

12-1-2019

An experimental model of chemically-induced ulceration of the buccal mucosa of *Mus musculus*

Erik Idrus

Faculty of Dentistry Universitas Indonesia, Jakarta, Indonesia, erik.idrus@gmail.com

Pratiwi Dwi Hartanti

Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia

Dewi Fatma Suniarti

Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia, dewi.fatma@ui.ac.id

Sri Redjeki Prasetyo

Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia

Yuniardini Septorini Wimardhani

Department of Oral Medicine, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia

See next page for additional authors

Follow this and additional works at: <https://scholarhub.ui.ac.id/mjhr>



Part of the [Epidemiology Commons](#), and the [Public Health Education and Promotion Commons](#)

Recommended Citation

Idrus E, Hartanti PD, Suniarti DF, Prasetyo SR, Wimardhani YS, Subarnbhesaj A. An experimental model of chemically-induced ulceration of the buccal mucosa of *Mus musculus*. *Makara J Health Res.* 2019;23(3):181–187.

An experimental model of chemically-induced ulceration of the buccal mucosa of *Mus musculus*

Authors

Erik Idrus, Pratiwi Dwi Hartanti, Dewi Fatma Suniarti, Sri Redjeki Prasetyo, Yuniardini Septorini Wimardhani, and Ajiravudh Subarnbhesaj

An experimental model of chemically-induced ulceration of the buccal mucosa of *Mus musculus*

Erik Idrus¹, Pratiwi Dwi Hartanti², Dewi Fatma Suniarti^{1*}, Sri Redjeki Prasetyo¹,
Yuniardini Septorini Wimardhani³, Ajiravudh Subarnbhesaj⁴

1. Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia

2. Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia

3. Department of Oral Medicine, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia

4. Department of Oral Biomedical Sciences, Faculty of Dentistry, Khon Kaen University, 40002 Khon Kaen Province, Thailand

*E-mail: dewi.fatma@ui.ac.id

Abstract

Background: Ulceration caused by chemical agents used in dental practice for in-office or home-used is a common event, resulting in discomfort and pain. Treatments for such conditions are still being developed, requiring extensive experiments both in vitro and in vivo studies. At present, a standardized experimental mouse model for mucosal ulceration caused by a chemical inducer to study the pathogenesis of ulceration and to develop medications for treatment of ulceration is still not available. The aim of this study was to create a chemically induced model of ulceration of the buccal mucosa of mice.

Methods: An in vivo study model of ulceration using a total of 9 mice (Swiss Webster) was performed. All mice received 70% acetic acid application on the left buccal mucosa, while the right buccal mucosa received only saline. Clinical and histological observations of ulcer formation and healing were performed, including the presence of redness and swelling, ulcer diameter, bodyweight as well as epithelial disintegration, dilation of blood vessels, and infiltration of inflammatory cells. **Results:** Buccal mucosa application of 70% acetic acid generated ulcers on day 2, reached its peak on day 3 and recovered by day 14. The histological features of inflammation were also seen in the ulcer model, and the degree of inflammation was consistent with the day of ulcers. **Conclusion:** Chemical trauma by the administration of 70% acetic acid effectively induce ulceration on buccal mucosa in mice, and this method can be considered as a novel, reproducible, and clinically relevant model to study pathogenesis and therapeutic approach for treating oral mucosal ulceration.

Keywords: acetic acid, mice, oral mucosa, ulcer

Introduction

An oral ulcer is defined as the breakdown and disintegration of the mucous membrane or skin with the loss of the epithelial tissue. Oral ulcers can be caused by mechanical, iatrogenic, chemical, or thermal trauma. Furthermore, trauma-induced ulcers in the oral cavity are relatively common and usually result from mechanical injury to the surface of non-keratinized tissue, such as the cheek mucosa, tip of the tongue, gingiva, hard palate, and soft palate.¹ Recent studies have reported that oral ulcers are typically not severe.² However, symptoms of pain during eating, swallowing, and speaking can adversely affect the patient's daily activities and quality of life. Moreover, oral ulcers can also impact nutritional intake and oral hygiene and promote secondary infections.³ Therefore, experimental studies, both in vitro and in vivo to produce standardized and reproducible oral ulcer model are needed, as the number of studies to develop drugs and treatment regimens to speed up the healing process of oral lesions are increasing.³⁻⁵

In an *in vivo* study of drug efficacy, animal models of disease and pathological conditions in the human mouth are needed. In a previous study a New Zealand white rabbit model of oral ulceration was reported with the use of 15 µL of 50% acetic acid applied to the maxillary labial mucosa, which resulted in the formation of uniform circular ulcers.³ Acetic acid was shown to produce ulceration by both histopathological and clinical analyses. The ulcer area reached a maximum size one day after exposure to acetic acid and then gradually decreased in size until day 14.⁴

Besides the use of chemical agents, ulcer models can also be produced by mechanical trauma or by thermally-induced trauma.^{6,7} For example, De Barros Silva, et al. created a model of a mechanical trauma-induced ulcer in a Wistar-type mouse using a no.15 scalpel knife to form an 8 mm-wide ulcer to the buccal mucosa.⁸ Shortcomings of previous studies include the procedures for the formation of non-standardized ulcers as well as the

choice and use of tools, the use of chemicals, and the means of exposure of the experimental animals.^{8,9} Thus, at present, there are no models that can be used as references in terms of procedures and methods for ulcer formation.^{5,10}

Mice are generally used as animal models owing to several beneficial properties, such as a short gestational period, relatively smaller body size than other types of experimental animals, ability to maintain large numbers of animals, simple observation of anatomical and physiological characteristics, and long reproductive periods (2–14 months).¹¹ Although acetic acid is often used to induce ulceration, acidic agents can cause coagulation necrosis by protein denaturation and coagulum formation.¹² This study aimed to create a standard and uniform *in vivo* ulcer model of ulcer in the oral mucosa that is chemically induced using of 70% acetic acid.

Methods

In this split-mouth study, a total of 9 male outbred strain Swiss Webster mice (bodyweight, 2–35 gr; 8–12 weeks) were used, and all mice were assigned to receive a single application of 70% acetic acid and saline on their left and right oral buccal mucosa, respectively. Three mice were used for clinical observation on the onset and progress of the ulceration, and six mice were used for histology examination of oral buccal mucosa on day 2, day 3, and day 14 (consisted of 2 mice each designated day) after the initiation day (day 0).

Mice were anesthetized intraperitoneally by the injection of 10% ketamine and 2% xylazine (2:1) at 0.12 mL/100 gr of bodyweight. The administration of 70% acetic acid for 60s on the dry oral mucosa was performed using an applicator (microbrush® regular size 2.0 mm, Microbrush International, Wisconsin, USA) soaked in 70% acetic acid for 3–5s. On the control site, saline was applied on the right buccal mucosa using the same procedure (Figure 1). The study protocol was approved by the Ethics Review Committee of the Faculty of Medicine, Universitas Indonesia (approval no.17-06-0546). All mice were treated by following the procedures and guidelines according to the institutional and national guideline for the care and use of animals.

Clinical examination. The examination was performed every day from day 0 to day 14. All mice underwent observations and measurements, including bodyweight, presence of redness and swelling on the oral mucosa, and ulcer size.

Histological examination. On days 2, 3, and 14, tissue specimens of the buccal mucosa were excised, fixed with 10% formalin, sectioned, and stained with hematoxylin and eosin. The epithelial disintegration, blood vessel

dilation, and infiltration of inflammatory cells were observed under a light microscope.

Results

In the clinical observation of the left buccal mucosa after application of acetic acid, two of three mice experienced redness from day 0 up to day 14, with one mouse showed no redness by day 12. All six mice showed swelling in the first seven days, and on day 14, no swelling was observed in all mice. In all mice, swelling reached its peak on day three and retained its condition until day 4–5. On day 7, the redness and swelling were significantly reduced compared to the previous day. On the control side (right buccal mucosa) after saline administration, no sign of inflammation (including redness and swelling), was observed from day 0 in all mice (Figure 1).

All treated mice experienced bodyweight loss compared to the untreated mice. Reduced bodyweight reached its peak around day 3–4. On day 7, the bodyweight started to increase, and on day 14, the body weight from all treated mice was comparable to the untreated mice (Figure 2).

Clinical examination on the buccal oral mucosa treated with saline and 70% acetic acid, is presented in Figure 3. The area treated with saline remained intact and showed a normal healthy mucosa (Figure 3A).

Immediate observation on the buccal mucosa post-treatment with 70% acetic acid showed a slightly red area with an intact epithelial layer (Figure 3B). The buccal mucosa treated with 70% acetic-acid exhibited ulceration limited to the treated area starting on day two and reached its maximum size on day three post-treatment (Figure 3C, 3D). By day 7, the ulceration was still present, with reduced size and sign of inflammation compared to the previous days post-treatment (Figure 3E).

As shown in Table 1, the size of the ulcer in the acetic acid group ranged 2–3 mm in diameter, starting from day 2 to day 3. On day 7, the ulcer started to shrink and the size progressively decreased. By day 14, the ulcer was diminished, and the appearance of the previously inflamed area was similar to the surrounding tissue (Figure 3F).

Histological examination from buccal oral mucosa treated with saline showed normal tissue appearance (Figure 4), while 70% acetic acid-treated mucosa on day 2, day 3, and day 14 post-treatment, showed different degrees of inflammation and ulceration.

On day 2, the epithelial disintegration was observed with blood vessel dilation (Figure 5). The epithelial layer appeared desquamated on day 3 after acetic acid treatment, along with profound dilation of blood vessels

in the surrounding area (Figure 6). By day 14, the epithelial layer was restored to the normal condition, with

a minor blood vessel dilation in the submucosal area (Figure 7).

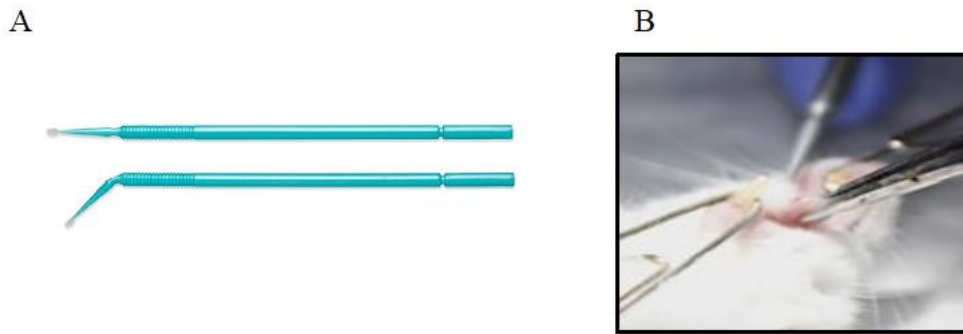


Figure 1. (A) Microbrush® applicators used to apply the 70% acetic acid on the oral mucosa (courtesy of Microbrush® International); (B) Application of 70% acetic acid on the oral mucosa

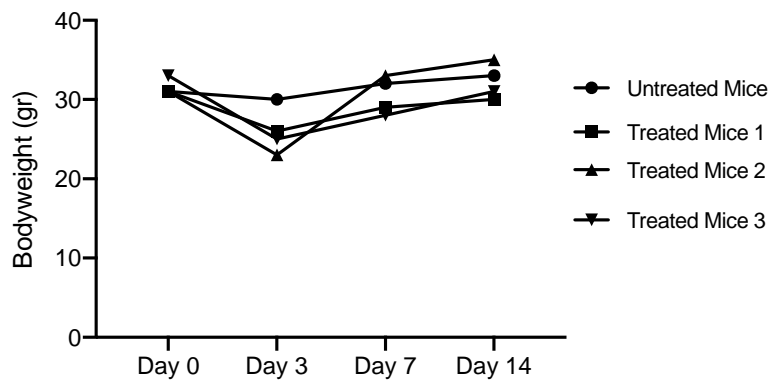


Figure 2. Bodyweight measurement of the mice treated with 70% acetic acid in comparison to the untreated ones. The measurement performed on day 0 prior to the treatment, day 3, day 7, and day 14 post-treatment (sacrifice day)

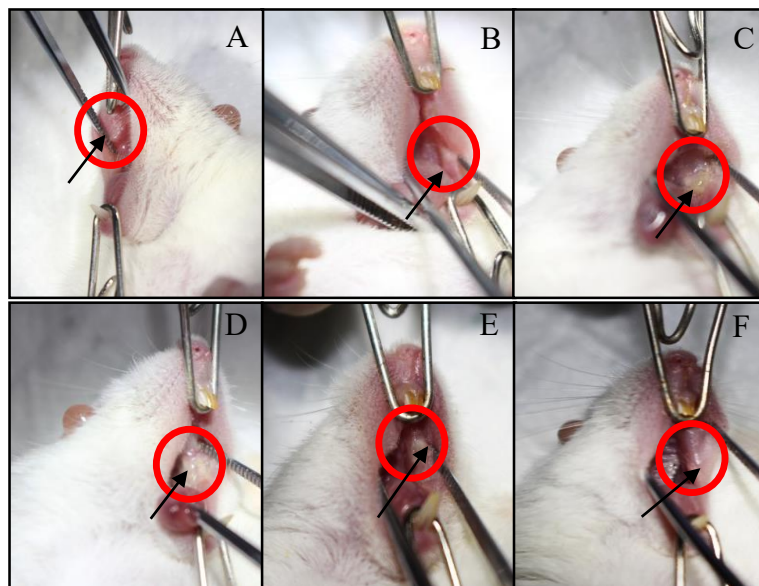


Figure 3. Clinical images of buccal mucosa from (A) healthy buccal mucosa and, (B-F) 70% acetic acid-treated buccal mucosa on (B) day 0/immediate post-treatment, (C) day 2, (D) day 3, (E) day 7, and (F) day 14 post-treatment

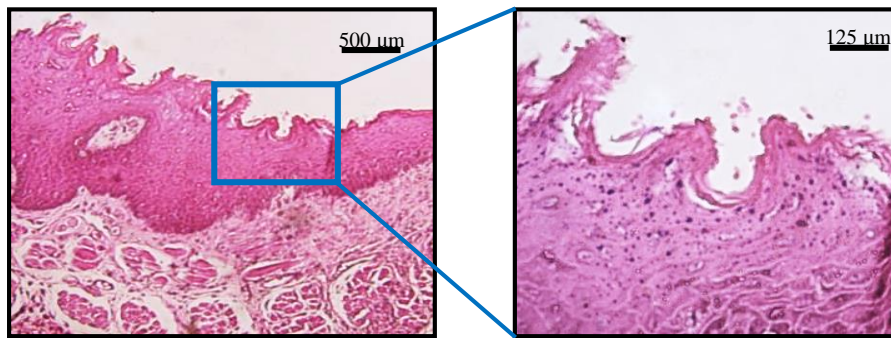


Figure 4. Histological sections of buccal mucosa from control group stained with hematoxylin and eosin, presents a piece of soft tissue covered by normal non-keratinized stratified squamous epithelium

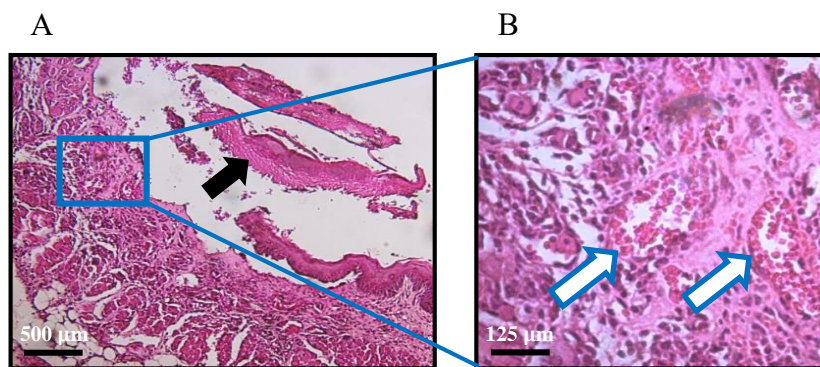


Figure 5. 70% acetic acid-treated buccal mucosa on day 2 after application stained with hematoxylin and eosin, presents detachment and disintegration of the overlying epithelium from the underlying connective tissue with prominent submucosal vasodilation. (A) black arrow: disintegrated epithelium; (B) white arrow: capillary dilation

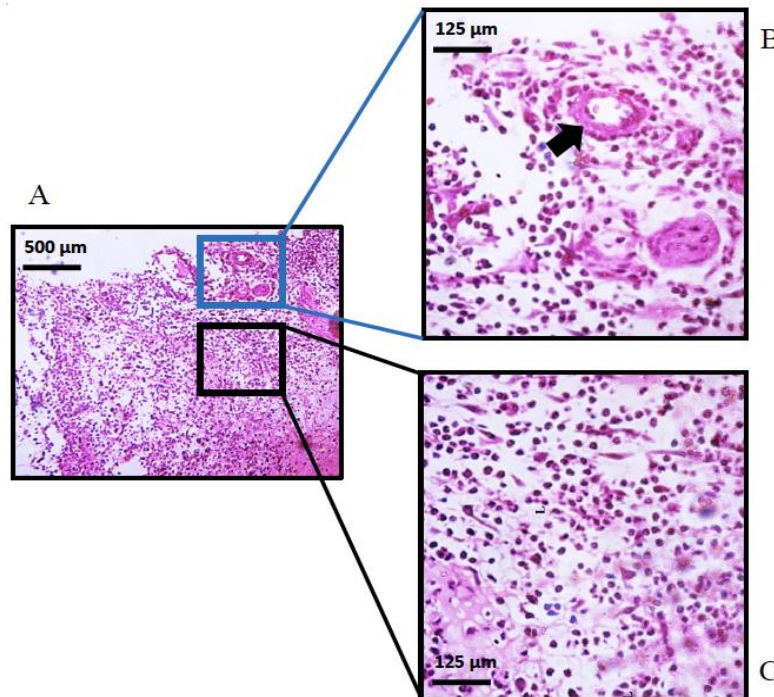


Figure 6. 70% acetic acid-treated buccal mucosa on day 3 after application stained with hematoxylin and eosin, presents the highest degree of tissue destruction as shown by the epithelial desquamation along with capillary dilation. (A) Disintegrated epithelium; (B) A mixed inflammatory cell infiltration in the underlying connective tissue, especially in the perivascular area (black arrow); and (C) around the desquamated epithelial cells

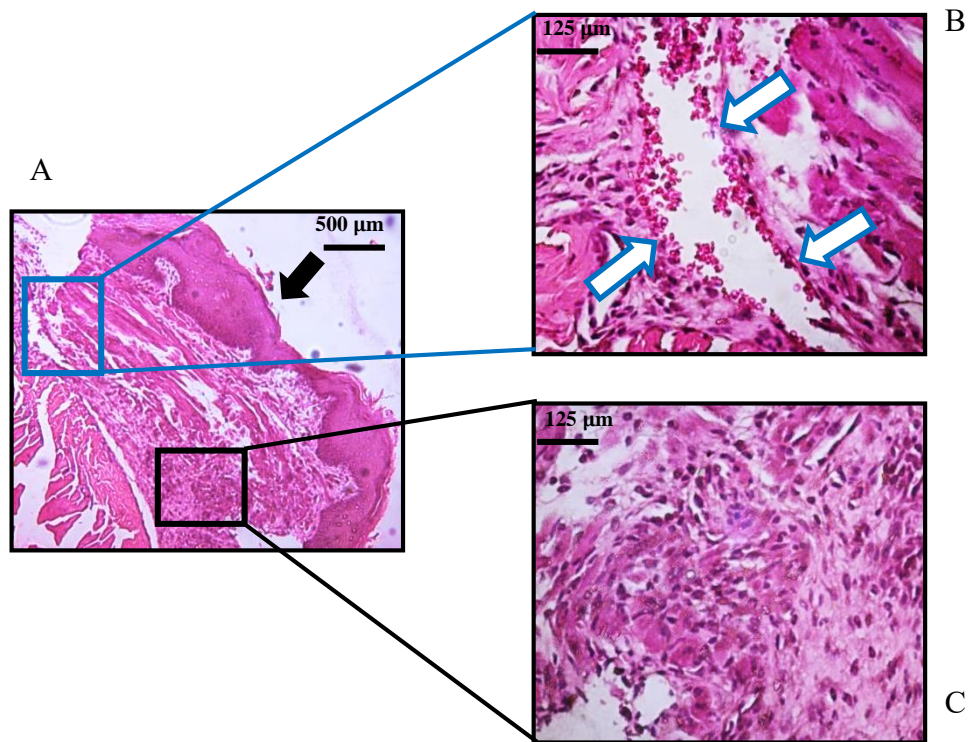


Figure 7. 70% acetic acid-treated buccal mucosa on day 14 after application stained with hematoxylin and eosin, presents the almost fully recovered buccal mucosa with intact epithelial layer, vasodilation and a mild inflammatory infiltrate. (A) Intact epithelial layer (Black arrow); (B) white arrow: Dilatation of blood vessels. (C) A mixed inflammatory cell infiltration in the underlying muscular layer

Table 1. Observation of buccal mucosa ulcer size treated with saline and acetic acid on five different days.

Group	Ulcer diameter (mm)				
	Day 2	Day 3	Day 7	Day 11	Day 14
Saline	0	0	0	0	0
	2	3	2	1	0
70% Acetic acid	3	3	1.5	0	0
	2	3	2	1	0

Discussion

Our study successfully developed an animal model to produce a uniform and reproducible ulcer formation to mimic condition of chemical exposure to the human oral cavity that could lead to the formation of ulcer. The model would allow its further usage to study the effects of many prospectus therapeutic agents/substances to promote the healing of oral ulcer. The model would be useful for initial study of the substances which are being developed to treat oral ulcers before they are commercially available.

The acetic acid model has been previously used in peptic ulcer model.⁹ Acetic acid is a common chemical agent used to induce inflammation/ulcers of various tissues and organs with different concentrations and methods being introduced from previous reports.^{3,4,13,14} This study using the application of 70% acetic acid to induce inflammation

offers a practical and straightforward method to create ulceration. While many studies had tested the effectiveness of many substances to treat oral ulcers, an established standardized experimental model of oral ulcer was still lacking.^{5,9} This study has proven that by applying acetic acid at a specific duration using a standardized tool (microbrush[®]) provides reproducible results.

In our study, 70% of acetic acid was applied with microbrush[®] for 60s to ensure that the acetic acid would not be over-applied and spread to the undesired area. Hence, uniform ulceration would be more achievable using microbrush[®]. A 70% concentration of acetic acid was selected from our previous pilot study that observes the effect of acetic acid application by different concentration techniques. The result found in this pilot study showed that the use of 70% acetic acid for 60s could create ulceration. Furthermore, the ulceration

created by this method could persist for approximately 14 days, as has been found in humans.

We observed the degree of inflammation on day one post-acetic acid treatment shown by redness and swelling, due to the ability of acetic acid to induce the inflammation on the underneath tissue. As the inflammation progressed, the ulceration was clearly observed on day two and reached its peak on day three post-treatment (Figure 3).

Ulceration on oral mucosa would propel pain and discomfort. This condition would lead to loss of weight in all treated mice in the first three days after 70% acetic acid application, as observed in this study. All mice started to gain weight on day seven as the healing process progressed (Figure 2). Same results were also found from the previous studies related to oral mucosa ulceration.^{6,7}

Ulcer on the oral mucosa can be observed as the lesions with the disintegration and loss of the epithelial tissue.¹⁵ These parameters were also applied in observing the ulceration in our study, besides clinical sign of inflammation (redness and swelling).

In the acetic acid-treated group, there were variations in ulcer diameter and healing time (Table 1). The ulcers in mice 1 and 3 had healed within 14 days, while those in mice 2 had recovered within 11 days. These results are consistent with those of previous studies, which found that oral mucous ulcers had healed within 10–14 days.¹⁶ Moreover, this variation is thought to be caused by several factors that influence the healing process. The ulcer recovery is defined as complete wound healing and the return of normal anatomical structure and function.^{17,18} Successful healing of wounds is dependent on time, an optimal healing process, cell type, molecular mediators, and elemental structures.

The wound healing process can also be influenced by the type, depth, and location of the wound. In general, the factors that influence wound healing are classified as either local or systematic.^{18,19} Local factors directly affect the wound itself, while systemic factors include the virulence of the organism, which can affect wound healing.²⁰ Local factors include bacterial infection, mechanical pressure, necrosis, the presence of foreign objects around the wound, and vascular supply. Systemic factors include metabolism, hormone production, and the presence of a chronic disease, such as malabsorption syndrome.^{18,19} Since there were no differences in the systemic conditions or sex of the experimental animals, the variations in the healing times among the groups were most likely caused by local factors, especially wound depth and variations in wound healing ability.

There were differences in the histopathological features between the acetic acid-treated and control mice (Figure 4–7). Based on histological appearance, the ulceration

was initially evident on day two after exposure of 70% acetic acid and had recovered by day 14, although there was still vasodilation of the arteries and infiltration of inflammatory cells. According to the clinical and histological observations, it can be concluded that the ulceration peaked on day three and recovered by day 14. These results are generally in line with those of previous studies that found the ulcers had healed, and the tissue had returned to normal by day 14. However, there were some differences from the previous studies. For example, the ulceration size was maximum on day one after the administration of acetic acid, which is a useful information to determine the best time to test the efficacy of anti-ulcer drugs.⁴

Conclusion

In conclusion, a model of ulceration of mice oral buccal mucosa was successfully created with the use of 70% acetic acid. The method used in this experiment is efficient and easily repeatable. The timing of ulcer formation and recovery can be determined by clinical and histological examinations. The model developed in the present study is expected to be used as a standard to induce ulcer formation of the oral mucosa to test the efficacy of anti-ulcer drugs in subsequent studies.

Acknowledgments

We thank R.V. Chikita, N. Sabrina, I.A.M. Pramutama, A. Tjahajani for discussion and technical assistance.

Funding

This work was supported in part by a grant from Penelitian Dasar Kemenristek Dikti Republik Indonesia No.: NKB-1766/UN.2.R3.1/HKP.05.00/2019.

Conflict of Interest Statement

The authors declare no conflict of interest.

Received: May 13th, 2019 Accepted: September 25th, 2019

References

1. Hitomi S, Ujihara I, Ono K. Pain mechanism of oral ulcerative mucositis and the therapeutic traditional herbal medicine hangeshashinto. *J Oral Biosci.* 2019;61:12–5.
2. Scully C, Shotts R. Mouth ulcers and other causes of orofacial soreness and pain. *West J Med* 2001;174:421–4.
3. Lim YS, Kwon SK, Park JH, Cho CG, Park SW, Kim WK. Enhanced mucosal healing with curcumin in animal oral ulcer model. *Laryngoscope.* 2016;126:E68–73.
4. Nodai T, Hitomi S, Ono K, Masaki C, Harano N, Morii A, et al. Endothelin-1 elicits TRP-mediated pain in an acid-induced oral ulcer model. *J Dent Res* 2018;97:901–8.
5. Jayantini R, Suniarti DF, Sarwono AT. Efficacy of a standardized ethanol extract of roselle calyx in the

- treatment of oral mucosa ulceration (in vivo). *Asian J Pharm Clin Res.* 2017;10:183–6.
6. Cavalcante GM, de Paula RJS, de Souza LP, Sousa FB, Mota MRL, Alves APNN. Experimental model of traumatic ulcer in the cheek mucosa of rats. *Acta Cir Bras.* 2011;26:227–34.
 7. Idrus E, Pramutama IAM, Suniarti DF, Wimardhani YS, Yuniastuti M. Experimental model of thermally induced-tongue ulcer in mice. *J Int Dent Med Res.* 2019;12:929–34.
 8. De Barros Silva PG, de Code EBB, Freitas MO, de Lima Martin JO, Alves APNN, Sousa FB. Experimental model of oral ulcer in mice: Comparing wound healing in three immunologically distinct animal lines. *J Oral Maxillofac Pathol.* 2018;22:444.
 9. El-Batal AI, Ahmed SF. Therapeutic effect of Aloe vera and silver nanoparticles on acid-induced oral ulcer in gamma-irradiated mice. *Braz Oral Res.* 2018;32:1–9.
 10. Builders PF, Kabele-Toge B, Builders M, Chindo BA, Anwunobi PA, Isimi YC, *et al.* Wound healing potential of formulated extract from hibiscus sabdariffa calyx. *Indian J Pharm Sci.* 2013;75:45–52.
 11. Vandamme T. Use of rodents as models of human diseases. *J Pharm Bioallied Sci.* 2014;6:2–9.
 12. Okabe S, Amagase K. An overview of acetic acid ulcer models: The history and state of the art of peptic ulcer research. *Biol Pharm Bull* 2005;28:1321–41.
 13. Amirshahrokhi K. Febuxostat attenuates ulcerative colitis by the inhibition of NF- κ B, proinflammatory cytokines, and oxidative stress in mice. *Int Immunopharmacol.* 2019;76:105884.
 14. Oliveira NV de M, Souza BDS, Moita LA, Oliveira LES, Brito FC, Magalhaes DA, *et al.* Proteins from *Plumeria pudica* latex exhibit protective effect in acetic acid induced colitis in mice by inhibition of pro-inflammatory mechanisms and oxidative stress. *Life Sci.* 2019;15: 116535.
 15. Muñoz-Corcuera M, Esparza-Gómez G, González-Moles MA, Bascones-Martínez A. Oral ulcers: Clinical aspects, a tool for dermatologists, part II chronic ulcers. *Clin Exp Dermatol.* 2009;34:456–61.
 16. Yilmaz N, Nisbet O, Nisbet C, Ceylan G, Hosgor F, Dede OD. Biochemical evaluation of the therapeutic effectiveness of honey in oral mucosal ulcers. *Bosn J Basic Med Sci.* 2009;9:290–5.
 17. Velnar T, Bailey T, Smrkolj V. The wound healing process: An overview of the cellular and molecular mechanisms. *J Int Med Res.* 2009;37:1528–42.
 18. Fitzpatrick SG, Cohen DM, Clark AN. Ulcerated Lesions of the Oral Mucosa: Clinical and Histologic Review. *Head Neck Pathol.* 2019;13:91–102.
 19. Young A, Mcnaught C, Young A. The physiology of wound healing The physiology of wound healing. *Wound Care.* 2000;9:299–300.
 20. Guo S, DiPietro LA. Factors affecting wound healing. *J Dent Res.* 2010;89:219–29.