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Effects of Blackcurrant Blend On Formalin-Induced Or Facial Pain In Rats

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ABSTRACT:- The purpose of this study was to investigate the effect of black currant administered intraperitoneally on the pain induced by formalin injected into the facial area of experimental animals. Male Sprague-Dawley rats weighing 260～280 g were used. The experimental group was subdivided into 4 groups based on the treatment method (n=6, each group): formalin (5%), formalin after distilled water (vehicle), formalin after black currant blend. To establish two models of acute orofacial pain, formalin was injected into the articular cavity(30 μl) or vibrissa pad(50 μl) under ether inhalation anesthesia, respectively. The number of noxious behavioral responses of scratching the facial region proximal to the injection site was recorded for 9 successive 5-min intervals following formalin injection. The black currant blend treatment significantly reduced the acute orofacial pain compared to the vehicle treated group at a concentration of 30mg/kg ($p < 0.05$). In particular, it was confirmed that the pain behavioral response was significantly reduced and at 25-30 minutes. These results indicate that blackcurrant is effective in controlling orofacial pain.

Key Words: Orofacial pain, Blackcurrant, acute inflammatory pain, rat

I. INTRODUCTION

Facial pain occurs due to various causes. The cause may disappear after treatment, or pain recurring over months progresses through diverse processes. Recently, research on its therapy using natural products or fruits has been conducted actively[1].Among them, the blackcurrant (Ribes nigrum L.) is a deciduous shrub in the family Saxifragaceae of the order Rosales, and the nutrition and efficacy of its fruit and leaf is so widely recognized that it is called the 'king of berries.' Its color is a deep purple similar to that of grapes, and it has a sweet tart flavor. It is known that the antioxidative potency of blackcurrant fruits, which are rich in vitamin C, anthocyanins, carotenoids, and polyphenols, can have effects on the prevention of lifestyle diseases by improving immunity and blood circulation within the body physiologically[2]. The blackcurrant is particularly rich in anthocyanin components, compared with other berries, and it has been reported that anthocyanin in the retina has the function of protecting vision by facilitating the regeneration of rhodopsin protein. A number of studies have reported that taking anthocyanin in large amounts is associated with the anti-diabetic, anti-oxidative, anti-inflammatory, anti-cancer, and anti-obesity action. Most of previous studies on the blackcurrant relate to antioxidant activity, and articles that confirm its effects on cell membrane damage, using cell models, and the effects of preventing urolithiasis and nephrolithiasis have been reported on the basis thereof[3]. The development of the processed products of the blackcurrant or the component analysis of the blackcurrant is being carried out actively at home and abroad. In this context, this study aimed to investigate the effects of intraperitoneally administered blackcurrant on pain induced by formalin injected into the facial area of laboratory animals.

II. RESEARCH METHOD

2.1 Animals

Male Sprague-Dawley white rats (230-250 g) were used for this study. Stress on the laboratory animals was minimized as far as possible, by maintaining the 12-hour light cycle of alternating day and night and the constant environment of 23-24℃ and freely providing feed and water for the laboratory animals. This study was carried out in accordance with IASP Ethical Guidelines for Investigations of Experimental Pain in Conscious Animal (1982). And pre-experimental testing by means of behavioral oppression was minimized.

2.2 Inducing Pain in the Facial Area

Formalin response was carried out in the same way as the method used in the previous study. By accumulating responses every five minutes directly after 5% formalin was subcutaneously injected into the right vibrissa padsand TMJ(Temp- oromandibular joint)of the laboratory animals, their behavioral responses were observed for 45 minutes in total. And the behaviors of rubbing or scratching the facial area into which the drug

was injected were considered as pain markers[4].

2.3 Evaluation of Nociceptive Responses Induced by Blackcurrant Administration

The effects of the blackcurrant on the formalin-induced nociceptive responses of the laboratory animal were evaluated. 1 ml of saline and 1 ml of blackcurrant were injected into the left lower abdomens of laboratory animals in the control group and the treatment group, respectively, and after 30 minutes, 5% formalin was injected into their right facial areas. Nociceptive responses were observed for 45 minutes at an interval of 5 minutes directly after the injection[5].

2.4 Statistics

One-way ANOVA was performed to test the significance of the results of the nociceptive response experiment. For statistical comparison, the significance level was set to $P<0.05$. All the results were indicated as mean ± standard error (SEM).

III. RESULTS

3. 1. 5% formalin-induced orofacial pain

Figure 1 illustrates the number of scratches produced by formalin before the subcutaneous injection of Blackcurrant blend. The nociceptive responses were reduced in 2nd phase (11～45 minutes), following administration of Blackcurrant blend extracts into rat's vibrissa pad 30 min before formalin injection .The subcutaneous injections of the control group affect the number of scratches. 15, 30 mg/kg of Blackcurrant blend injected significant nociceptive effects ($p < 0.05$).

3.2.Pain control effect according to time after administration of baobab extract in facial area

Figure 2 illustrates the number of scratches produced by formalin before the TMJ injection of Blackcurrant blend. The TMJ injections of the control group affect the number of scratches. 15, 30 mg/kg of Blackcurrant blend injected significant nociceptive effects ($p < 0.05$).

3. 3. Pain control effect according to TMJ treatment

Figure 3 illustrates the number of scratches produced by formalin before the TMJ injection of Blackcurrant blend. The TMJ injections of the control group affect the number of scratches. The nociceptive responses were reduced in 2nd phase (11～45 minutes), following administration of Blackcurrant blend extracts into rat's TMJ 30 min before formalin injection. 15, 30 mg/kg of Blackcurrant blend injected significant nociceptive effects ($p < 0.05$).

3. 4. Pain control effect according to time after TMJ administration

Figure 4 illustrates the number of scratches produced by formalin before the TMJ injection of Blackcurrant blend. Administration of Blackcurrant blend extracts into rat's TMJ significantly reduced the nociceptive responses 15～25 minutes after induction of pain. There were 6 animals in each group. The TMJ injections of the control group affect the number of scratches. 15, 30 mg/kg of Blackcurrant blend injected significant nociceptive effects ($p < 0.05$).

IV. DISCUSSION

There are a wide variety of causes of facial pain, and extensive research is being carried out to identify the causes. It has been reported that natural products and fruits contribute to the prevention and decrease of diseases thanks to their anti-inflammatory, anti-cancer, analgesic, and anti-oxidative effects. Currant is a deciduous shrub of the dicotyledonous rosewood spideraceae. It is native to northwestern europe and is distinguished by the color of the fruit as red currant and black currant. Currents contains a lot of vitamin C and calcium, phosphorus and iron, etc., and it shows various pharmacological effects including antioxidation. In particular, black currant is rich in anthocyanin, and it is known to prevent antioxidation, vision loss, and cataract[6]. In addition, it is effective in preventing various vascular diseases such as myocardial infarction and hypertension and treating diabetes. Although it has proven effective in the development and treatment of various diseases, there have been few studies on the control of orofacial pain. Among them, the blackcurrant is widely used for food, and is known to contain vitamin C and polyphenolic compounds, particularly anthocyanin. Research on the chemical composition and anti-oxidative activity of the blackcurrant found that the blackcurrant contains vitamin C and polyphenol compounds in large amounts, and reported that it shows excellent effects in neuron protection and anti-oxidative action[7]. However, reports on the blackcurrant in connection with pain are insufficient. This study intended to investigate the efficacy of the blackcurrant in connection with oral and maxillofacial pain. To sum up findings from the above experiment, in case of injecting 5% formalin into the facial area, significantly increased nociceptive responses were observed compared with the control group. And it

was found that such nociceptive responses were reduced by the blackcurrant, which shows that the blackcurrant played an important part in controlling pain in the facial area. Therefore, findings of this experiment serve as the basis for understanding the treatment and prognosis of patients with facial pain, and it is deemed that further studies will be needed to reveal components contained in the blackcurrant.

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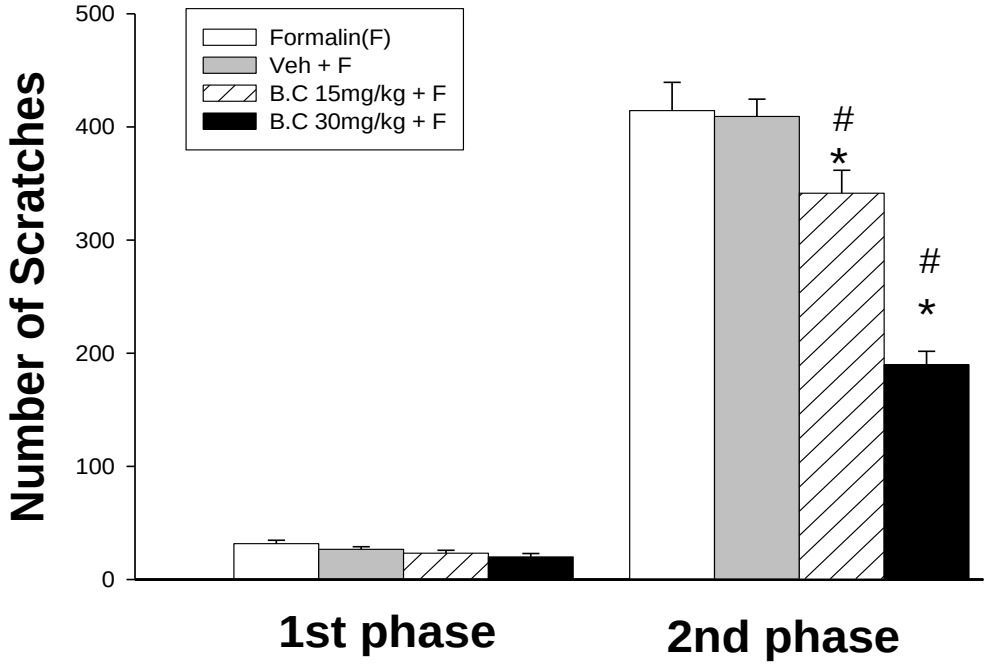
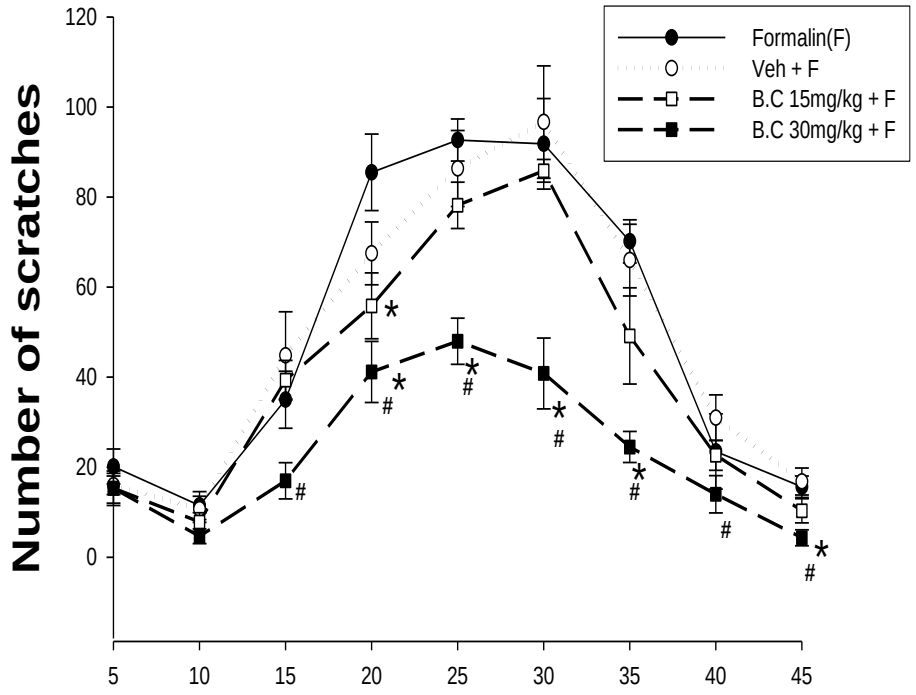
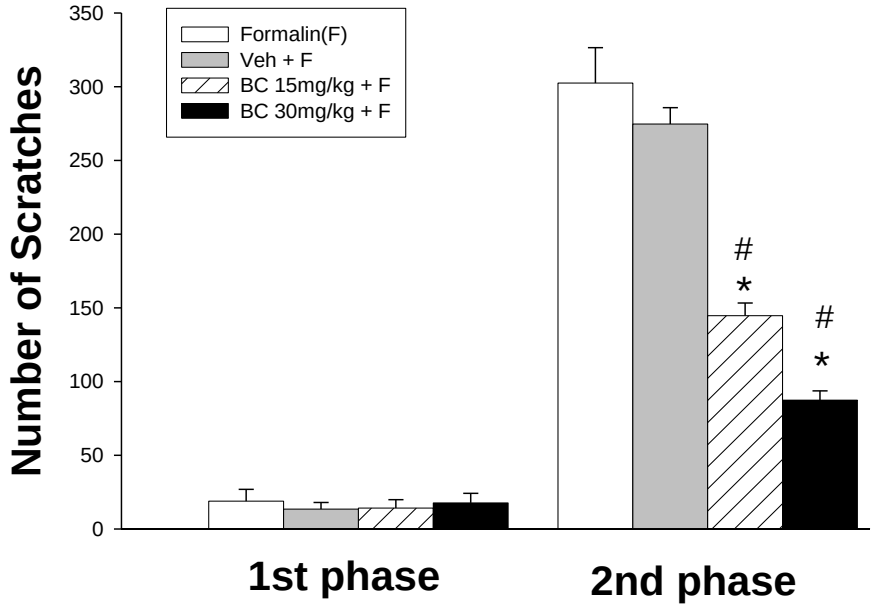


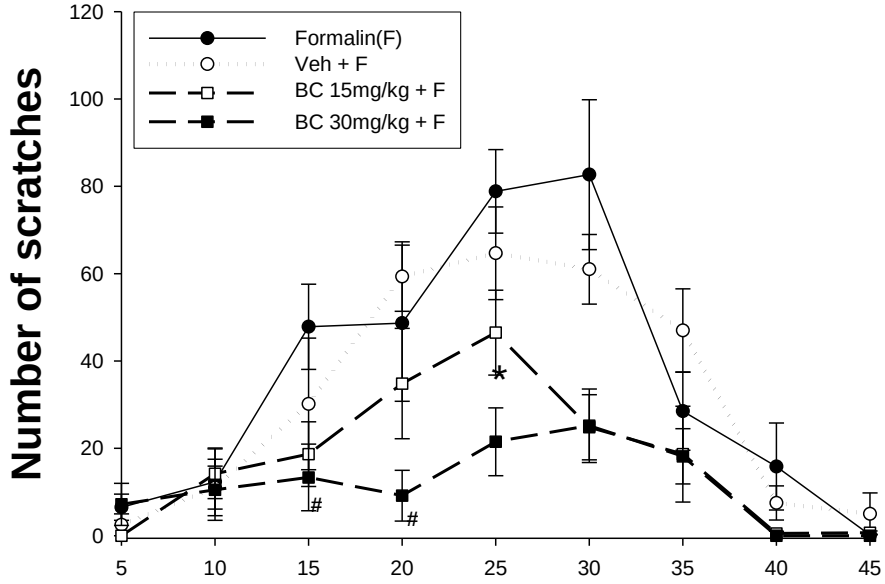
<Fig. 1> Effects of Blackcurrant blend extracts on nociceptive behavior(n=6). *p<0.05, Formalin vs Blackcurrant blend 15 mg/kg+ Formalin, *p<0.05, Formalin vs Blackcurrant blend 30 mg/kg + Formalin(n=6). #p<0.05, Veh vs Blackcurrant blend 15 mg/kg+ Formalin, *p<0.05, Veh vs Blackcurrant blend 30 mg/kg + Formalin(n=6).



<Fig. 2> Changes in nociceptive responses following administration of Blackcurrant blend extracts. #p<0.05, Formalin vs Blackcurrant blend 15 mg/kg+ Formalin(n=6). *p<0.05, Formalin vs Blackcurrant blend 30 mg/kg + Formalin(n=6).



<Fig. 3> Effects of Blackcurrant blend extracts on nociceptive behavior in TMJ(n=6). *p<0.05, F vs Blackcurrant blend 30 mg/kg + Formalin(n=6). #*p*<0.05, Veh vs Blackcurrant blend 15 mg/kg+ Formalin, **p*<0.05, Veh vs Blackcurrant blend 30 mg/kg + Formalin(n=6).



<Fig 4.> Changes in nociceptive responses following administration of Blackcurrant blend extracts into TMJ(n=6). #*p*<0.05, Formalin vs Blackcurrant blend 15 mg/kg+ Formalin(n=6). **p*<0.05, Formalin vs Blackcurrant blend 30 mg/kg + Formalin(n=6).