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Review Article

ORAL MUCOSAL ULCER INDUCTION METHODS IN RATS: A SYSTEMATIC REVIEW

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ABSTRACT

Oral mucosal ulcers are a prevalent condition, but there are still limited drugs available to treat them. Varieties of induction techniques to obtain oral mucosal ulcer models in rats have frequently been used. This systematic review aimed to describe different approaches and to recommend the most effective method for oral mucosal ulcer induction methods in rats for anti-oral mucosal ulcer drug discovery. The PRISMA guidelines were used in the framework regarding this systematic review. The electronic databases PubMed, Science Direct, SCOPUS, and EBSCOhost-CINAHL Plus were used for article searching using specific keywords. The Risk of Bias Tool from Syrcle was used to undertake the evaluation of bias risk. Based on the analysis of 14 articles, the following findings were gathered: Wistar rats were frequently used mouse strains at an average of 8 w old and weighed between 120 and 300 g**.** Induction methods used to obtain ulcer models were acetic acid, biopsy punch, scalpel blade, thermal, and phenol. Acetic acid induction was the most commonly used compared to the other induction techniques. The ulcers were obtained by acetic acid identical to those that occur on the human oral mucosa and available at a reasonable price. However, the ulcer formation takes longer compared with biopsy punch and scalpel blade induction. The systematic review found that there are various methods for inducing oral ulcers in rats, with acetic acid being the recommended method to produce a suitable mucosal ulcer model in rats.

Keywords: Induction methods, Oral mucosal ulcers, *In vivo* study, Rat

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INTRODUCTION

Oral mucosal ulcers are the most common oral cavity lesions caused by various factors [1-3]. Ulcer lesions are typically circular sores with a yellow-white appearance resulting from an epithelial defect covered by fibrin [4, 5]. Diagnosing lesions in the oral mucosa can be challenging for dentists due to the overlapping clinical and histological features among different types of ulcerated lesions. The majority of ulcerative lesions in the oral mucosa are associated with infection, immunity, trauma, or neoplastic disease [1, 6]. In addition, anti-oral mucosal ulcer drugs are still very limited in drug stores, even synthetic drugs or herbal materials. Therefore, further research is still needed regarding the drug discovery for oral mucosal ulceration therapy.

Oral mucosal ulceration modeling in experimental animals was needed to conduct a good research method for drug discovery. One of which is the rat, especially white rats (Rattus norvegicus), which are the most commonly used animal models in research [7]. Rats have many advantages over other animals because of their relatively short life cycle (2-3.5 y), the large number of offspring per birth, ease of handling, larger body size compared to mice, ease of providing treatment and intervention, and less afraid of light. Rat's activity is not disturbed by the presence of humans in the vicinity, it is resistant to arsenic trioxide, has reproductive characteristics similar to other mammals, has anatomical structure, physiology, and genetics similar to humans, it is also easy to maintain under controlled environmental and sanitary conditions, as well as with special diets [7-9].

A valid experimental model is needed to evaluate pharmacological effects such as anti-oral mucosal ulcer effects, especially for new drug discovery or research. A good ulcer model is a useful tool in order to acquire a more comprehensive comprehension of the pathophysiology, wound healing, and pharmacology mechanisms of the disease, especially for anti-oral mucosal ulcer properties [10]. Therefore, several induction methods are carried out to obtain an ulcer model, such as using chemical, mechanical, or thermal agents. Because each method has several advantages and disadvantages, it is necessary to analyze and select the most appropriate method based on consideration of the availability of local sources, research objectives, hypothesis testing, or other factors related to the research questions [11].

Inexperienced researchers are likely to make mistakes in selecting test animal species, induction methods, and evaluation methods of test results. This may lead to ineffective research time and costs [12]. To the best of our knowledge, we have not been able to find a review article that discusses the various induction methods for oral mucosal ulceration, their comparison, and the most commonly performed by researchers in scientific journals, as well as analyses of their effectiveness. Therefore, this review article will discuss the induction methods of oral mucosal ulceration that are often used and considered effective, as well as the characteristics of rats as animal models for oral mucosal ulceration.

Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed when developing this systematic review and executing the literature search. Keywords were determined using the Population, Intervention, Comparison, and Outcome (PICO) method [13]. The populations that this systematic review covered are "Rats or Animal Studies or Animal Experimental or *In vivo*"; with intervention is "the creation of an oral ulcer or oral mucosal ulcer model". Comparisons and outcomes in this review are found in the Results section. Inclusion criteria were articles published during the last 10 y (from 2013 to 2023), written in English with full text available, research study limited to *in vivo* studies, research conducted on rats with ulcers, and treatments done on non-keratinized mucosa. The exclusion criteria for this article were articles that are not relevant to the research question, not written in English, duplicate articles, *in vitro* studies, review articles, studies on animals other than rats, studies conducted on keratinized oral mucosa or oral hard tissue, studies on other than the oral mucosa, and studies on other diseases induction models such as induction for oral mucositis. PubMed, ScienceDirect, SCOPUS, and EBSCOhost-CINAHL Plus were used as electronic databases for literature searches from May to June 2023. The keywords being used were Oral Ulcer or Oral Mucosal Ulcer, and Animal Experimental or Animal Study or *in vivo*, and Rats.

Syrcle's Risk of Bias tool was utilized to evaluate article quality in this systematic review. There are 10 domains proposed to assess the

risk of bias; 1. Sequence Generation; 2. Baseline Characteristics; 3. Allocation Concealment; 4. Random Housing; 5. Blinding Intervention; 6. Random Outcome Data; 7. Blinding Outcome; 8. Incomplete Outcome Data; 9. Selection Outcome Reporting; 10. Other Source of Bias. There is no scoring system for the cycle's risk of bias. The author's assessment was carried out by answering specific questions for each item. There are three possible responses: "Yes" for low risk of bias, "No" for high risk of bias, and "Unclear" for inadequate data [14].

RESULTS AND DISCUSSION

The results obtained were 23,002 pieces of articles and another form of literature in four electronic databases using a combination of keywords. Furthermore, based on the inclusion criteria, 22,449 articles were excluded, leaving 553 articles. After reviewing the titles and abstracts of various articles, a total of 14 were selected for the final analysis. The eligibility of each article was determined using Syrcle's risk of bias assessment. You can refer to fig. 1 for an overview of the literature search process and table 2 for the assessment of risk of bias.

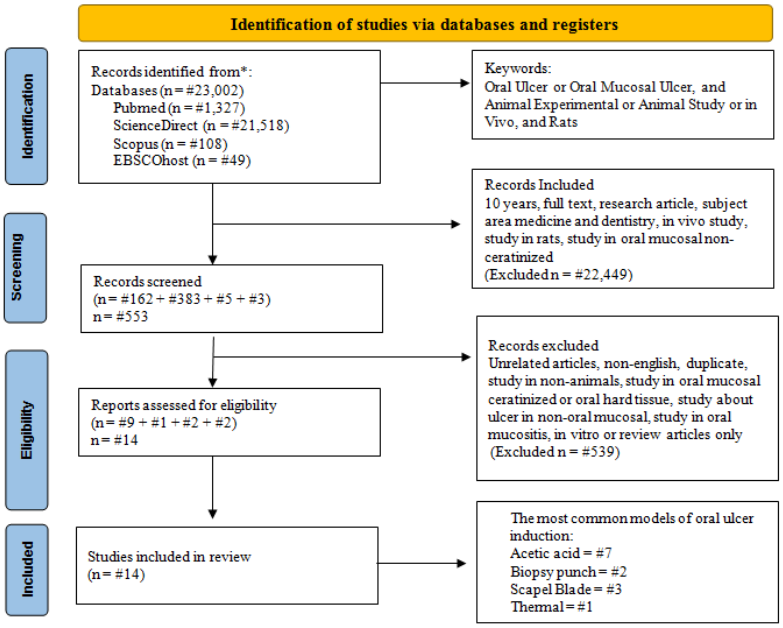

Fig. 1: PRISMA flow chart

Table 1: Assessment of the risk of bias

S. No.	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding (Intervention)	Random outcome assessments	Blinding (outcome)	Incomplete outcome data	Selective outcome reporting	Other sources of Bias	References
1	☻	☺	☻	☻	☻	☻	☻	☺	☺	☺	[15]
2	☺	☺	☻	☺	☻	☺	☻	☺	☺	☺	[16]
3	☺	☺	☻	☺	☺	☺	☺	☺	☺	☺	[17]
4	☺	☺	☻	☺	☺	☺	☺	☺	☺	☺	[18]
5	☻	☺	☻	☻	☻	☻	☻	☺	☺	☺	[19]
6	☺	☺	☻	☺	☻	☺	☻	☺	☺	☺	[20]
7	☺	☺	☻	☺	☻	☺	☻	☺	☺	☺	[21]
8	☺	☺	☻	☺	☻	☺	☻	☺	☺	☺	[22]
9	☺	☺	☻	☺	☻	☺	☻	☺	☺	☺	[23]
10	☻	☺	☻	☻	☻	☻	☻	☺	☺	☺	[24]
11	☺	☺	☻	☺	☻	☺	☻	☺	☺	☺	[25]
12	☻	☺	☻	☻	☻	☻	☻	☺	☺	☺	[26]
13	☻	☺	☻	☻	☻	☻	☻	☺	☺	☺	[27]
14	☻	☺	☻	☻	☻	☻	☻	☺	☺	☺	[28]

Notes: ☺: Yes; ☻: Unclear

Table 2: Characteristics of the Animals

No	Animal	Quantity	Method of quantity	Weight (gram)	Age (Weeks)	Reference
1	Wistar Albino Rats	60	Not Mentioned	210-270	Not Mentioned	[15]
2	Wistar Rats	72	Not Mentioned	260-310	Not Mentioned	[16]
3	Sprague–Dawley Rats	45	Not Mentioned	250-200	Not Mentioned	[17]
4	Wistar Rats	30	Not Mentioned	120-160	8	[18]
5	Wistar Rats	20	Lemeshow's formula	250-300	4-8	[19]
6	Sprague-Dawley Rats	35	Not Mentioned	Not mentioned	8	[20]
7	Sprague-Dawley Rats	24	Not Mentioned	200-250	5	[21]
8	Wistar rats	73	Not Mentioned	210	Not Mentioned	[22]
9	Sprague Dawley rats	10	Animal Research: Reporting *in vivo* experiments (Arrive) guideline	Not mentioned	8	[23]
10	Wistar rats	30	Not Mentioned	200-250	8-12	[24]

No	Animal	Quantity	Method of quantity	Weight (gram)	Age (Weeks)	Reference
11	Sprague-Dawley rats	30	Not Mentioned	200-300	7	[25]
12	Sprague–Dawley rats	50	Animal Research: Reporting of *in vivo* experiments (Arrive)	180-220	8	[26]
13	Wistar rats	316	Not Mentioned	Not Mentioned	8-9	[27]
14	Wistar rats		Lemeshow's formula	150-200	Not Mentioned	[28]

Table 3: Characteristics of the induction method

S. No.	Treatments of the animals	Induction	Induction procedure	Time (s)	Doses	Site	Results	Time (H)	Examiner	Reference
1	The animals were kept at a temperature of 22±2 °C with 12 h of automatic lighting per day. They had ad libitum access to commercial rat food containing 20% protein.	Acetic acid	Filter papers with a 5.0 mm diameter were soaked in acetic acid and pressed onto the surface.	60	15 ml of 50%	Buccal	Chronic ulceration with well-defined borders developed	48	Not Mentioned	[15]
2	The animals were kept in plastic cages with pine-sawdust floors at a temperature of 24 °C, a 12-hour cycle of light and dark. They were provided unrestricted access to water and commercial food.	Punch biopsy	Not explained			Buccal	A circular excisional wound with a diameter of 8 mm and a depth of 1 mm	24	Pathological Examinations	[16]
3	Not explained	Acetic acid	Cotton tips soaked in acetic acid (5 mm diameter) pressed onto the surface.	60	15 ml of 50%	Labial	A round ulcer with necrotic tissue	Not mentioned	Not Mentioned	[17]
4	The Wistar rats were kept in cages with two rats per cage and in a room with a temperature of 27 °C. The environment was artificially lit for 12 h a day and dark for the other 12 h. The rats had unlimited access to a standard diet and water.	Scalpel blade	An incision with a round stainless steel blade about 10 mm			Labial	There is a yellowish-white ulcer with a red halo surrounding it.	24	Not Mentioned	[18]
5	Not explained	Punch biopsy and incise	The tissue was cut at its base with surgical blade no. 15 after an 8g/3 mm punch biopsy.			Labial	An ulcer lesion with a diameter of 3 mm surrounded by a white color and an erythematous arc.	24	Not Mentioned	[19]
6	The animal was kept in a 12/12-hour light-dark cycle and had unrestricted access to food and water.	Phenol	Not explained			Buccal	An almost uniform round ulcer	144	Not Mentioned	[20]
7	At a room temperature of 21±2 °C and a relative humidity of 60±5 percent, the rats were fed on a 12 h light/dark cycle.	Acetic acid	A glass tube was used to apply acetic acid (D: 6 mm, depth: 30 mm)	30	200 µL of 30%	Buccal	Ulcer wounds	72	Not Mentioned	[21]
8	Animals are housed in sawdust-filled boxes. For a photoperiod of 12 h, the animals were housed at room temperature with controlled humidity, and they received industrial feed (Bio-base®, Guas Frias, SC, Brazil) according on their nutritional requirements as well as water at will.	Scalpel blade	A marker with an 8 mm diameter was utilized to standardize the lesion area after the ulceration was done using an 8 mm scalpel blade.			Buccal	Not mentioned	Not mentioned	Not Mentioned	[22]
9	Rats were housed at 22 °C, 50–60 % relative humidity, and a 12-hour light–dark cycle.	Acetic acid	A circular filter paper with a 3 mm diameter that had been soaked in 50% acetic acid was applied to the surface.	30	50%	Labial	Oral mucosal lesions that are clearly defined and are 2.5–3 mm in diameter. They have a central depression, a yellow–gray pseudo membrane, and a red edge.	48	Two Experienced Dentists.	[23]
10	For a week, the animals adjusted to a temperature of 22±2 °C.	Thermal burn	Thermal damage was used to cause ulceration by applying a hot ball burnisher tip with no pressure (diameter: 2-3 mm).	60		Labial	Ulcer lesions with a diameter average of 4–5 mm	24	Not Mentioned	[24]
11	The animals were housed on a 12-hour cycle of light and dark (lights on from 6:00 to 8:00), and they were kept in a room that was kept at a consistent temperature and humidity level (22–25 0C and 40–60 percent), with access to food pellets and water on demand.	Acetic acid	In 15 ml of 50% acetic acid, a round filter paper (d: 5 mm, Whatman No. 1) was soaked. This piece of acid-soaked paper was placed on top.	30	15 ml of 50%	Labial	Tissue necrosis and ulcer wounds with a diameter of 5 mm	72	Not Mentioned	[25]
12	Not Mentioned	Acetic acid	A 6 mm by 5 mm filter paper soaked in 70% acetic acid was used to create oral ulcers (Sigma-Aldrich, St. Louis, MO)	180 s	70%	Buccal	Uniform fashion of ulcer size and depth	48	Not Mentioned	[26]
13	The rats were maintained on a light-dark cycle (L: D, 12:12 h) in a	Acetic acid	Acetic acid diluted with water was	30 s	50%	Labial	Not Mentioned	48	Not Mentioned	[27]

S. No.	Treatments of the animals	Induction	Induction procedure	Time (s)	Doses	Site	Results	Time (H)	Examiner	Reference
	temperature-and humidity-controlled environment at a constant temperature of 21-23 0C and 40%-60%, respectively. They were housed in pairs in transparent cages with wood chips under particular pathogen-free conditions. Water and food pellets were available at all times.		applied to a 9-mm2 piece of filter paper.							
14	For the first seven days prior to the experiment, the animals are housed in the same cage at a constant temperature of 25 2 °C with access to food pellets and purified water as needed.	Scalpel blade	With a scalpel blade no. 15 and an 8 mm cross-sectional wound diameter, the ulceration is produced.			Buccal	Not Mentioned	Not Mentioned	Not Mentioned	[28]

Regarding the results of the bias assessment, It was discovered that there were issues with allocation concealment; all articles showed a risk of bias with inadequate information, while almost the majority of blinding domains, both interventions and outcomes, showed a risk of bias with inadequate information as well. The risk of bias was low for domains such as sequence generation, incomplete outcome data, and selective outcome reporting.

Table 2 shows the characteristics of the rats used in the articles reviewed. There are two types of rats used in these studies related to oral mucosal ulcer models: Wistar rats in 8 articles [15, 16, 18, 19, 22, 24, 27, 28] and Sprague-Dawley rats in 6 articles [17, 20, 21, 23, 25, 26]. The number of rat samples used varied in each article, but most articles (10 articles) did not mention the method of determining the number, while others mentioned using the Animal Research Guidelines: Reporting *In vivo* Experiments (ARRIVE) in 2 articles and using Lemeshow's formula in 2 articles [19, 23, 26, 28]. The weight of the rats used ranged from 120 to 310 grams, and 8 w of age were used in 7 articles.

Table 3 shows the characteristics of the study and the induction methods. Most rats were housed at room temperature of 21-27 °C with a 12-hour light-dark cycle and had access to commercial rat food and water in 12 articles, and 2 articles did not mention the details. Induction methods used to obtain ulcer models were acetic acid in 7 articles [15, 17, 21, 23, 25-27], biopsy punch in 2 articles [16, 19], scalpel blade in 3 articles [18, 22, 28], thermal in 1 article [24], and phenol in 1 article [20]. The induction method using acetic acid was done in several ways: apply 15 ml of 50% acetic acid on cotton tips or round filter paper with a diameter of 3-9 mm, and then place them on the buccal and labial mucosa for 30-60 seconds, as listed in five articles [15, 17, 23, 25, 27]. In addition, 1 article suggests applying a 6 x 5 mm filter paper soaked in 70% acetic acid to the labial mucosa for 180 seconds [26], and another article utilized 200 microliters of 30% acetic acid in a glass tube measuring 6 mm in diameter and 30 mm in depth. It was then applied to the labial mucosa for 30 seconds [21]. Within 48 to 72 h, the ulcers were characterized by a central depression, yellow-white or gray pseudomembrane, and erythematous border, arising from all doses of acetic acid [15, 17, 21, 23, 25-27].

Another induction method was using a 3 mm punch biopsy tool (8 grams) on the labial mucosa, followed by cutting the base of the tissue using a No. 15 scalpel, as described in one article. The results obtained were white ulcer lesions surrounded by erythema borders with a diameter of 3 mm [19]. While another article using punch biopsy did not describe the procedure in detail, the excised lesions formed round results measuring 8 mm in diameter and 1 mm in depth [16]. All ulcers that were caused by punch biopsy were obtained within 24 h.

Another method of induction was using a scalpel, described in 3 articles. An 8-10 mm diameter incision or abrasion made with a No. 15 scalpel on the buccal mucosa [22, 28] and labial mucosa [18]. A yellowish-white ulcer lesion surrounded by an erythema halo was formed within 24 h and was only mentioned in one of those three articles. In addition, one article used thermal induction procedures. A 2–3 mm-diameter burnisher tip was heated and applied without pressure for 60 seconds on the labial mucosa. The ulcer formed in a diameter of 4-5 mm within 24 h [24]. The detailed induction procedure using phenol was not described in the article, but it was mentioned that it was carried out on the buccal mucosa, and an almost uniform round ulcer was formed after 144 h [20]. Furthermore, the experts who assessed ulcer shape were only mentioned in two of the 14 articles reviewed. One article that used the punch biopsy induction method was assessed by a pathologist [16], and another article that used the acetic acid induction method was assessed by two dentists [23]. Most of the ulcer modeling (13 articles) was conducted in the research to determine the efficacy of a particular drug or treatment, while one article was not related to therapeutic efficacy. All articles reviewed mentioned that both a control group and an experimental group were used. Most of the articles mentioned the negative control group (13 articles), and there was only 1 article that did not mention it, but still used a normal control group to make a comparison.

Based on the 14 articles reviewed, the characteristics of the most widely used rats in the study were from the 8-week-old Wistar rat, with the lowest weight of 120 grams and the largest of 310 grams. Rats have many advantages over other model animals. Rats were easy to control, had a short life cycle (2-3.5 y), had a large number of offspring per birth, had an active reproductive period of one year and a gestation period of 20-22 d, easy to handle, calmer and easier to do some interventions, larger body size compared to mice, less afraid of light, and less inclined to gather with other species. The activities of rats are also not disturbed by the presence of humans in the vicinity; they are resistant to arsenic trioxide; they have reproductive characteristics similar to other mammals and anatomical structure, physiology, and genetics similar to humans; these animals are kept in carefully controlled environments and fed special diets. Additionally, their genome is similar to that of humans. (99%), their good genetic and molecular tools are available for studies, the small body size can facilitate large-scale, high-output studies, and making it a cost-effective animal model [7-9]. Hence, the potential to make medical research and, particularly, drug development more efficient and scalable. During studies focused on the oral mucosa, rats are often utilized in studies because their microscopic and macroscopic anatomical structures are similar to those of the human oral mucosa. The oral mucosa comprises surface epithelial tissue and connective tissue beneath it, with the lamina basalis providing support and nutrition to the epithelium [29].

The animal welfare standards in the Update of the Guide for the Care and Use of Laboratory Animals by the National Research Council of the United States must be obeyed, to conduct good research procedures. Encompass setting the cage temperature, ranging from 21 to 26 °C, conditioning a 12-hour light-dark cycle, acclimatizing the animals in the experimental room for 5-7 d pre-treatment, and randomizing the rats into groups. Randomization resulted in the even distribution of body weight for all groups, with a variation in body weight not more than 20% of the average body weight [30, 31]. All of the articles included in this review met these requirements.

The most widely used induction method to model ulcers is acetic acid. Acetic acid is often used to create ulcers in different organs using varying concentrations and techniques [32]. The advantages of using acetic acid in inducing ulcers are easily available materials, easy application, uniform ulcer shape, and easy repeatability, and

also can save costs. Topical application of 50% acetic acid causes strong acid contact with the oral mucosa for several minutes and causes infiltration of inflammatory cells accompanied by swelling, which is an early sign of inflammation [27]. The process of inducing ulceration in rat oral mucosa with acetic acid resembles the natural process of ulcer formation in human oral mucosa, both clinically and histologically. After the mucosa is contacted with acetic acid, a reddish color is formed on the surface of the mucosa, indicating continuous inflammation in the area. Total ulcer formation occurs in 48 to 72 h, as indicated by the disintegration and desquamation of epithelial tissue [15, 17, 21, 23, 25-27]. The healing process of oral mucosal ulcers in the negative control group of rats will occur between days 7 and 14. This group is not treated with drug test materials or any therapies. This natural healing period resembles that occurs in humans without any drug or therapy intervention [32].

This systematic review revealed that the most common induction method for oral mucosal ulcer models in rats apply 15 ml of 50% acetic acid to the oral mucosa for a period of 30 to 60 seconds. The ulcer formed within 48–72 h, with characteristics of white or yellowish-gray color, and surrounded by erythema clear border [15, 17, 23, 25, 27]. In contrast, induction using punch biopsy or scalpel blade requires special tools and techniques, as well as the expertise of trained operators, to obtain a uniform ulcer that resembles the natural shape of ulcers [29]. Likewise, the thermal induction method also requires special techniques and expertise to obtain the appropriate ulcer results. The application of a burnisher without pressure at 800 °C can produce ulcer models that resemble natural ulcers in humans. However, the advantage of oral mucosal ulcer induction using punch biopsy, scalpel blade, and thermal, compared to acetic acid, ulcers can be formed immediately after the procedure [33]. Meanwhile, induction methods using phenol were rarely used in studies.

Our analysis in this systematic review showed slight differences when compared to a study applied 70% acetic acid to the ventral tongue mucosa of Wistar rats for 2 min. Ulcers can form faster, within 24 h, presumably due to the use of a higher concentration of acid and longer contact time of acid with the mucosa. Moreover, the fact that the acid is applied to the ventral mucosa of the tongue, which contains more blood vessels than the buccal or labial mucosa, allows the inflammatory reaction to occur more quickly [34].

The use of rats as experimental animals is also advantageous in terms of drug discovery efforts for very rare diseases because the number of patients is small and it is difficult to conduct clinical trials. Research regarding the effectiveness and safety of drugs which was done in rats (in vivo study) can obtain information about the effective doses of the new drugs for rats, which then can be converted to the human dose [7, 35]. Therefore, most of the research articles reviewed used rat models as they related to new drug discovery efforts.

Another insight that can be obtained from this systematic review is related to research reporting, which was not considered in detail by the researchers. The risk of bias assessment results clearly indicate that every article has an "unclear" component in domain assessment. Clinical researchers will have difficulty obtaining preclinical data from poorly reported animal studies. So then, it can be recommended for preclinical studies that will be used as the basis for clinical trials to follow the instructions according to the "Gold Standard Publication Checklist (GSPC)". The animal study reporting standards also encourage the application of the 3R principle (reduce, refine, and replace) and the 5 Freedoms (freedom of hunger and thirst, freedom from discomfort, freedom from pain, injury, or disease, freedom to express normal behavior, and freedom from fear and distress) [8, 36, 37]. The International Council for Laboratory Animal Science (ICLAS) has created eight Harmonized Animal Research Reporting Principles in addition to these GSPC reporting guidelines (HARRP). Ethics, context and goals, study design, animal information, experimental technique, housing information, and research environment are the six fundamental concepts that have been agreed upon [37].

CONCLUSION

Based on the qualitative analysis in this systematic review article, there are various methods for inducing oral ulcers in rats. Induction methods used to obtain ulcer models were acetic acid, biopsy punch, scalpel blade, thermal, and phenol. We can recommend that the rats with the best characteristics that can be used as experimental animals for oral mucosal ulcer models are Wistar rats, 8 w old, weighing 120–310 grams. The best induction method for the oral mucosal ulcer model is 50% acetic acid, applied for 30–60 seconds on the buccal/labial mucosa. The treatment of rats must follow ethical standards and applicable animal welfare principles, and research reporting must also be written following the guidelines for writing research reports on animals.

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AUTHORS CONTRIBUTIONS

The contribution of all the authors is equal.

CONFLICT OF INTERESTS

There are no conflicts of interest present.

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