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Research Report

The effect of watermelon frost on prostaglandin E_2 (PGE_2) in inflamed pulp tissue (in vitro study)

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ABSTRACT

Background: *Pulp inflammation can be marked by the increase of prostaglandin E_2 (PGE_2) level compared to normal pulp. The increase of PGE_2 may lead to vasodilatation, increase of vascular permeability, pain and bone resorption. Watermelon frost has been well known in Chinese society for pain relief and inflammation in oral cavity and teeth.* ***Purpose:*** *The aim of this study was to investigate that watermelon frost can be used to decrease the PGE_2 level.* ***Method:*** *27 samples of pulp tissues used in this in-vitro study, were extirpated from the patients' teeth with symptomatic irreversible pulpitis referred to clinic of Conservative Dentistry, RSPGM Faculty of Dentistry, USU. Trial materials were applied to 27 samples i.e. watermelon frost as a trial material and commercial watermelon frost and eugenol to observe their effect on PGE_2. PGE_2 level of each material was detected through ELISA method by measuring and comparing the absorbance reading of the wells of the samples against standards with a micro plate reader at W_1 = 650 nm and W_2 = 490 nm.* ***Result:*** *The result showed the biggest effect was found in the third group (eugenol), mean 4.6933, followed by the first group (watermelon frost as a trial material), mean 18,1578 then the second group (commercial watermelon frost), mean 82,2689. One-Way ANOVA revealed that there were significant differences among all trial materials ($p < 0.001$) on PGE_2 level.* ***Conclusion:*** *This study demonstrated that watermelon frost can be used to decrease the PGE_2 level in inflamed pulp tissue and led to the acceptance of traditional medicine and natural products as an alternative form of dental care.*

Key words: *watermelon frost, prostaglandin-E_2, inflammation, pain*

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INTRODUCTION

Pulp inflammation is a case which is often found in dental practice. This inflammation has caused pain to the patients.

The inflamed pulp will cause pain that is obviously seen by the increase of prostaglandin E_2 (PGE_2) if it is compared with normal pulp. The increase of PGE_2 will trigger pain on teeth, vein vasodilatation, vascular permeability, and bone resorption.[1-7]

Hospitals and dental clinics with patients of symptomatic irreversible pulpitis and periodontitis need intradental pain relief to decrease inflammation. Generally, the pain relief, which is often used, is eugenol (clove oil) as sedative. Apparently, most of Indonesians have used clove oil as traditional medicine to relieve intradental pain for a long time. They unconsciously have known eugenol as local sedative but they have not precisely figured out how much eugenol should be consumed.[8] A study by Olsson *et al*.,[9] showed that any materials containing eugenol had more serious reaction of tissues than those without eugenol.[10]

Another pain relief commonly used is pulperyl containing creosate, procaine, phenol and chloroform. Several corticosteroids can be used to reduce intradental pain for acute pulpitis patients. Practically their use is combined with polyantibiotic. Because polyantibiotic is imported, its market price is expensive and it is not widely sold.

Watermelon frost, a traditional medicine for therapy, has been widely known by the Chinese community for ages. Clinical experiment has proven that watermelon frost affects inflammation treatment of oral cavity, tonsilitis, laryngitis,

pharyngitis, dental pain caused by high fever, inflamed gums, scald, etc.[11,12] This indicates that inflammation can be reduced or healed after being given watermelon frost as traditional medicine.

Research dealing with watermelon frost which will be used as pain relief should be increased more because Indonesia is enriched by tropical fruit like watermelon. In endodontic treatment the materials used should be biocompatible (nontoxic in human tissues).

However, concerning the use of watermelon frost as pain relief, a question arises as the following: Can watermelon frost that has been widely known for medical treatments of oral cavity, pharyngitis, laryngitis, gingivitis be used as intradental pain relief? The objective of the study was to figure out how watermelon frost affects PGE_2 which is one of inflammatory mediators.

It is expected that the study can be seen as one consideration to develop the use of watermelon frost as traditional medicine for the inflammation and the pain relief besides eugenol, miswak and corticosteroid.[13,14] Furthermore, the study can familiarize the use of watermelon frost so that people can utilize watermelon which is not expensive. In addition, the study will encourage primary health services to provide economical material used as pain relief which is nontoxic and biocompatible enough in dental tissues.

MATERIALS AND METHODS

Type of this study was comparative experimental research. It involved watermelon frost as the material. The steps to make watermelon frost were: chose a watermelon weighing 2.5 kg, sliced its top, and part of the flesh was removed. Put 500 grams of glauber's salt (sodium sulfate decahydrate) on it (Figure 1). Then a small stick of bamboo to retained it and put the top back so the hole was covered. Afterwards the watermelon was hung in fridge (not a part of freezer) approximately 7–10 days or until frost (white powder) was formed in outer part of watermelon rind. This frost has a therapeutic effect in reducing pain and inflammation.[15]

Figure 1. Watermelon which is given glauber's salt.

Watermelon as a trial material produced white powder (frost) by adding glauber's salt ($Na_2SO_4.10H_2O$) for several days and frost was dissolved in aquadest (Figure 2).

Figure 2. Watermelon frost found in the rind surface of watermelon.

Sample was divided based on the materials: Group I (9 samples) was given watermelon frost with concentration 50% (dissolved with aquadest), Group II (9 samples) was given watermelon frost with commercial 50% (content based on the supply of factory) and Group III (9 samples) was given eugenol 50% (dissolved with dimethyl sulfoxide/DMSO).

Pulp tissues were obtained from the teeth which were chosen by opening the cavity and extirpating pulp with extirpating needle (barbed-broach). The teeth rooted with more than one pulp were gained from the largest root canal. Then, the pulp was placed in Eppendorf containing 0.5 ml of phosphate buffer saline (PBS) and 10^{-3} M of nimesulide; centrifuge was applied at 2400 rpm for 10 minutes; after that, it was restored at temperature –23° C. Each Eppendorf was given watermelon frost as trial material, commercial watermelon frost and eugenol. Analysis of PGE_2 quantitative level was seen by using Enzyme-Linked Immunosorbent Assay kit (Neogen, USA) through an equipment called "ELISA reader" that showed the absorbance level of color at the wave length W_1 = 650 nm and W_2 = 490 nm.

RESULT

The result from reading the absorbance level of color on each sample by ELISA reader showed the largest decrease of PGE_2 level in eugenol group then followed by watermelon as trial material. Meanwhile, the group of

commercial watermelon frost showed the smallest decrease of PGE_2 level (Table 1).

Table 1. The PGE_2 level from watermelon frost as trial material, commercial watermelon frost and eugenol (ng/mL)

Number Sample	Concentration of PGE_2 (ng/mL)		
	Group I (Watermelon frost as trial material)	Group II (Commercial watermelon frost)	Group III (Eugenol)
1.	17.87	86.62	4.30
2.	14.96	98.95	6.51
3.	28.35	68.99	12.27
4.	17.87	108.05	5.68
5.	15.95	68.65	3.76
6.	15.80	71.25	2.92
7.	13.59	83.50	3.80
8.	26.15	92.78	1.95
9.	12.88	61.63	1.05

Table 2. The average level and standard of deviation from the decrease of PGE_2 level (ng/mL)

Type of Materials	n	$\overline{X}$	SD
Watermelon frost as trial material	9	18.16	5.45
Commercial watermelon frost	9	82.27	15.74
Eugenol	9	4.69	3.31
Total	27	35.04	35.75

Table 2 indicated that the average level and standard of deviation at group of watermelon frost as trial material were 18.16 ± 5.45 ng/mL. At commercial watermelon frost group, the average level and standard of deviation were 82.23 ± 15.74 ng/mL. At eugenol group, they were 4.69 ± 3.31 ng/mL. These implied that effect of eugenol in reducing the PGE_2 level is higher than that in watermelon frost (as trial material and commercial). In the meantime, the effect of watermelon as trial material in reducing the PGE_2 level is higher than that of commercial watermelon frost.

Table 3. Analysis of variant for the groups: watermelon frost as trial material, commercial watermelon frost, and eugenol the PGE_2 level (ng/mL)

	df	F	P.
Between Groups	2	160.821	.000*
Within Groups	24		
Total	26		

Explanation : P: ANOVA test
* : Significance
F : Frequency
df : degree of freedom

To see the difference of PGE_2 level in watermelon frost as trial material, commercial watermelon frost and eugenol, One-Way ANOVA was applied with $\alpha = 0.001$ (Table 3).

The result of ANOVA showed that there was statistically significant difference ($p < 0.001$) among groups of watermelon frost as trial material, commercial watermelon frost and eugenol in the decrease of PGE_2 level.

DISCUSSION

In this study, the effect of watermelon frost as trial material was compared to the effect of commercial watermelon frost and eugenol. Of those three materials, it was eugenol that had the greatest effect on PGE_2. This was followed by watermelon frost as trial material and commercial watermelon frost.

However, the decrease of PGE_2 in commercial watermelon frost was lower than in watermelon frost as trial material. This was probably caused by the content of pure watermelon frost in commercial product as much as 50% and the mixture with other materials; *Rhizoma Belamcandae* (5%), *Bulbus Fritillariae* (15%), *Radix Sophorae Tonkinensis* (10%), *Mentholum* (5%). Indigo Naturalis (5%) and Borneolum (10%). The content of watermelon frost as much as 50% has caused the decrease of PGE_2 lower than the watermelon frost as trial material where the content is pure. Besides, other contents found in commercial watermelon frost can influence the effectiveness of watermelon frost in reducing the PGE_2 level.

According to Cohen *et al. cit* Hashimoto *et al.*,[8] the PGE_2 level on the tissue of symptomatic inflamed pulp was significantly higher than that of asymptomatic inflamed pulp. Isett J *et al.*[17] in his study stated that higher PGE_2 level is found in irreversible pulpitis and it leads to vein vasodilatation, the increase of vascular permeability, chemotaxis, pain, and bone resorption.[16]

The tables in this study implied that each group had several samples showing higher PGE_2 level (out layer) even though it was given trial material. In table 1, it was seen that even though the pulp tissue was taken from symptomic irreversible pulpitis, the PGE_2 level in each case could be different. In severe inflammation, the PGE_2 level would be high. Consequently, in group I the PGE_2 level of the third sample was 28.55 ng/mL; in group II the fourth sample was 108.05 ng/mL; in group III the third sample was 12.27 ng/mL. These cases are possibly caused by the PGE_2 level that was higher than that found on other samples.

Eugenol has the greatest capability to reduce the PGE_2 level. It was shown from a study by Dewhirst and Hirafuji *cit.* Hashimoto *et al.*,[8] where eugenol can inhibit biosynthesis of PGE_2 through in vitro. In contrary, a study by Prashar *et al.*[18] showed that eugenol is very cytotoxic in fibroblast and endothelium cells. The toxicity effect has caused the cell inactive through apoptosis and necrosis. Finally, if the inflamed cells in the pulp tissue are inactive, the PGE_2 level will be significantly decreased.

According to the study by Ho *et al*.,[10] it was mentioned that eugenol can inhibit the growth and proliferation of osteoblastic cell line U2OS. Therefore, eugenol has significant role on periapical toxicity and destroys the cell in the pulp tissue.

Watermelon frost is a traditional/herbal medicine which has been empirically known by the Chinese community as one of therapies against inflammation. The use of watermelon frost has been approved by Chinese Drugs Administration Department. Watermelon frost is obtained from watermelon by using glauber's salt. Since watermelon frost is naturally gained from watermelon, it is biocompatible enough in oral tissues. As consequence, the cells in the pulp tissue including the inflamed cells after being given watermelon frost are probably vital.[19]

From the data analysis and the discussion, it can be concluded that there were differences among the decrease of PGE_2 level found in commercial watermelon frost, watermelon frost as trial material, and eugenol. Based on the analysis above, it can be seen that eugenol has bigger effect of the PGE_2 decrease 4 times than watermelon frost as trial material and it is bigger 17 times than commercial watermelon frost. Meanwhile the effect of the PGE_2 decrease is bigger 5 times than commercial watermelon frost. Eugenol is very cytotoxic in fibroblast and endothelium cells. The effect of cytotoxicity can lead to the death of cells through apoptosis and necrosis. If the inflamed cells in the pulp tissue are inactive, the PGE_2 level will be significantly decreased. Because watermelon frost is naturally gained from the watermelon, watermelon frost is biocompatible and nontoxic in oral tissues, so the cells in the pulp tissue including the inflamed cells after being given watermelon frost are vital. To identify the active substance which functions as anti PGE_2 in watermelon frost, fraction and characterization tests need to be carried out. The tests refer to the physical and chemical characteristics of watermelon frost. Besides, cell culture needs to be applied to observe the condition of cells in the pulp tissue after being given watermelon frost.

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