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The Use of an Ambroxol Solution to Assess Acute Dermal Irritation on Rabbit Skin

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ABSTRACT

According to in vitro research, ambroxol can significantly inhibit biofilms made by biofilm-forming bacteria, depending on the concentration of ambroxol. As an anti-biofilm, ambroxol can be administered in topical dosage forms. One of the conditions for a good topical is not to irritate the skin. This study aimed to determine the presence of an irritating effect on the skin and assess and evaluate the characteristics of topical ambroxol when exposed to the skin. The method used refers to BPOM 2020, namely by applying 0.5 ml of ambroxol solution at a dose of 15 mg/5 ml and 30 mg/ml on the skin of the rabbit's back. Covering it with gauze and a non-irritant plaster after 4 hours, the residue is cleaned and then observed at hours 1, 24, 48, and 72 to see whether or not an erythema and edema effect was arising from the influence of the experiment, and at the end of the experiment, a histopathological test was carried out. The results showed that macroscopically, the ambroxol solution did not show any erythema or edema, so the primary irritation index score was obtained for all test solutions with a score of 0. In contrast, in the microscopic irritation test, the erythema score was four and the edema score was three. This study concludes that the irritation effect from the ambroxol solution is very light with a primary irritation index of 0; no injuries were caused during exposure to ambroxol solution, and histopathologically, the irritation effect caused by ambroxol solution is of a moderate degree.

Keywords*: Ambroxol solutions, Acute dermal irritation test, Histopatologic test, Erythema, Edema*

INTRODUCTION

Ambroxol is a mucolytic drug used to treat cough symptoms such as the common cold, pharyngitis, acute bronchitis, and chronic obstructive pulmonary disease. Ambroxol functions to overcome the appearance of excessive mucus in the respiratory system. Ambroxol has secretolytic and secretomotor properties that promote mucus clearance in the respiratory tract. Ambroxol can also make type II pneumocyte cells make and release

surfactant substances, which reduces the amount of mucus that sticks to the bronchial walls (Reddy KS, 2022; Kristen, Schimmel, and Henk, 2004).

Ambroxol, with the chemical name Trans-4-[(2-Amino-3,5dibromobenzyl) amino] cyclohexanol hydrochloride, is a drug that functions to thin phlegm. Ambroxol also has anti-inflammatory and antioxidant properties. (Beeh, 2018) Ambroxol is a derivative of the bromhexine compound, which has almost the same effect. However, there are differences in its chemical structure, such as the addition of hydroxyl groups and the deletion of methyl groups. This difference in chemical structure makes ambroxol have additional effects compared to bromhexine, such as anti-inflammatory, antioxidant, and local anesthetic effects, as well as antiviral and antibacterial effects at certain levels. (Weiser, 2008). Ambroxol is often used as a first-line therapy for acute cough symptoms in patients with acute bronchitis or the common cold. Ambroxol has also been found to help treat Gaucher's and Parkinson's disease because it is reported to increase the glucocerebrosidase enzyme (Kantar A, 2020; de Azevedo Queiroz O, 2021).

Ambroxol was also reported to interfere with the formation of adherent biofilms and reduce biofilm production. It has been proven in several previous studies. Ambroxol effectively treats respiratory tract infections by inhibiting the formation of mature oral biofilms. Ambroxol can also inhibit Candida albicans bacteria by exerting its fungicidal activity against fungal cells interspersed in preformed biofilms (Delgadillo, 2014). Candida species can also cause skin infections. In a study of the effect of ambroxol on pneumonia with biofilm formation in a rat model of endotracheal intubation. Demonstrated that ambroxol can damage the structure of the Pseudomonas aeruginosa biofilm in intubation tubes and reduce bacteria (Li F., 2011). It was also found that ambroxol could inhibit Serratia marcescens from forming biofilms (Abbas, 2017). Ambroxol is also known to have the potential to be used for the treatment of the diabetic foot infection Proteus mirabilis because ambroxol can significantly inhibit biofilm formation and remove biofilms that have previously formed (Abbas H. A., 2014).

The study of ten potential anti-biofilm agents such as N-acetylcysteine, ambroxol, piroxicam, diclofenac sodium, ketoprofen, 4-nitropyrdidine-N-oxide, sodium ascorbate, sucralose, xylitol, and sorbitol showed varying activity against previously formed biofilms. The results showed that 4NPO was the most active; diclofenac sodium,

ketoprofen, N-acetylcysteine, ambroxol, sodium ascorbate, and piroxicam showed moderate activity, while sucralose, xylitol, and sorbitol showed weak activity. (Abbas, 2012)

Biofilm formation also complicates infectious diseases and increases the number of biofilm-forming bacteria resistant to antibiotics. Ambroxol is not yet available in topical preparations. However, in several studies, it has been proven that ambroxol can be used as an anti-biofilm by inhibiting the formation of biofilms on pathogens, so it has the potential to be applied to topical preparations. (Jamal, 2015)

A dermal acute irritation or corrosion test is a test on animals (albino rabbits) to detect toxic effects that appear after exposure to the test preparation on the dermis for up to 4 hours. The principle of the acute dermal irritation/corrosion test is the exposure of the test preparation in a single dose to the skin of the test animal, with the untreated skin area functioning as a control. The dermal acute irritation/corrosion test aims to determine the presence of an irritating effect on the skin and to assess and evaluate the characteristics of a substance when exposed to the skin (Control, 2020).

Skin irritation reactions are not only local to the damaged skin surface but can also cause toxic effects that can be harmful and life-threatening to the sufferer. Common symptoms that can occur if irritation occurs, such as heat, are caused by the dilation of blood vessels in the affected area, which can be seen by the appearance of redness in the area of the skin (erythema). Besides that, it can also cause edema, which can be observed by the enlargement of frozen plasma in the injured area and accelerated by the presence of fibrous tissue covering the area (Control, 2020).

Based on this, an irritation test must be carried out before use in humans to prevent hypersensitivity reactions. If there are signs of irritation on the skin of experimental animals, there is a possibility of irritation on human skin. This research was conducted to determine the safety of ambroxol solution through an acute dermal irritation test on albino rabbit test animals. Thus, the utilization of ambroxol can be maximized to be used as a treatment for infections caused by biofilm. This study is an early-stage test for topical ambroxol solution, previously marketed only for oral use. With this research, the ambroxol solution's safety data can be obtained for topical preparations.

MATERAL AND METHODS

Preparation of test Animals

The test animals were acclimated to the experimental room for 5 days and placed in individual cages. 24 hours before testing, the animal's fur was shaved on a 2x3 cm2 area on the back for the test substance exposure site.

Preparation of the Test

A 60-ml ambroxol solution was prepared. Ambroxol is mixed with 10% propylene glycol. Propylene glycol is used as a co-solvent. Propylene glycol has high penetration-enhancing activity and is considered the safest because of its low irritation effect on the skin. After the Ambroxol and propylene dissolve, they are mixed with distilled water as a solvent mixture and stirred until homogeneous. This study used doses of 15 mg/5 ml and 30 mg/5 ml.

Administering the Test of Preparations

The test preparation was exposed to a skin area of ± 6 (2 x 3) cm2 at the exposure site. The exposure site was covered with gauze and plastered with a non-irritant plaster. The plaster should be loosened using an appropriate semi-occlusive dressing during exposure. When the test preparation or test substance is applied to an application, the bandage must adhere to the skin to ensure good contact and uniform distribution of the test substance on the skin. Animals must be prevented from inhaling or swallowing the test material on the plaster (Control, 2020).

Acute Dermal Irritation Test

In the acute dermal irritation test study, experimental animals will be given ambroxol solution. Giving the solution by applying it to the animal's skin, which is determined with the test preparation, is rubbed first on gauze and then affixed to the test animal. The exposure site is then covered with gauze and wrapped with non-irritating plaster. After 4 hours, the residue of the preparation is cleaned using distilled water. At the end of the toxicity test experiment, a histopathological examination was carried out to see if there were any abnormalities in the organs caused by the administration of the test preparation. In this study, the backs of the rabbits were marked for each group to be given the test sample. Group I was the test sample of 30 mg/5 ml ambroxol solution; group II was the negative control, namely propylene glycol; group III was the positive control, namely ambroxol syrup 30 mg/5 ml; and group IV was the test sample of 15 mg/5 ml ambroxol solution. Observations were made at 1, 24, 48, and 72 hours after opening the patch. In histopathological testing, sampling, and observation, only 30 mg/5 ml ambroxol solution was used (Control, 2020).

Observation

All test animals must be observed for the presence or absence of erythema and edema; response assessment is carried out at 1, 24, 48, and 72 hours after opening the patch (for test preparations that are not corrosive or irritant). For preliminary tests on one animal, observations are made immediately after the opening of the patch. If skin damage cannot be identified as irritation or corrosion at 72 hours, statements can be continued until day 14 to determine reversibility. In addition to reports of anger, local toxic effects, such as defatting of the skin and the impact of other toxicities and body weight, should be described and recorded. The histopathological examination should be considered to clarify a questionable response. (Control, 2020)

To avoid subjectivity in assessing skin reactions or scoring, it should be carried out by trained individuals who, if necessary, can use illustrations of scoring or scoring images. Skin reactions were evaluated in the table below, with a maximum score of 4 (Control, 2020).

Table. 1 Assessment of skin reactions

Erythema Formation	**Score**
Erythema Formation	
No erythema……	0
Very slight erythema (barely distinguishable)…..	1
Visible erythema……..…..	2
Moderate to severe erythema……	
Severe erythema (flesh red) to the formation of eschar inhibits erythema assessment….	3 4
Formation of Udema	
No edema……	0
Very small edema (almostindistinguishable)……	1
Small edema (clearly visible boundaries)	2
A moderate degree of edema (approximately 1mm in the area)…….	3
Severe edema (area increased by more than 1 mm extending beyond the scope of exposure by the test substance)	4

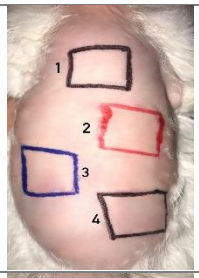
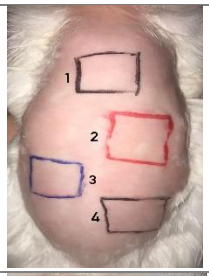
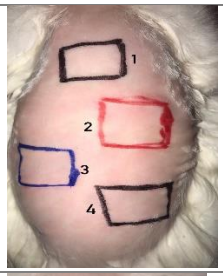
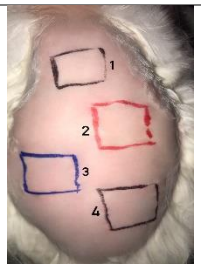
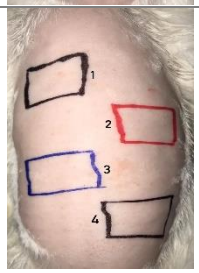
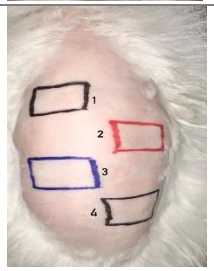
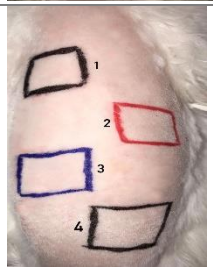
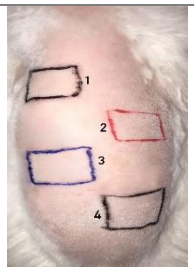
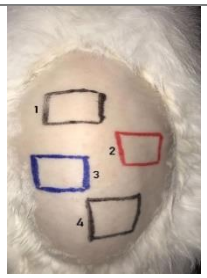
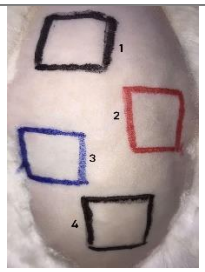
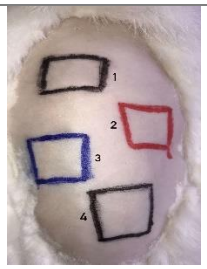
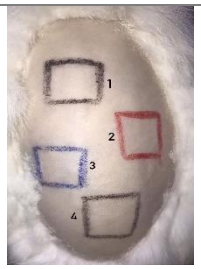
Table 2. Response category

Average value	**Response category**
0,0-0,4	Neglible
0,5-1,9	Slight
2,0-4,9	Moderate
5,0-8,0	Severe

RESULTS AND DISCUSSION

In this study, the irritation test of ambroxol solution was carried out on three rabbits. Observations are presented in the table 3. below.

Table 3. The results of the macroscopic acute dermal irritation test

Rabbit	Time			
	1 hour	24 hours	48 hours	72 hours
R1				
R2				
R3				

Information:
1: Ambroxol Solution (30mg)
2: Negative Control (Propylene Glycol)
3: Positive Control (Ambroxol® Syrup)
4: Ambroxol Solution (15mg)

Based on Table 3, the results obtained for samples 1 and 2 did not show erythema or edema, according to the positive control. The danger from exposure to a substance in humans can be determined by studying the cumulative effects, doses that can cause toxic effects on humans, carcinogenic, teratogenic, mutagenic effects, and others. In general, this information can be obtained from experiments using test animals as models in a series of toxicity tests, which include acute oral toxicity tests, oral subchronic toxicity, chronic oral

toxicity, teratogenicity, skin sensitization, eye irritation, acute dermal irritation, vaginal mucosal irritation, acute dermal toxicity, subchronic dermal toxicity, and carcinogenicity tests. Toxicity tests on test animals are one of the supporting pieces of evidence for the safety of test preparation. The choice of the test depends on the intended use of a substance and the possible risk of exposure to humans (Control, 2020).

Dermal acute irritation is a test on animals (albino rabbits) to detect toxic effects that appear after exposure to the test preparation on the dermis for up to 4 hours. The principle of acute dermal irritation is the exposure of the test preparation in a single dose to the skin of the test animal, with the untreated skin area functioning as a control. Dermal acute irritation aims to determine the presence of an irritating effect on the skin and to assess and evaluate the characteristics of a substance when exposed to the skin (Control, 2020). (Nur, 2019; S, I, & Susanti, 2009)

Table 4. The result of scoring dermal acute irritation test

Formula	Resulting Erythema and Edema							
	1 hour		24 hours		48 hours		72 hours	
	E*	O*	E	O	E	O	E	O
Positive Control (Ambroxol syrup)	0	0	0	0	0	0	0	0
Negative Control (Propilen glikol)	0	0	0	0	0	0	0	0
Sample 1 (Ambroxol solution 15 mg /5 ml)	0	0	0	0	0	0	0	0
Sample 2 (Ambroxol solution 30 mg /5 ml)	0	0	0	0	0	0	0	0
Average								0

*E= Erythema O:Oedema

An irritation test is a method used to evaluate the irritation potential of a chemical on the skin or mucous membranes. This method assesses whether a material is safe for humans or animals. Chemicals such as cosmetics, drugs, pesticides, and cleaning agents are generally tested using this method before being sold to the public. The irritation test is carried out by exposing the skin or mucous membranes to the chemical test for a certain period of time. During this period, possible reactions such as redness, swelling, and itching of the skin or mucous membranes were observed. The response is then assessed and categorized as non-irritant (non-irritating), mild irritation, moderate irritation, or severe irritation.

This study aims to determine the irritating effect of the prepared solution after it is used on rabbits so that the safety level of the

ambroxol solution can be determined. Irritation is an inflammatory phenomenon in the skin due to foreign compounds (S, I, & Susanti, 2009). The symptoms that can occur are erythema and edema. Erythema is a reddish reaction on the skin that arises. At the same time, edema is a reaction to swelling of the skin due to the side effects of topical preparations (Nur, 2019).

The results of the acute dermal irritation test of ambroxol solution showed that in the first rabbit, second rabbit, and third rabbit, there was no erythema or edema at each observation point at 1 hour, 24 hours, 48 hours, and 72 hours, both in the treatment sample and control treatment, so that the calculation of the primary irritation index obtained the results of scoring erythema and edema at 1 hour, 24 hours, 48 hours, and 72 hours was 0. When viewed from the response category of the primary irritation index, this value is included in the category as non-irritating to the skin. These results indicate that the ambroxol solution is classified as harmless when used topically.

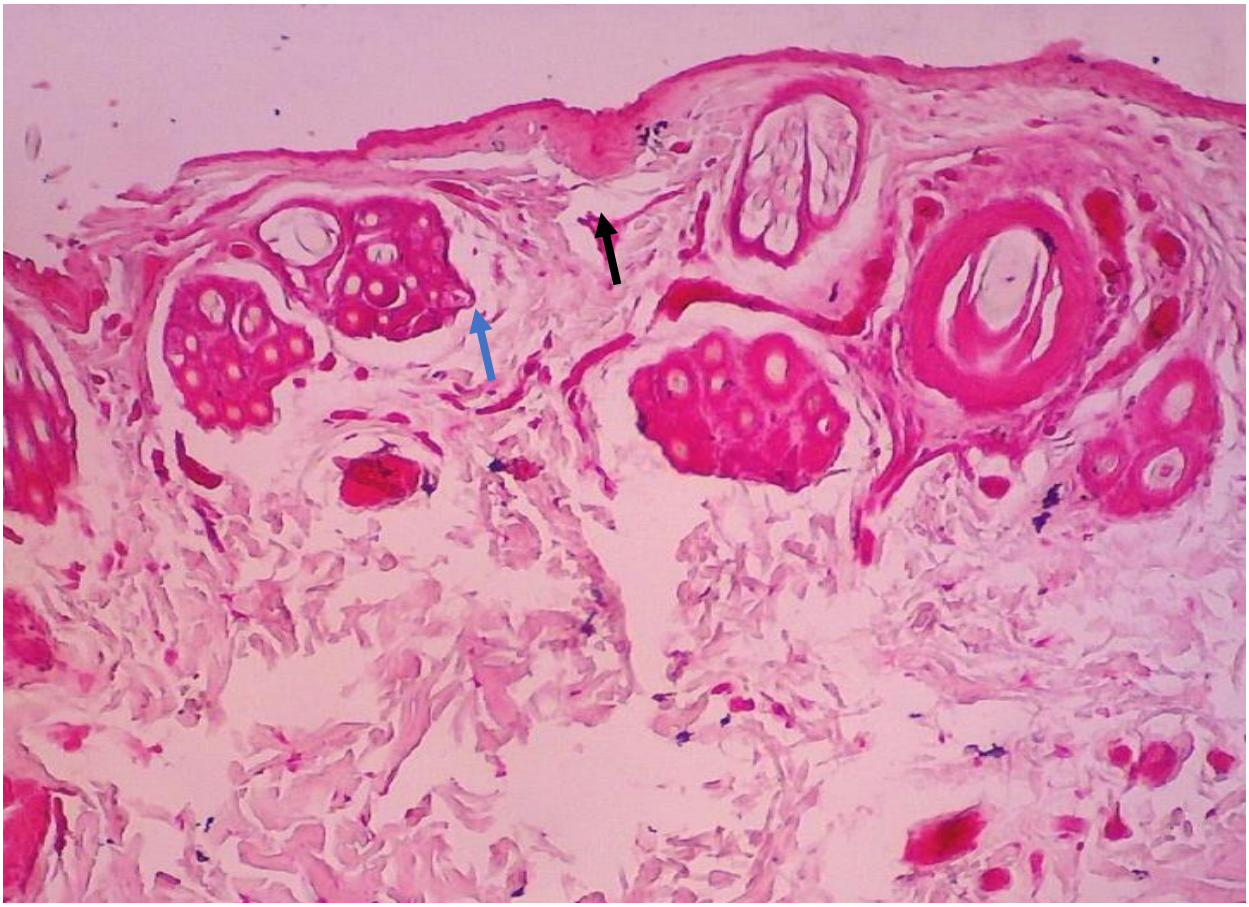


Figure 1. Histopatological Staining Results Hematoxylin-Eosin; edema (blue arrow), erythema (black arrow). Scale bar 100µm.

Different from histopathological testing, a histopathological examination was also carried out to see if there were any abnormalities in the organs caused by the administration of the test preparation. This test uses ambroxol solution at a dose of 30 mg/5 ml. The histopathological examination results showed that the erythema formed was in a severe category; when seen from the skin reaction assessment table, it could be given a score of "4". From the results of the scoring that has been done, the results of the irritation test microscopically on ambroxol solution at a dose of 30 mg/5 ml are irritating. The presence of erythema and edema appearing on the rabbit skin samples that were carried out in histopathological tests in this test could occur due to the shaving performed on the experimental animals; the rabbit's skin was scratched so that the skin was injured; this means that the first barrier of the skin is disturbed, and the skin that is chafed does cause permeability to increase. The sensitivity of the skin of experimental animals is slightly different from human skin. Especially for rabbits, the level of irritation is very easy to see. The irritation index in humans is still uncertain, as is the value in experimental animals (Fatmawaty, 2016).

Ambroxol is a new agent for the treatment of biofilm-associated infections by inhibiting the formation of biofilms on pathogens, so it has the potential to be applied in topical preparations to treat biofilm-related infections (Hidayati, 2019). One of the infectious diseases caused by biofilms is diabetic foot ulcers caused by Proteus mirabilis. By inhibiting the formation of biofilms and removing the previously formed biofilms, ambroxol can be used to treat diabetic foot infections caused by Proteus mirabilis. Although ambroxol is not available in topical preparations, as a first step, it is necessary to carry out an acute dermal irritation test to see the skin's response (Abbas H. A., 2014).

CONCLUSION

Ambroxol solution with a dose of 15 mg/5 ml and a dose of 30mg/ml in the macroscopic irritation test did not appear to irritate the skin of albino rabbits with a primary irritation index result of 0. In contrast, in the microscopic irritation test, it could be concluded that the ambroxol solution with a dose of 30 mg/5 ml was irritating.

SUGGESTIONS

The researcher hopes this research can be continued by making formulations of ambroxol preparations into creams to facilitate ambroxol penetration into the wound tissues.

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