Effect of *Eriobotrya japonica* Seed Extract on 5-Fluorouracil-Induced Mucositis in Hamsters

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In this study, we investigated the effects of an *Eriobotrya japonica* seed extract (ESE) on mucositis using a 5-fluorouracil (5-FU)-induced mucositis hamster model. This model was prepared by intraperitoneally administering 90 mg/kg of 5-FU to hamsters on Day 1, scratching 1 cm² of the left cheek pouch of hamsters with a wire brush on Days 2, 3, and 4, and intraperitoneally administering 60 mg/kg of 5-FU on Day 5. Mucositis was evaluated based on the mucositis score at the mucositis site, left cheek pouch thickness, histological findings on HE staining, and plasma lipid peroxide levels. On Day 10, the mucositis score and left cheek pouch thickness in the ESE group were significantly lower than those in the tap water group. Histologically, the two groups showed a defect of the cheek pouch epithelium on Day 6. On Day 10, epithelial injury and bacterial infection were noted in the tap water group. However, in the ESE group, similar findings were not observed. On Day 6, the plasma lipid peroxide level in the tap water group was significantly higher than that in the normal group. In the ESE group, the plasma lipid peroxide level was significantly lower than that in the tap water group. These results suggest that ESE is useful for treating chemotherapy-induced mucositis.

Key words *Eriobotrya japonica* seed extract; chemotherapy; mucositis; oxidative stress; bacterial infection

Frequent/massive administration of anticancer agents frequently causes mucositis. This condition involves the entire oral cavity in many cases, inducing pain and hemorrhage. Therefore, it decreases dietary intake, markedly reducing the quality of life (QOL). First, free radicals produced by anticancer agents act on the oral mucosa as oxidative stress, destroying the mucosal tissue and causing mucositis. Secondly, the oral cavity enters an infection-prone state via the bone marrow-suppressing effects of anticancer agents, resulting in mucositis via bacterial infection. However, actually, these two factors may be intricately involved. Therefore, it is important to inhibit excessive anticancer agent-related oxidative stress and prevent bacterial infection in the oral cavity, for the prevention and treatment of mucositis.

Currently, chemotherapy-induced mucositis is being investigated in clinical practice. Several studies have reported that gargling with antioxidants, cryotherapy in which the development of free radicals in the oral cavity is prevented by reducing oral mucosal transfer of anticancer agents, and maintenance of a clean oral cavity achieved specific effects. However, it is difficult to prevent and treat chemotherapy-induced mucositis, and symptomatic therapy is mainly performed in clinical practice. Thus, strategies against chemotherapy-induced mucositis should be established.

*Eriobotrya japonica* seed extract (ESE), which was used in this study, was extracted from *Eriobotrya japonica* seeds with 70% EtOH. We previously confirmed that this extract contained various substances such as polyphenols, amino acids, and unsaturated fatty acids (Fig. 1). Furthermore, an *in vitro* study demonstrated the radical-scavenging actions of ESE. In several *in vivo* studies, ESE was useful for treating rats with dimethylnitrosamine-induced hepatopathy, a rat nephropathy model prepared by administering adriamycin, an anticancer agent, and a rat inflammation model prepared by administering lipopolysaccharide comprising Gram-negative bacteria. We confirmed that these effects were achieved via the antioxidant actions of ESE. These results suggest that ESE exhibits antioxidant and anti-inflammatory actions, and that this extract is useful for treating chemotherapy-induced mucositis. In this study, we investigated the effects of ESE using a hamster mucositis model prepared by 5-fluorouracil (5-FU) administration.

**MATERIALS AND METHODS**

Materials Sufficiently sun-dried seeds of Mogi-loquant collected at Muroto and Susaki cities in Kochi Prefecture and Shimotsucho in Wakayama Prefecture of Japan were used. 5-Fluorouracil (5-FU) was provided by Kyowahakko (Japan). All other chemicals were of reagent grade.

**Preparation of ESE** *Eriobotrya japonica* seeds were extracted by 70% ethanol. Briefly, 1.0 kg of seeds were crushed in a blender equipped with a refrigerator at 1000 rpm, and then continuously stirred by a mixer at 300 rpm for 7 d after being dissolved in 70% ethanol. The supernatant was then collected and evaporated to dryness to prepare the dried extracts. The final yield of 70% ethanol extracts was 108 g. The dried extracts were emulsified in 201 distilled water.

**Animals** Male Syrian Golden Hamsters, aged six weeks, were purchased from SLC Japan. Animals were acclimatized for 7 d at 23±2 °C with free access to pellet food (CE-2, Clea, Osaka, Japan) and water. Healthy hamsters were then selected and randomly divided into two groups: normal
group and mucositis model group. In addition, the mucositis model group was divided into a tap water group and ESE group. All animal experiments were performed according to the guideline for the care and use of laboratory animal of Kochi University and were approved by our local ethics committee for experimental animal use.

Preparation of 5-FU-Induced Mucositis Model Hamsters The animal model of mucositis was based on a modified method of Sonis et al.15) This model was prepared by intraperitoneally administering 90 mg/kg of 5-FU to hamsters on Day 1, scratching 1 cm² of the left cheek pouch with a wire brush on Days 2, 3, and 4, and intraperitoneally administering 60 mg/kg of 5-FU on Day 5. The normal group did not undergo rubbing of the cheek pouch, and was intraperitoneally administered saline.

Administration of ESE to Mucositis Model Hamsters ESE was administered to the mucositis model hamsters using a water-supply bottle at a dose of 10 ml/d from 7 d before model preparation until the end of this experiment.

Measurement of the Leukocyte Count On Days 6 and 10 of the experiment, blood was collected from 6 hamsters per group. Turck's reagent at 900 μl was added to 100 μl of blood, and leukocytes were counted using a blood cell counter.

Evaluation of Mucositis On Days 6, 8, and 10, mucositis was photographed using a digital camera. As described by Lima et al.,16) mucositis was evaluated using the single blind method. Furthermore, the cheek pouch mucosal thickness was measured on Day 10.

- Score 0: normal cheek pouch
- Score 1: moderate erythema and hyperemia; no hemorrhagic areas, ulcerations or abscess.
- Score 2: severe erythema and hyperemia; the presence of hemorrhagic areas, small ulcerations or scarred tissue, but no abscess.
- Score 3: severe erythema and hyperemia; the presence of hemorrhagic areas, extensive ulcerations and abscess.

For histological examination of mucositis, the left cheek pouch was extirpated after 6 hamsters per group were anesthetized with pentobarbital at 50 mg/kg on Days 6 and 10. Specimens were fixed in 10% formalin for 48 h, and paraffin-embedded. Then, hematoxylin and eosin (HE) staining was performed.

Measurement of Plasma Lipid Peroxide Levels On Days 6 and 10, the plasma lipid peroxide level was measured as a thiobarubarbituric acid reactive substance (TBARS), as described by Yagi.17)

Statistical Analysis The data are expressed as the mean±S.E.M. Statistical analysis was performed by analysis of variance (ANOVA), followed by the unpaired t-test was used for comparison of two-group, and Turkey test was used for comparison of more than two groups, \( p<0.05 \) indicated a significant difference among groups.

RESULTS

Effect of ESE on the Leukocyte Count Figure 2 shows the leukocyte Count. The leukocyte counts on Days 6 and 10 in the tap water and ESE groups were significantly lower than those in the normal group. The value on Day 10 was lower than that on Day 6. On Days 6 and 10, the values were similar between the tap water and ESE groups.

Effect of ESE on Mucositis Figure 3 shows the macroscopic findings of mucositis. Macroscopically, cheek pouch mucosal injury and hemorrhage at the injured site were ob-
served on Day 6 in all 6 animals in the tap water and ESE groups. On Day 10, abscess in the lesion site was noted in 5 of the 6 animals in the tap water group. In the ESE group, there was no abscess.

Figure 4 shows the mucositis score. The mucositis scores on Days 6 and 8 were similar between the tap water and ESE groups. In the tap water group, the score reached a maximum on Day 10. In the ESE group, it simultaneously reached a minimum. In addition, there was a significant difference between the two groups.

Figure 5 shows the cheek pouch mucosal thickness. In the tap water and ESE groups, the values of mucosal thickness at the lesion site on Day 10 were significantly higher than that in the normal group. Furthermore, the value in the ESE group was significantly lower than that in the tap water group.

**Histological Findings of Mucositis**  
Figure 6 shows the histological findings of mucositis. Histologically, on Day 6, defects of the buccal mucosal epithelial tissue and muscle layer were noted in the tap water and ESE groups. On Day 10, an epithelial tissue defect and bacterial infection with indigenous bacteria in the oral cavity at the local site of mucositis were confirmed in the tap water group. In the ESE group, there was no epithelial tissue defect nor bacterial infection. In addition, thin epithelial tissue/muscle layer formation was observed in the ESE group.

**Plasma Lipid Peroxide Level**  
Figure 7 shows the plasma lipid peroxide levels. On Day 6, the plasma lipid peroxide level in the tap water group was significantly higher than that in the normal group. Furthermore, the value in the ESE group was significantly lower than that in the tap water group.
There was no significant difference between the normal and ESE groups. On Day 10, there were no significant differences among the normal, tap water, and ESE groups.

**DISCUSSION**

A following review could be referred. In particular, gargling with antioxidants is effective, and is employed in many hospitals. However, problems with regurgitation after gargling and their bitter taste may reduce compliance. Furthermore, a study indicated that a xanthine oxidase inhibitor, allopurinol, was not effective for 5-FU-related mucositis. Therefore, in the future, various substances with antioxidant actions should be investigated to treat chemotherapy-induced mucositis.

In this experiment, we used a model prepared by Sonis et al. This model is commonly used to investigate chemotherapy-induced mucositis. In this model, mucositis was induced by scraping the oral mucosa after bone marrow suppression was induced by massive 5-FU administration. Therefore, it has been reported that the leukocyte count after 5-FU administration decreased. In this experiment, there was also a decrease in the leukocyte count in the 5-FU-treated group. In addition, the leukocyte counts were similar between the tap water and ESE groups. Based on these findings, ESE administration may not influence the effects of 5-FU.

On Days 6 and 8, the state of mucositis was similar between the tap water and ESE groups. On Day 10, the mucositis score and mucosal thickness at the mucositis site were higher in the tap water group. In the ESE group, these parameters were low. As mucositis was induced in our experimental model, the process of healing after onset was mainly observed. Therefore, there was a difference in the state of mucositis on Day 10 between the two groups, possibly because ESE administration promoted the healing of mucositis. In addition, we histologically confirmed a difference in thickness on Day 10 between the two groups, as demonstrated by macroscopic findings. In the tap water group, an epithelial tissue defect and bacterial infection at the mucositis site were noted, suggesting the delayed healing of mucositis. In the ESE group, at the mucositis site, the epithelial tissue and muscle layer had regenerated, and bacterial infection was not observed. Thus, we confirmed that ESE administration promoted the healing of mucositis.

The results regarding the plasma lipid peroxide level showed the in vivo development of excessive oxidative stress immediately after 5-FU administration in the tap water group. In the ESE group, excessive oxidative stress may have been inhibited. These findings suggest that ESE eliminates free radicals excessively produced in vivo, that is, an important factor for chemotherapy-related mucositis.

This experiment demonstrated that ESE promoted the healing of mucositis in a hamster 5-FU-induced mucositis model. The action mechanism may involve the prevention of oxidative stress, leading to the suppression of mucosal injury.
bacterial infection in local mucositis sites and inhibition of excessive in vivo oxidative stress immediately after the administration of anticancer agents. We previously confirmed that ESE contained various substances. In this experiment, bacterial infection was possibly inhibited in local mucositis sites via the bactericidal actions of benzoic and caffeic acids contained in this extract. Furthermore, the antioxidant actions of ESE may be associated with a fat-soluble fraction, β-sitosterol. In the future, further examination is needed. In addition, ingestion of ESE, which promotes the healing of mucositis, before the administration of anticancer agents may be useful for preventing mucositis. Therefore, the preventive effects of ESE on mucositis should also be further investigated in the future.

Conklin reported that the administration of substances with antioxidant actions during chemotherapy enhanced the effects of anticancer agents. In this experiment, we examined the influence of ESE administration on 5-FU effects using the leukocyte count as an index, because the meaning is uncertain, author should describe it clearly. There was no influence of ESE administration on 5-FU effects. However, ESE administration may enhance the anticancer effects of anticancer agents, as reported by Conklin. Therefore, this issue should be further investigated using cancer models in the future.

In this study, we confirmed that ESE promoted the healing of chemotherapy-induced mucositis, suggesting its usefulness in the prevention/treatment of mucositis during chemotherapy.

REFERENCES