27. Mangosteen rind on oral mucositis

by Ismi Rajiani

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CONTENTS

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Mangosteen Rind on Oral Mucositis Caused by Radio and Chemotherapy in Cancer Treatment (In Vivo Study on Rats)

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ABSTRACT

Background: Cancer malignancy is often treated by using medication supplements using radio and chemotherapy. However, these treatments have adverse effects on oral which cause difficulties in talking, eating, and even opening the mouth. Therefore, herbal medicine like mangosteen rind gel may become the alternative therapy for this condition.

Method: This research was quantitative and experimental randomized pre-post test with control group design. Samples were 15 Sprague Dawley female rats divided into three groups where group 1 was applicated with mangosteen rind gel intervention, unit 2 was with standard gel intervention (positive control) and the last group was untreated group (negative control). Effect of the mangosteen rind gel of treatment group and post-test of the control group were performed with the ANOVA test.

Results: The usage of mangosteen rind gel on oral mucositis accelerated the reduction of oral mucositis diameter compared to the standard gel and untreated group. On the 14th day of intervention, the recovery rate of oral mucositis on team 1 reached 99.80%, on unit 2 was 67.70% and only 1.20% in an untreated group. Cell signaling examination showed an average increase of TNF-α from day one until day 14. The lowest difference of TNF-α was group 1 (8.88 pg/mL or 14.50%), then followed by group 2 was 12.71 pg/mL (19.25%), and the highest difference was group 3 (21.43 pg/mL or 32.55%).

Conclusion: Mangosteen rind gel may accelerate healing of oral mucositis caused by radio and chemotherapy in cancer treatment. However, further study is necessary to determine the applicability to human being.

Keywords: *cancer, mangosteen, oral mucositis, rats*

INTRODUCTION

Cancer is a killing illness that causes prime death in over the world. Based on International Agency for Research on Cancer (IARC), there were 8.201.575 cancer death and found 14.067.894 new cancer cases in the world [1]. Treatment of cancer is usually followed either by chemotherapy or radiotherapy, but adverse effect of this therapy is oral mucositis inflammation of oral mucosa with ulcer [2, 3]. This kind of inflammation will be followed by burning sensation, so the patients causing difficulties in talking, eating and even opening the mouth (3rd grade of cancer therapy). Furthermore, the advance condition (4th grade of cancer therapy) shows that the patients aren't able to consume delicious food [4, 5].

Therapy of oral mucositis depends on the symptoms which are usually used to calm down the pain due to the inflammation and cure the oral infections. Topical application is one of therapy used for oral mucositis. Nowadays, the standard gel is mostly used for cancer patients with oral mucositis. Unfortunately, sometimes this gel causes hypersensitivity reaction such as skin eruption, itchiness, edema, etc. The researcher reported among 30 patients with oral and oropharynx lesions treated by standard gel generated 83% with pain

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reduction, 13% with no change condition, 3% with early repairment but then become worse [6]. Besides, the gel is no more effective than therapy with *Sucralfate* and *Mucaine* in relieving the pain associated with radiotherapy including *Oral Mucositis* [7]. Due to this condition, herbal treatment could be an alternative solution.

Mangosteen is Indonesian fruit usually used as traditional medicine to cure a stomachache, chronical ulcer, skin infection. Besides, it also has the effect of analgesic and anti-inflammation [8, 9]. It contains fenol derivative such as xanthones or xanthan-9H-ones [8]. Mangosteen rind safe to be used [10] includes of variation of xanthone: α-, β-, γ-mangosteens. They have the ability as the antioxidant, antitumor, anti-bacterial, anti-virus, anti fungi, anti-allergic and anti-inflammation [11,12,13]. Besides, α-Mangosteen can prevent hypoxia due to Reactive Oxygen Species (ROS) of cancer cell [14]. According to some researchers, the diameter of tumor became decrease, and complete regression happened on rats after treated by high doses of xanthone (3.0 mg per tumor) [15].

The initial study [16] showed that mangosteen rind as anti-inflammation gave an excellent impact for male Wistar rats on the recovery of oral ulcer caused by trauma. During inflammation phase in wound healing process, some cytokines have an essential role especially such as IL-1, IL-1β, IL-6, and TNF-α [17]. This study would like to investigate the ability of mangosteen rind in healing oral mucositis happening during cancer treatment with chemo and radiotherapy.

METHODOLOGY

Material

1. Carcinogenic agent 7,12-dimetilbenz[a] antrasen (DMBA), corn oil, prepared as test compound mixture.

2. Elisa Kit from Biolegend Company.

Method

1. The extract of mangosteen rind (18) :

a. Identification of the type of mangosteen: Garcinia mangostana L.

b. Prime active substance: xanthone and α-mangosteen.

c. Mangosteen rinds were cut, dried in oven 50°C in 72 hours, ground to become powder and stored in air retention bowl.

d. Then the powder of mangosteen rind was extracted by Soxhlet method.

2. The formula of mangosteen rind gel:

a. Mangosteen rind extract and other additional material were mixed homogeneously.

b. Added in sequence: Na Benzoid, Carbopol, HPMC (Hidroksi Propyl Metil Selulosa), TEA (Triethanolamine) and mixture homogenously when attached to each material.

c. Put it in a tube.

3. Cancer induction with 7,12-DMBA (Dimethylbene (a) anthracene) (19):

a. DMBA solution was made by mixing it with corn oil and vortex in 15 minutes.

b. Given doses were 20 mg/kg weight, twice a week in 5 weeks (frequency: 10 times).

c. Injected with the oral cannula through the oesophagus.

d. Observed from the first week after DMBA initiation. Palpation its breast 1-2 times a week till there was a lump.

4. Oral mucositis model:

a. Make a wound on the lower oral labial mucosa.

b. Treated with H2O2 (hydrogen peroxide) 3% using cotton bud in 90 seconds.

c. Observed till there was an ulcer (around 48 hours).

Identification of oral mucositis recovery:

1. Measured the diameter of the wound (mm) every day from the 1st day until the 14th day.

2. Measured TNF α on the 1st and 14th day:

a. Centrifuged 5 ml blood taken from a retro-orbital sinus (20).

b. Elisa (Enzyme-linked immunosorbent assay) kit was used to detect the amount of TNF α in blood serum (pg/mL).

Subjects:

1. Female Sprague Dawley rats :

a. 160-180 g in weight,

b. 5 – 6 weeks old,

c. Healthy condition and never used in research before.

2. Sample

With simple random sampling, 15 rats were divided into three groups:

a. Group 1: the wound was treated with mangosteen rind gel.

b. Group 2: the wound was treated with standard gel (positive control).

c. Group 3: untreated/without treatment (negative control).

Each group consisted of 5 (five) rats and 1 (one) additional rat to anticipate death rat (21). Data were analyzed with repeated ANOVA test and presented in tables and graphics.

RESULTS

Formula of Mangosteen Rind Gel

Table 1. Formula of Mangosteen Rind Gel

Material	Amount
Mangosteen Rind Extract	60% x 20 g = 12 g
HPMC	
TEA	1.1 g
Carbopol	
Na Benzoate	
Aquadest	20 g – (12 g + 1.1 g) = 6,9 g

Diameter of Oral Mucositis Recovery Process

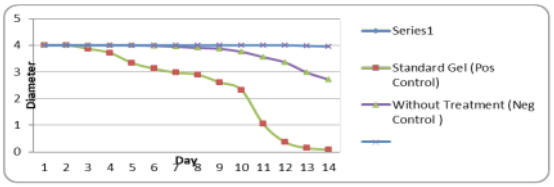


Figure 1. Diameter of Oral Mucositis Recovery Process

Figure 1 showed that diameter of oral mucositis became decreased. On the 14th day, group 1 (treated by mangosteen rind gel) healed (99.8%) with diameter 0.08 ± 0.08 mm. Group 2 (treated by standard gel) only healed 67.7% with diameter 2.71 ± 0.41 mm. Group 3 (untreated) only 1.2% healed with diameter 3.95 ± 0.10 mm.

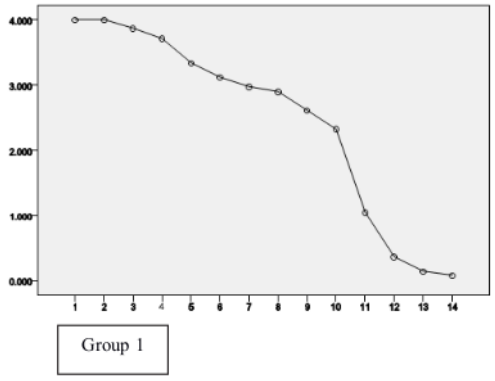
TNF-α

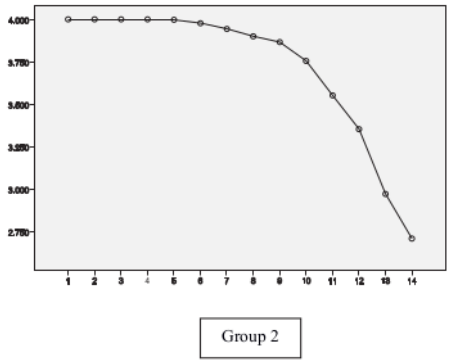
Table 2. Measurement of TNF-α on Oral Mucositis Recovery Process

No.	Intervention	Mean of TNF-α ± SD (pg/mL)		Δ TNF-α	
		Day-1	Day-14	pg/mL	%
1.	Mangosteen Rind Gel	52.40 ± 13.92	61.29 ± 14.25	8.89	14.50
2.	Standard Gel (Positive Control)	53.34 ± 8.52	66.06 ± 8.85	12.71	19.25
3.	Without Treatment (Negative Control)	44.40 ± 6.48	65.83 ± 22.86	21.43	32.55

Table 2 showed that the amount of TNF-α was increased from day 1 to day 14. The lowest difference in each group was on the 1st I which was treated by mangosteen rind gel (8.89 pg/mL or 14.50%), followed by group 2 was 12.71 pg/mL (19.25%) and the highest difference was group 3 (21.43 pg/mL or 32.55%).

Inferential Analysis:





Measurement of Oral Mucositis Diameter

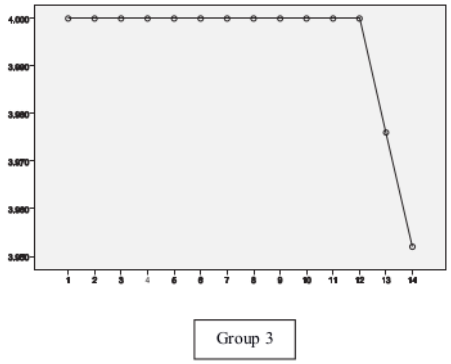


Figure 2. Measurement of Oral Mucositis Diameter Treated by Mangosteen Rind Gel (Group 1), Standard Gel (Group 2) and Untreated Group (Group 3)

There were significant differences analyzed by ANOVA test. ($p_{value} < 0{,}001$). The wound healing process on group 1 happened on day 2 and day five on group 2, but no healing process happened until day 12 in group 3.

Measurement of Oral Mucositis Diameter among Groups

Table 3. Differential Test of Oral Mucositis Diameter Inter Groups

Pairwise Comparison		p value
Group 1*)	Group 2	.000
	Group 3	.000
Group 2*)	Group 1	.000
	Group 3	.001
Group 3*)	Group 1	.000
	Group 2	.001

*) Group 1: treated by mangosteen rind gel; Group 2: treated by standard gel ; Group 3: without treatment

Table 3 showed there was significant difference on measurement of oral mucositis diameter ($p_{value} \leq 0{,}001$) in inter groups.

DISCUSSION

This study used mangosteen Garcinia mangostana which contains xanthone dan α mangosteen [18]. Mangosteen rind gel (20 g) only comprised 60% (12 g) of its active substance due to the research that mangosteen rind extract with concentration more than 40% could inhibit the bacteria growth [16]. The result showed that the decrease in the size of diameter oral mucositis treated by mangosteen rind gel was faster than others. Wound healing process happened on the 2nd day while others were on the 5th day (positive control group) and 12th day (untreated group). On the 14th day, wound healing process was almost complete (99.8%) with the diameter 0.08 ± 0.08 mm. Meanwhile, the healing process on the group treated by standard gel was only 67.7% with diameter 2.71 ± 0.41 mm and just 1.2% with diameter 3.95 ± 0.10 mm on the untreated group. Wound healing process would be optimal if the material contained with subsequences that could protect the cells from bacteria infection, decreased inflammation process and induced cell proliferation to reconstruct the broken cells [22]. Besides, antioxidant subsequences could help in reducing inflammation process [23].

Xanthone has biochemistry effects such as antioxidant, anti-bacterial, anti-inflammation so it could be used to treat wounds. Some researchers had proved the role of xanthone in wound healing, tumor or carcinogenic activities [24]. Another research had shown that extract of mangosteen rind had the ability as oral anti-bacteria to inhibit the growth of Streptococcus pyogenes, Streptococcus mutans, Staphylococcus aureus, and Porphyromonas gingivalis [13,25]. Xanthone had also effect on the maturation of collagen, it was primary protein (70-80%) in the extracellular matrix that had an essential role in wound healing process [26]. This healing process consists of 4 (four) phases hemostatic, inflammation, cell proliferation and maturation. It's started with the formation of fibrin and infiltration of the neutrophil. This process happened precisely and orderly. The interruption occurred in the process would cause chronical wound and prolong the recovery time [27].

The result showed that there was an increase of TNF-α from day one until day 14 in wound healing process of oral mucositis. The lowest difference of TNF-α was on group 1 with mangosteen rind gel (14.50%), then followed by group 2 with standard gel (19.25%) and the highest difference was on team 3 (32.55%). TNF-α was released by neutrophil and macrophage cells. This TNF-α had roles in inflammation and reepithelialization process, and it would increase on the acute and chronical wound [17]. It was produced at the beginning of healing process, and its concentration was essential to the result of wound healing. When its concentration was low, it could support indirectly on inflammation process and increase growth factors which produced macrophage cell. On the other side, the higher the level of its concentration, it could depress synthesis or matrix cellular protein (ECM), promoted the production of natural tissue inhibitors (TIMPs) and increased matrix metalloproiteinases (MMPs) [28]. So the process of wound healing might be delayed.

CONCLUSION

There was an effect of mangosteen rind gel on wound healing process of oral mucositis due to cancer therapy – chemotherapy and radiotherapy. It could accelerate the healing of oral mucositis within 14 days. Amount of mangosteen rind extract 60% on gel formula could heal this oral mucositis.

Measurement of oral mucositis diameter on the 14th day, group 1 (mangosteen rind gel intervention) healed 99.80% with diameter 0.08 ± 0.08 mm, group 2 (standard gel intervention) only healed 67.70% with diameter 2.71 ± 0.41 mm and group 3 (without intervention) only 1.20% healed with diameter 3.95 ± 0.10 mm.

There was an increase of TNF-α from day one until day 14 in wound healing process of oral mucositis. The lowest difference of TNF-α was on group 1 treated by mangosteen rind gel was 8.89 pg/mL; 14.50%, on group 2 treated by standard gel was 12.71 pg/mL; 19.25% and on unit 3 without intervention was 21,43 pg/mL; 32,55%.

Conflict of Interest: The authors have no conflict of interests related to the conduct and reporting of this research.

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Ethical Clearance: Before conducting the study, written permission was obtained from Politeknik Kesehatan Kementrian Kesehatan Semarang, Indonesia.

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