 25 minutes. That of 12.5 mg/ml C.A group peaked at 30 and began to decrease from 35 minutes. That of 25.0 mg/ml C.A group peaked at 20 minutes and significantly decreased the pain behavior response induced by formalin from 25 minutes.

3. Effects of ginger extract on regulating inflammatory temporomandibular joint's pain behavior response

The inflammatory TMJ pain was induced by administrating 5% formalin (30 $\mu\ell$) into the right articular cavity of experimental animals, and the pain behavior response control effects of ginger were observed (Fig. 5). The first phase pain behavior response in the TMJ part was 19.33 ± 4.42 and 2.25 ± 1.65 times for the formalin group and the vehicle group, respectively, and it was 4.67 ± 3.13, 11.50 ± 4.82, and 11.29 ± 5.41 times for 6.25, 12.5, and 25.0 mg/ml of Z.O group. There was no significant difference between the vehicle group and the experimental groups. The second phase pain behavior response was 178.00 ± 9.57 and 166.75 ± 12.10 times for the formalin group and the vehicle group, respectively, which were high. The 6.25 and 12.5 mg/ml Z.O groups showed 114.17 ± 4.17 and 100.50 ± 21.21 times, which were significantly lower than the formalin group and the vehicle group. Moreover, it was 56.43 ± 10.68 times for the 25 mg/ml of Z.O group, indicating that it significantly reduced pain behavior response and the magnitude of decrease was concentration dependent ($p<0.05$).

The temporal dynamics of pain behavior response were observed (Fig. 6). Pain behavior response induced by formalin injection increased after 20 minutes and peaked at 30. The oral administration of 6.25 mg/ml Z.O group

was high between 25 and 30 minutes after formalin injection and began to decrease from 35 minutes. The 12.5 and 25 mg/ml Z.O groups significantly decreased the pain behavior response induced by formalin from 20 minutes, when it generally increased.

4. Effects of turmeric root, turmeric, and ginger extract mixture on regulating inflammatory temporomandibular joint's pain behavior response

The inflammatory TMJ pain was induced by administrating 5% formalin (30 $\mu\ell$) into the right articular cavity of experimental animals, and the pain behavior response control effects of turmeric root, turmeric, and ginger extract mixture were examined (Fig. 7). The first phase pain behavior response in the facial region was 21.17 ± 3.20, 10.50 ± 6.18, 9.17 ± 6.92, 9.67 ± 3.02, and 4.67 ± 3.13 times for the formalin group, the vehicle group, the 6.25 mg/ml C.L group, the 6.25 mg/ml C.A group, and the 6.25 mg/ml Z.O group, respectively. The 6.25 mg/ml turmeric root, turmeric, and ginger mixture group showed 0.83 ± 0.83 times. The second phase pain behavior response was 176.67 ± 10.12, 164.00 ± 18.16, 120.17 ± 7.67, 123.83 ± 2.23, and 114.17 ± 4.17 times for formalin group, the vehicle group, the 6.25 mg/ml C.L group, the 6.25 mg/ml C.A group, and the 6.25 mg/ml Z.O group, respectively, showing that the pain behavior response of the experimental groups was lower than that of the vehicle group. Moreover, the second phase pain behavior response of the mixture group was 60.50 ± 2.88 times, which was significantly lower than that of the 6.25 mg/ml C.L group, that of the 6.25 mg/ml C.A group, and that of the 6.25 mg/ml Z.O group ($p<0.05$).

The temporal dynamics of pain behavior response were examined (Fig. 8). Pain behavior response induced by formalin injection increased after 20 minutes and peaked at 30 minutes. The oral administration of 6.25 mg/ml C.L group, that of 6.25 mg/ml C.A group, and that of 6.25 mg/ml Z.O group significantly decreased pain behavior response between 20 and 35minutes after formalin injection. The oral administration of the mixture group significantly decreased the pain behavior response induced by formalin since 20 minutes persistently.

5. Effects of repeated turmeric root, turmeric, and ginger extract treatment on regulating inflammatory temporomandibular joint's pain behavior response

The inflammatory TMJ pain was induced by administrating 5% formalin (30 $\mu\ell$) into the right articular cavity of experimental animals, and the pain behavior response control effects of repeatedly administrated turmeric root, turmeric, and ginger were examined (Fig. 9). The first phase pain behavior response in the TMJ region was 17.50 ± 4.42, 2.17 ± 1.17, 19.00 ± 4.74, 7.83 ± 4.35, and 10.17 ± 4.29 for the formalin group, the vehicle group, the 6.25 mg/ml repeated C.L group, the 6.25 mg/ml repeated C.A group, and the 6.25 mg/ml repeated Z.O group, respectively. The vehicle group and the experimental groups were not significant difference. The second phase pain behavior response was 168.83 ± 8.11 and 163.00 ± 9.20 times for the formalin group and the vehicle group, respectively, which were high. It was 54.83 ± 21.12, 56.00 ± 13.16, and 48.50 ± 7.29 for the 6.25mg/ml repeated C.L group, the 6.25 mg/ml repeated C.A group, and the 6.25 mg/ml repeated Z.O group, respectively, indicating that pain behavior response significantly decreased. Among these repeated administration groups, pain behavior response was the lowest in the Z.O group, followed by the C.L group and the C.A group, and the results showed that the pain behavior response of the repeated administration groups was significantly lower than that of the single administration groups ($p<0.05$).

The temporal dynamics of pain behavior response were observed (Fig. 10). Pain behavior response induced by formalin injection increased after 20 minutes and peaked at 30 minutes. It was found that the 6.25 mg/ml repeated administration groups (C.L group, C.A group, and Z.O group) were significantly reduced pain behavior response after 25 minutes after formalin injection, while pain behavior response was high between 20 and 25 minutes.

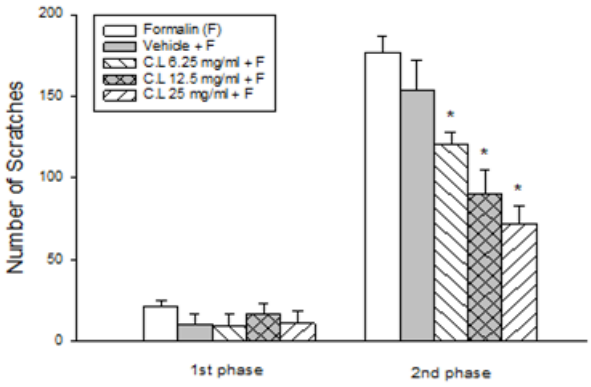

Fig. 1. Effects of C.L on nociceptive behavior at TMJ. Following administration of C.L, the nociceptive responses were reduced in 2nd phase in a dose-dependent manner. There were 6 animals in each group. *p<0.05, Formalin vs C.L 6.25, 12.5, 25 mg/ml + F.

C.L: *Curcuma longa L.*

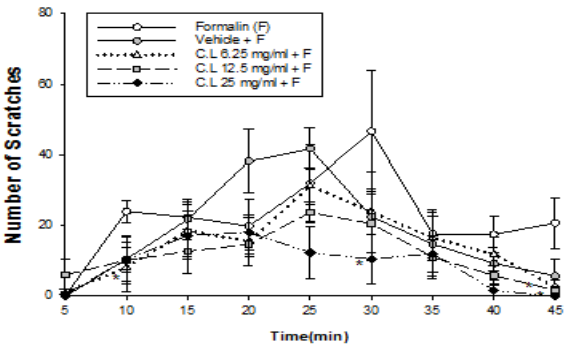

Fig. 2. Effects of C.L on nociceptive behavior at TMJ. Following administration of C.L, the nociceptive responses were reduced in 2nd phase in a dose-

dependent manner. There were 6 animals in each group. *p<0.05, Formalin vs C.L 6.25, 12.5, 25 mg/ml + F.
C.L: *Curcuma longa L.*

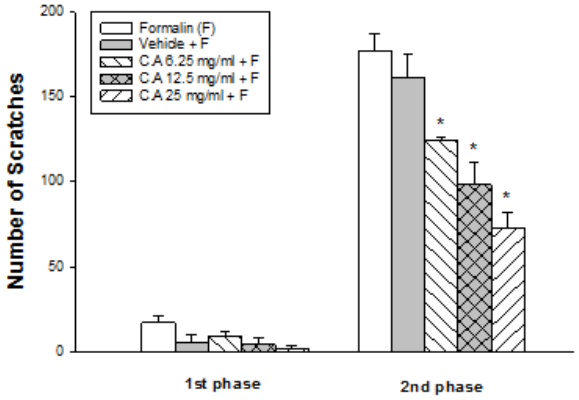

Fig. 3. Changes in nociceptive responses at TMJ following administration of C.A. Oral administration of C.A, significantly reduced the nociceptive responses 20~30 mim after induction of pain. There were 6 animals in each group. *p<0.05, Formalin vs C.A 6.25, 12.5, 25 mg/ml + F.
C.A: Curcuma aromatic Salisb.

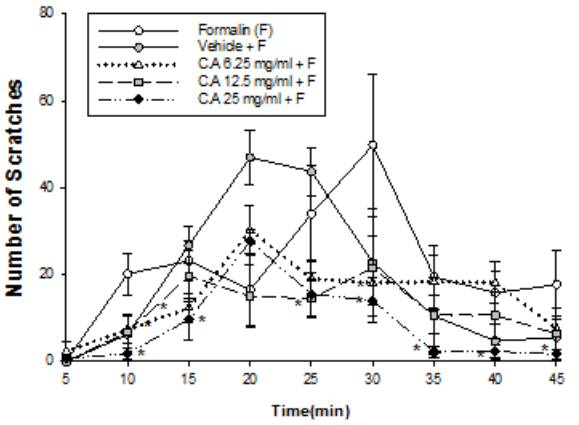

Fig. 4. Changes in nociceptive responses at TMJ following administration of C.A. Oral administration of C.A, significantly reduced the nociceptive responses 20~30 mim after induction of pain. There were 6 animals in each group. *p<0.05, Formalin vs C.A 6.25, 12.5, 25 mg/ml + F.
C.A: Curcuma aromatica Salisb.

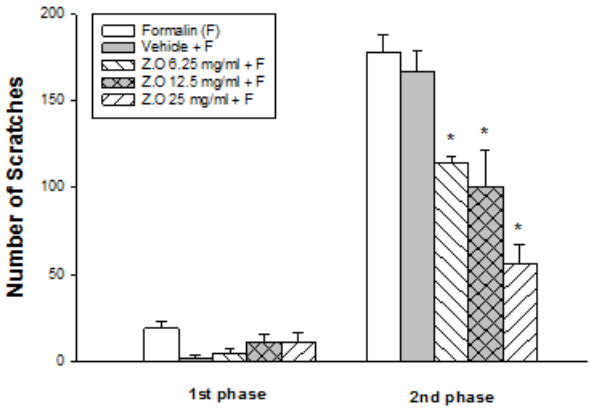

Fig. 5. Effects of Z.O on nociceptive behavior at TMJ. Following administration of Z.O, the nociceptive responses were reduced in 2nd phase in a dose-dependent manner. There were 6 animals in each group. *p<0.05, Formalin vs Z.O 6.25, 12.5, 25 mg/ml + F.
Z.O: *Zingiber officinale Rosc.*

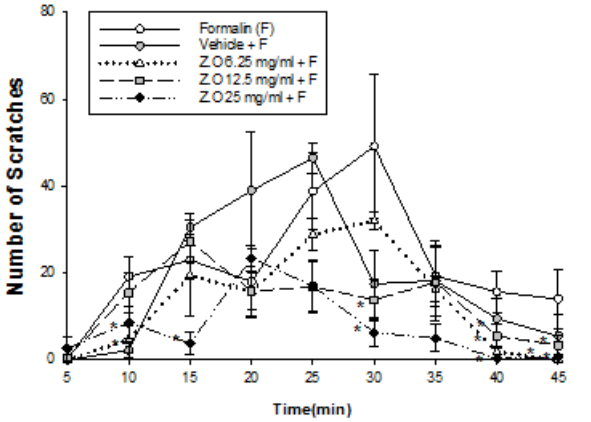

Fig. 6. Effects of Z.O on nociceptive behavior at TMJ. Following administration of Z.O, the nociceptive responses were reduced in 2nd phase in a dose-dependent manner. There were 6 animals in each group. *p<0.05, Formalin vs Z.O 6.25, 12.5, 25 mg/ml + F.
Z.O: *Zingiber officinale Rosc.*

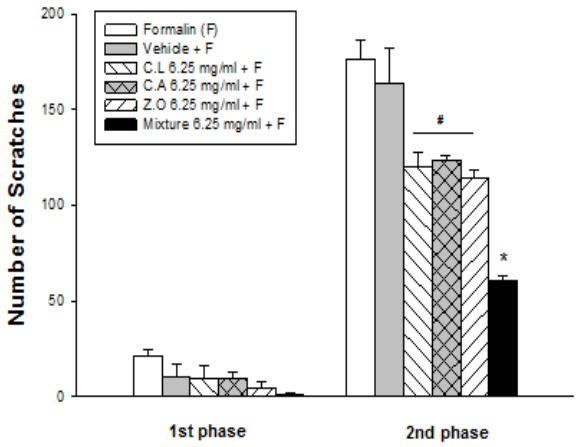

Fig. 7. Effects of mixture on nociceptive behavior at TMJ. Following administration of mixture, the nociceptive responses were reduced in 2nd phase in a dose-dependent manner. There were 6 animals in each group. #p<0.05, formalin vs C.L, C.A, Z.O, *p<0.05, C.L, C.A, Z.O vs mixture 6.25 mg/ml + F.

C.L: *Curcuma longa L.*
C.A: Curcuma aromatica Salisb.
Z.O: *Zingiber officinale Rosc.*
Mixture: combine of C.L, C.A, Z.O

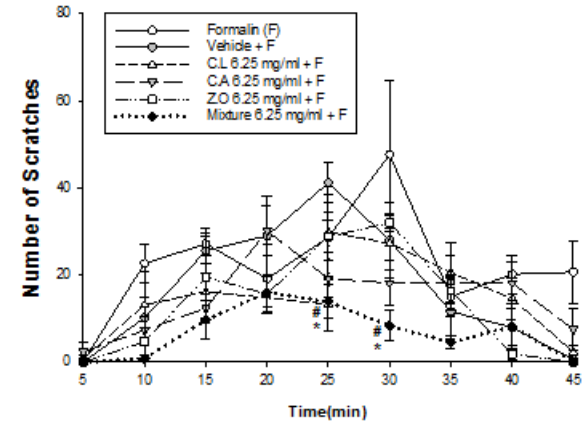

Fig. 8. Effects of mixture on nociceptive behavior at TMJ. Following administration of mixture, the nociceptive responses were reduced in 2nd phase in a dose-dependent manner. There were 6 animals in each group. *p<0.05, C.L vs mixture 6.25 mg/ml + F, #p<0.05, Z.O vs mixture 6.25 mg/ml + F.

C.L: *Curcuma longa L.*
C.A: Curcuma aroma tica Salisb.
Z.O: *Zingiber officinale Rosc.*
Mixture: combine of C.L, C.A, Z.O

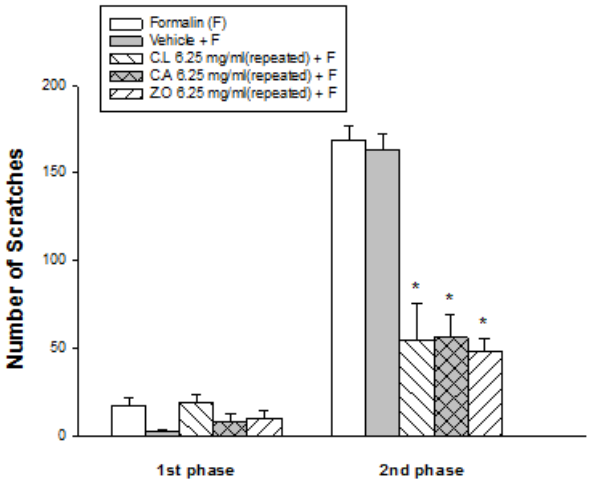

Fig. 9. Effects of repeated on nociceptive behavior at TMJ. Following administration of repeated, the nociceptive responses were reduced in 2nd phase in a dose-dependent manner. There were 6 animals in each group. *p<0.05, Formalin vs C.L, C.A, Z.O 6.25 mg/ml + F.

C.L: *Curcuma longa L.*
C.A: Curcuma aromatica Salisb.
Z.O: *Zingiber officinale Rosc.*

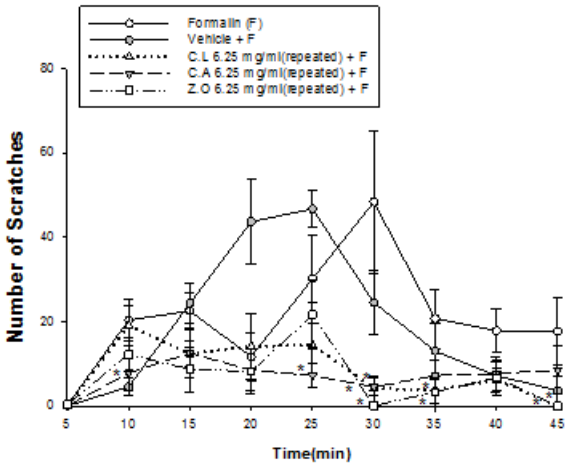

Fig. 10. Effects of repeated on nociceptive behavior at TMJ. Following administration of repeated, the nociceptive responses were reduced in 2nd phase in a dose-dependent manner. There were 6 animals in each group. *p<0.05, Formalin vs C.L, C.A, Z.O 6.25 mg/ml + F.

C.L: *Curcuma longa L.*
C.A: Curcuma aromatica Salisb.
Z.O: *Zingiber officinale Rosc.*

DISCUSSION

Previous studies have shown that the occurrence of facial pain including TMJ affects daily life, exercise activities, and socio-economic activities.[32] Recently, the TMJ has become an important disease not only dental medicine but also as in oriental medicine and rehabilitation medicine, so that various treatment methods have been developed. Due to the increased incidence and

prevalence of TMD, more dental clinics specialized in the TMJ have been established. Since people are able to access medical information through various routes, they have become more interested in health. TMJ pain can be induced by direct causes (e.g., tissue damage, trauma, and intense pressure) and oxidation stress due to various causal factors, and anti-inflammatory analgesics and physical therapy on a localized area are representative treatment methods. However, symptomatic therapy can also control pain owing to the action of various antioxidants, which are contained in natural products or foods.

A study recently examined the possibility of using BSASM (BioSpectrum Ato Soothing Max), which is made by mixing vegetable ingredients such as licorice, green tea, rosemary, Asiatic pennywort, chamomile, scutellaria, and Korean knotweed, as an alternative medicine replacing steroid hormone *in vitro*. The results of the study confirmed that BSASM demonstrated immunoregulation effects through inhibiting the IL-2 expression in the T-cell along with anti-inflammatory effects.[34] Moreover, inflammatory pain model induced by formalin injection in the hind paw of experimental animals showed that the injection of curcumin into the spinal cord cavity could effectively regulate inflammatory pain, which proved the possibility of using curcumin as a analgesics.[35] The results of this study clearly showed that orally administrating the extract of plants belong to Zingiberaceae could reduce formalin-induced inflammatory pain in the TMJ in a dose-dependent manner. The results indicate that the phytonutrients of Zingiberaceae plants are associated with the control of inflammatory pain and may be effective in controlling the pain in the inflammatory TMJ. Particularly, curcumin in turmeric root and turmeric, which belong to Zingiberaceae, are proven to be an effective antioxidant by many previous studies. Curcumin is a major component of turmeric root and turmeric (Zingiberaceae). It is widely used as an antioxidant, anticancer, and antiviral agent, and it is known to prevent DNA damage due to oxidation and to be good at eliminating free radicals.[40] Lee et al.(2015) reported that an enlarged prostate was alleviated after administering the hot water-soluble extract of turmeric root to rats, which had enlarged prostates induced by testosterone and the administration reduced IL-6, TNF-α, and IL-1β, and curcumin reduced the activation of COX-2, NF-κb. Among major components of ginger (Zingiberaceae), 6-gingerol exhibits anti-inflammatory, germicidal, and antioxidant effects.[42] In the ulcerative colitis-induced Balb/c male mice, concentrations of IFN-γ, IL-6, TNF-α, IL-12, INOS, and COX-2 decreased following administration of the ginger extract.[9] The results of previous studies using diverse experiment models have confirmed that Zingiberaceae plants have antioxidant and anti-inflammatory effects.

Our study showed that administrating the mixture of turmeric root, turmeric, and ginger extract decreased pain behavior response significantly more than administering the extract of each one. It is presumed that it is because of the synergistic effect of medicines that have similar pharmacological action. In the maxillofacial region of rat, after formalin-induced inflammation, administrating of resveratrol and sulforaphane more decreased the concentration of IL-6, IL-1β, Nrf2, iNOS, and Nox4 when they administered together than they administered separately.[43] Moreover, a study evaluating the antifungal action of chlorogenic acid and fluconazole against *C. albicans* revealed that the chlorogenic acid and fluconazole combination was more effective in treating skin candidiasis than the chlorogenic acid alone or fluconazole alone treatment. The result suggested that the combination of the two medications would decrease the use of fluconazole and reduce the side effects of fluconazole.[44] Therefore, the combination of the natural extract exhibiting similar pharmacological actions may increase the efficacy of extract and reduce the amount of a dose, resulting in reducing the side effects that may be caused by a medication.

This study examined the inflammatory pain control ability in the TMJ using Zingiberaceae plants, which have antioxidant and anti-inflammatory effects. Pain behavior response, which was significantly increased by formalin induction, decreased by turmeric root, turmeric, and ginger oral administration in a dose-dependent manner. The results implied that Zingiberaceae plants could be applied to the occurrence and control of inflammatory maxillofacial pain, which was induced by formalin, effectively. Additionally, this study confirmed that the mixture administration of low concentration turmeric root, turmeric, and ginger extract or the repeated administration of low concentration single extract was more effective in pain control than a single administration of one extract type. In conclusion, the results of this study implied that Zingiberaceae plants could be utilized as candidates for developing medications useful for controlling inflammatory pain in the TMJ.

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