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EPITHELIAL CELL REGENERATION OF GASTRIC MUCOSA IN RAT (*Rattus novergicus*) CAUSED BY A TRADITIONAL ALCOHOLIC DRINK, *SOPI* TREATED WITH RED FRUIT (*Pandanus conoideus Lam*)

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ABSTRACT

Red Fruit (*Pandanus conoideus* Lam) is one type of plant that contains **antioxidant of tocoferol, alfatocoferol, and betacaroten**, which supposedly netralizise free radical and heal **gastric mucosa** damaged in white rat (*Rattus novergicus*) which induced by a Maluku alcoholic drink called as *sopi*. This research was perfomed to investigate the effect of red fruit in **gastric mucosa** of rat which is induced by sopi. Mice with an average weight of 200 grams were given a dose of 2.5 ml/200g BB twice daily for 60 days. Then the *Rattus novergicus* was given the red fruit at a dose of 0.2 ml/200g BB, 0.4 ml/200g BB, and 0.6 ml/200g BB twice daily for 30 days, respectively. The results of this study indi`cate that the effect of red fruit was found to histology of the **mucosal gastric** of the mice. Provision of the *Pandanus conoideus* Lam with an effective dose of 0.6 ml/200g BB of **mucosal gastric was repaired. While ulcer gastric of the *Rattus novergicus*** were exposed to t**he type of *sopi*.**

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INTRODUCTION

In Indonesia, there are few regional alcoholic drinks such as liquid *brem*, *tuak*, *saguer*, and *ciu* [**1**]. One of Maluku province local alcoholic drink

fabricated traditionally was *sopi*. This sopi was produced by people living on the hills part of Ambon island, the capital city of Maluku province, Seram island,and South west of Maluku, respectively. In Maluku, *sopi* was normally produced for the traditional culture celebrations and parties in almost all part of Maluku areas. A long term consuming of alcoholic drink will cause a heart failure, hypertency, stroke, liver failure, stomach aches, gastric failures, and a decreased of reproducity system. Furthermore, the overdoses of alcohol consumption can damage brain system as a result of the decreasing of the ability to memorize something, and to concentrate [**2**]. It is wel-known that the fast absorption of alcohol was happened through gastric and smooth intestine to the blood flow, and then the whole body [**3]**. In addition, alcohol can damage mucosa gastric due to its fast penetration in it by relasing free radial substance so that there is a network broken of the gastric especially on blood vein and parietal cell inside the gastric with an indicator of damaging cell [**4-6**]. Gastric is an important organ to react with HCL, and protein enzymes. Not all free radial substances in the body are bad. During a certain conditions, such free radials are needed, for example: in killing bacteria. Therefore, the free radials must be controlled by an antioxidant of the body. Antioxidant is a wanted substance to the body to neutralize the free radials, and protect the broken cells in the body. The antioxidant could be an enzyme such as catalace. Beside of that, there are another non-enzymatic antioxidants. These antioxidants can be vitamins C, E, A, and beta-carotene. Most of these substances are exist in red fruit (*Pandanus conoideus*). The red fruit contains chemical active substances such as β-carotene, tocopherol, oleat acid, **linoleat acid of Omega-9, linoleat acid of Omega-3**, and decanoat [**7**]. Such active substances are important in improving the immune system of the body, intelligential memory, and cell repaired. Based on the major active substances of tocopherol, alpha-tocopherol, and β-carotene in red fruit, the fruit can function as cell regeneration. While its antioxidant can protect the attack of free radial subtances. By giving the extraction of red fruit (*Pandanus conoideus* Lam) to the body, it can regenerate **epithelial cell** of mucosa gastric in the white rat (*Rattus novergicus*) under *sopi* problem.

METHODS

The content dose decision of *sopi*

According to Louhenapessy [**8**], an initial dose used to a chronical sociaety decease is 100 mL (with a weight body of 50 kg). If it was used to human being with the weight of 70 kg, then the dose was calculated as folows 70/50 × 100 ml = 140 mL. The conversion from human weight to that in mouse (20 g) is 0.018 [**9**], so that rat dose is 0,018 × 140 ml = 2,5 mL. Therefore, from such results, the used dose was chosen to be 2.5 mL/200g BB.

The content dose decision of Red Fruit

As initial dose, a common used dose in society was taken ~8 ml (with a weight body of 50 kg). For human kind with the weight of (with a weight body of 50 kg), the dose was obtained as 70/50 × 8 ml = 11.2 mL. While the conversion factor from human being (70 kg) to be rat (200 gr) was 0.018, so that the rat dose was 0.018 × 11.2 ml = 0.2 ml. Based on the above mentioned calculations, the first used dose (dose I) was 0.2 ml/200gr BB. While the use of dose I, and dose III were 0.4 ml/200gr BB, and 0.6 ml/200gr BB, respectively. Acording to Revianti, et al. [**10**], they found the treated dose of 0.3 ml/BB had already give the protection effect to the increase of the **SGPT** level.

Treatment to tested animal

30 white male rats were divided into 5 groups randomly, and each test was consisted on 6 male white rats. While 20 of the rats were orally given 2.5 ml/200gr BB *sopi* every 2 days during 4 weeks (1 month). The following are the details of the whole 5 groups:

(1). Group I (negative control) is male white rats with only aquades treatment for 8 weeks as a normal control.

(2). Group II (possitive control) is male white rats with 2.5 ml/200gr BB *sopi* treatment every 2 days for 8 weeks.

(3). Group III is male white rats with 0.2 ml/200gr BB red fruit juice treatment every 2 days for 4 weeks.

(4). Group IV is male white rats with 0.4 ml/200gr BB red fruit juice treatment every 2 days for 4 weeks.

(5). Group V is male white rats with 0.6 ml/200gr BB red fruit juice treatment every 2 days for 4 weeks.

Fabrication of gastric preparate histology

Handari (1983) [**11**] stated that a condition of fixation gastric with 4% formaline, after washed with 5 minutes aquades, there was an increase of dehydration every 30 minutes from 30%, 50%, 70%, 80%, 90% to 100%. The remind alcohol was cleansed by clearing process. Gastric was put inside xylol I, and xylol II for 1 hour, respectively. Furthermore, infiltration process was

conducted by inserting the organ in the paraffin I, paraffin II, and paraffin III at 60 ^{0}C for 1 hour, respectively. Moreover, embedding process was carried out by putting gastric inside paraffin box for 24 hours, and then sectioning or cutting through few minutes waiting process inside the paraffin ox by using microtom with the thickness of 6 µm. The slicing result was put down on objective glass which has been lubricated with albumin gliserine, and then was heated up on hot plate at 40 ^{0}C. Stanning process in xylol I, and xylol II, for 15 to 30 minutes each, and following by inserting the preparate in alcohol 100%, 90%, 80%, 70%, and 30% for 3 minutes, respectively. In addition, the preparate was inserted inside hematoxilin 1% in aquades for 2 to 10 minutes, washed with water for 3 minutes, and then such preparate was put inside eosin I alcohol 50%, eosin II alcohol 100 % for 3 minutes each. Finally, the treated preparate was diluted inside xylol I, and xylol II for 3 minutes each, and then dried. In the last step, the preparate was closed with glass substrate, and observed under microscope.

RESULTS AND DISCUSSION

From the results as shown both in **Figure 1** and **Table 1**, the descriptive analyzation can be obtained from the gastric of micrograph photo. By giving sopi for 60 days to white rat (*Rattus novergicus*), her mucosa gastric may have been broken. However, after its treatment with red fruit for 30 days, we found there was a regeneration or repair of mucosa gastric. These indications are seen obviously in the micrograph photo of mucosa gastric in **Fig. 1. According to Fig. 1(A1), Fig. 1(A2), Fig. 1(B1), Fig. 1(B2), and Fig. 1(C1) provided the observation data of** the 30 days treatment of red fruit juice with dose of 0.6 ml/200g BB into white rat addicted due to *sopi* with dose of 2.5 ml/200g BB, there were regenerated of mucosa gastric indicated with more observed epithial cells in the gastric. Such regenerated cells were because of the content of **tocopherol** (natural vitamin E) with its ability to repair broken cells, and **alpha-tocopherol, and beta-carotene with their functions as antioxidant to handle free radials sourced by *sopi.***

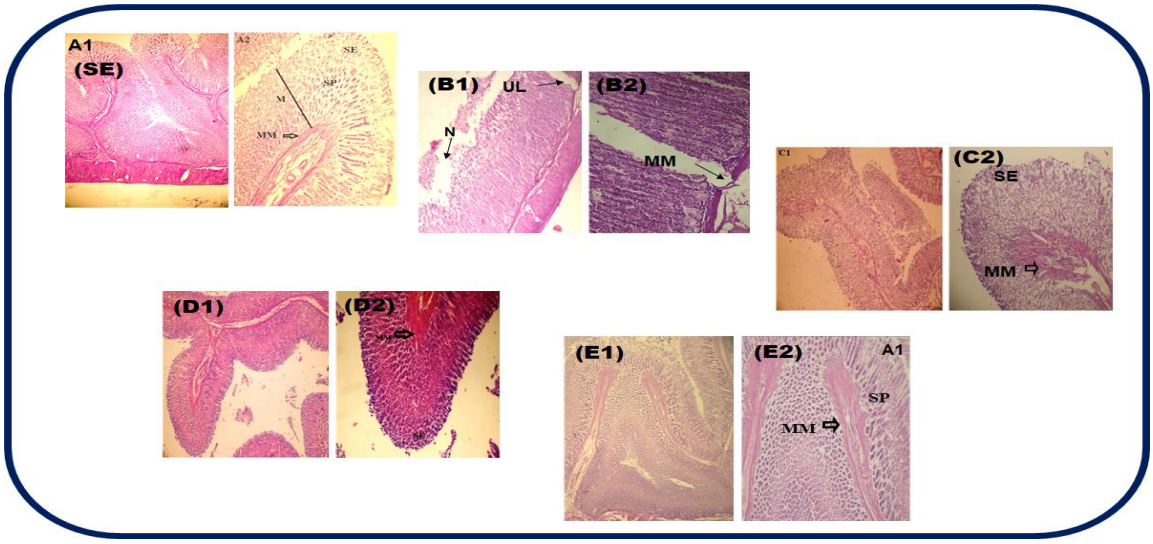

Figure 1. Micrograph photo of a controlled **gastric mucosa** of rat and its treatment. The folowing are the explanation of figure captures: **A1** (**SE**) is the mucosal gastric histology of controlled white rat with 40x enlargement; **(A2)** is the mucosal gastric histology of controlled white rat with 100x enlargement in which **SE, M, MM, and SP** are epithelial cell, gastric mucosa, muscularis mucosa (lamina of muscle), and parietal cell, respectively. **In (A2), there is no damage of mucosa gastric; (B1)** is 100x enlargement histology of white rat given by *sopi* with dose of 2.5 ml/200g BB for 60 days in which **N**, **UL** and **LP** are necrosis cell, ulcer (wound) gastric, and ***lamina propria* (a part of mucosa)**, respectively; **(B2)** is 100x enlargement histology of white rat given by *sopi* with dose of 2,5 ml/200g BB for 60 days; (C1) White rats treated with sopi for 30 days plus the red fruit juice with 0.2 ml/200g BB dose for 60 days (the picture with 40 x magnification), and (C2) White rats treated with sopi for 30 days plus the red fruit juice with 0.2 ml/200g BB dose for 60 days (the picture with 100 x magnification); **(D1)** is 40x enlargement histology of white rat given by *sopi* with dose of 0.4 ml/200g BB for 30 days; **(D2)** is is 100x enlargement histology of white rat given by *sopi* with dose of 0.4 ml/200g BB for 30 days; **(E1)** is is 40x enlargement mucosa gastric histology of white rat given by red fruit juice with dose of 0.6 ml/200g BB for 30 days; and **(E2)** is is 100x enlargement mucosa gastric histology of white rat given by red fruit juice with dose of 0.6 ml/200g BB for 30 days.

Table 1. The observation results of controlled white rat with its mucosal gastric micrograph photos treated with *sopi* plus red fruit.

Type of Treatment	Result of observation
Normal	No damage of mucosa gastric (epihtesial cell, parietal cell)
Sopi with dose of 2,5 ml/200g BB	Mocosa gastric (epihtesial cell, parietal cell) experienced necrosis (injured cell), and ulcer (wound) in gastric.
Red fruit juice with dose of 0,2 ml/200g BB	Mucosa gastric regeneration (epihtesial cell, parietal cell)
Red fruit juice with dose of 0,4 ml/200g BB	Mucosa gastric regeneration in which it looks like more epihtesial cell and parietal cell in the mucosa.
Red fruit juice with dose of 0,6 ml/200g BB	Mucosa gastric regeneration (epihtesial cell, parietal cell) until the same as a normal mucosa gastric.

The observation of gastric histology preparate in the controlled white rat group which was only treated with aquades shows no changing in mucosa gastric (**Fig. 1 A1 (SE) and Fig. 1(A2)**). While the white rat group treated with *sopi* of 2.5 ml/200g BB dose shows a big broken part of gastric histology structure as depicted in their microscopic picture of mucosa gastric (**Fig. 1(B1) and Fig. 1(B2)**). The mechanism behind such broken parts can be explained into the following few manners such as the irritation of mucosa gastric due to alcohol effect from sopi, and the blocking of prostlagandin synthesis related to the mucosa gastric defending besides of bikarbonat, mucosa resistancy, and blood circulation in mucosa [**14**]. Mucosa gastric needs a strong blood flow to protect its existancy.

If the blood supply is decreased, mucosa gastric will tend to experience necrosis and ulcus problem. The irritation due to sopi results the increase of permeability of mucosa gastric. By the increase of mucosa permeability, a reversed diffusion of HCl happened in the mucosa gastric because of the local histamine (by mast cell in the lamina propia of mucosa gastric). This histamine then links with its receptor in the parietal gastric cell, and finally increases gastric acid secration from parietal cell [**13**]. The decrease of prostlagandin synthesis causes a distruption of gastric protection factor due to its function as defensive factor which can enhance mucus secretion and bicarbonate ion, mucosa blood circulation, mocosa resistancy, as well as fasten the grow and separation of cell [**12**].

In this research, mucosa gastric was observed to be broken in epihtesial cells of gastric **and** parietal cell **experienced necrocis as well as the presence of** ulcus gastric. Necrocis is a death cell that caused the gastric network not fully perfect. While ulcus in gastric is a wound in gastric. However, ulcus histology is a vanishing of epihtesial cell which reaches or passes through **muskularis mukosa so that it looks like an erossion. Such opinion is in agreement with Ref.** [**4-6**] mentioned that alcohol can break mucosa gastric because it can release free radicals. This broken part is usually happened in blood cell, and parietal cell located in the gastric wall that caused ulcus/ wound. Gastric has its own self defence to protect her self from an irritation. In gastric, there is a defence of **mukosa gastro duodenal which protects the whole gastric and repair** mukosa gastric if there is a damage. This system consisted of three layers of pre-epihtesial, epihtesial, and post-epihtesial. The **pre-**epihtesial layer contains **muccus-bikarbonat working as a protection of physiochemical into a molecule such as hydrogen ion. The secretion muccus on the** surface epihtesial cell consists of 95% water, and a mixture between lipid and glikoprotein. The mucus secretion was at least stimulated by prostaglandin E (**PGE**). PGE improves mucus production, and reduces the production of gastric acid. This effect helps to protect mucosa gastric. Beside its function to realese free radicals, alcohol can destroy mucus gel, and fosfolipid layer that causes a reversed acid and damage in mucosa gastric. Bikarbonat mucus layer is the protected part of the main pre-epihtesial. However, such protection is not always protecting the gastric from its broken. If released free radicals have a large number more than its ability of protective function, then gastric will have a slightly damage, so that an additional antioxidant from the outter food of the body is necessary. In this work, the juice of red fruit is the antioxidant source for the body. By injecting the red fruit

juice with 0.2 ml/200g BB dose for 30 days to a group of white rats treated with *sopi* of 2.5 ml/200g BB dose, one can see a repair process on mucosa gastric cells (**Fig. 1(C1)** and **Fig. 1.(C2)**). Therefore, the treatment with red fruit juice could reduce the damage of mucosa gastric of white rat due to *sopi* treatment. This is because of the antioksidant contents in red fruit juice such as **tocopherol**, **alpha-tocopherol**, and **beta-carotene have ability to neutralize free radical, and repair broken cells so that it can handle the broken of** mucosa gastric, and ulcus gastric. **Tocopherol** (natural Vitamin E) that improves prolifersai / cell multiplication is a healing mechanism process of ulcus in gastric. Such prolifersai is important in the ulcus healing because this process supplies epihtesial cells which is very useful to reepihtesialisation of mucosa surface, and the repair of gastric digestive cells.

By giving red fruit juice with 0.4 ml/200g BB dose for 30 days to the group of white rats damaged due to *sopi* with 2.5 ml/200g BB dose, there is a little 886onsume886t due to not only a repair of mucosa gastric cells, but also more mucosa gastric cells observed in comparison with that for such treatment with 0.2 ml/200g BB dose (**Fig. 1(D1)** and **Fig. 1(D2)**). This reason is because of more concentrated of dose (2 times higher) in red fruit juice to the white rats realated to more antioxidant content in the treatment. While by increasing the red fruit juice to be 0.6 ml/200g BB dose for 30 days to the treated white rats with sopi of 2.5 ml/200g BB dose, the mucosa gastric has been fixed the same as the gastric histology of controlled white rats as depicted in **Fig. 1(E1)**, and **Fig. 1(E2)**. Therefore, there are obvious differences in gastric histology from those five groups of white rats: (1). Controlled white rats, (2). White rats treated with *sopi* of 2.5 ml/200g BB dose, (3). White rats treated with *sopi* of 2.5 ml/200g BB dose plus the injection of 0.2 ml/200g BB dose of red fruit juice. (4). White rats treated with *sopi* of 2.5 ml/200g BB dose plus the injection of 0.4 ml/200g BB dose of red fruit juice, and (5). White rats treated with *sopi* of 2.5 ml/200g BB dose plus the injection of 0.6 ml/200g BB dose of red fruit juice.

CONCLUSION

We conclude that there are 2 main findings as follows

1. With the treatment of red fruit (*Pandanus conoideus* Lam), mucosa gastric of white rat (*Rattus novergicus*) damaged due to sopi could be repaired.
2. By using the red fruit juice (*Pandanus conoideus* Lam) with dose of 0.6 ml/200g BB, an effective healing of mocosa gastric in the rat was observed.

The following are our two suggestions to extend this research:

1. People in society please do not 886onsume an alcoholic drink of sopi type due to its effect to mucosa gastric damage.
2. Red fruit can be used as an alternative medicine to heal a cronic decease in gastric.

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