

Safety Test of Agarwood Leaves Tea (*Aquilaria malaccensis* Lamk.) Through Skin Sensitization Test on Albino Rabbit

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Abstract

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BACKGROUND: Agarwood tree (*Aquilaria malaccensis* Lamk) is a tree from the Thymelaeaceae tribe that has a high selling value. The part that is often used is the sapwood of the agarwood tree, used as a base for perfumes and traditional medicines. Agarwood farmers in Langkat have used their leaves as tea drinks, brewing aloes leaves. Before being widely produced by the community, it is necessary to test the safety of agarwood leaf tea products through skin sensitization test.

AIM: To find out whether steeping aloes leaves irritate the skin or not.

METHODS: The test method using test animals namely albino rabbits as many as 15 tails, consisting of 4 test groups 1 group there were 3 test animals and 1 control group, induced with Freund's Complete Adjuvant (FCA) by intradermal and topical injection with a test position of 1.3%, 2.6%, 3.9% and 5.2% to form an immune response, then a Challenge test. The level and degree of skin reaction were assessed based on the Magnusson and Kligman scales.

RESULTS: From the observation for 72 hours there was no change in the skin of the test animals after exposure to the test doses, indicating that the agarwood leaf tea is safe to consume if the tea is on the skin, the skin will not experience irritation.

CONCLUSION: Testing on albino rabbits with four different doses did not show an irritating effect on the skin. Observation also shows that there is not a single bit that can affect intradermal to challenge testing so it was safe for consumption.

Introduction

Agarwood, known as gaharu, eaglewood or karas is wood that has a distinctive aroma and is of high selling value used for incense, perfume and traditional medicines. Agarwood comes from tropical trees in the genus *Aquilaria malaccensis* Lamk. (Thymelaeaceae) [1]. The genus *Aquilaria* is spread through South Asia from India to China and many in Indonesia.

All parts of the Agarwood tree can be used and are beneficial especially in the field of medicine. Agarwood has anti-cancer properties and anti-depression in its extract [2], [3]. In addition, agarwood leaves are also anti-inflammatory and

hepatoprotective [4]. Other studies also show leaf agarwood has the potential for anti-diabetes [5].

One of the uses of agarwood leaves are to be processed tea products. Agarwood farmers in Langkat (North Sumatra) have used their leaves as brewed tea drinks, so that tea products can be consumed by the community, so we need to test the level of product safety. Food safety in a food product is needed in improving food quality. Foods that are not safe can cause diseases called foodborne diseases, which are symptoms of diseases that arise as a result of consuming foods containing toxic substances or compounds or pathogenic organisms.

For this reason, it is necessary to conduct research on the safety test of gaharu tea products, to

determine the safety level of products from agarwood tea leaves through skin sensitization test.

This study aims to determine whether agarwood leaf tea contains substances that have the potential to cause sensitization to the skin and as information to the public about the safety of agarwood leaves tea products.

Material and Methods

Materials

Agarwood leaves (*Aquilaria malaccensis* Lamk) from Bahorok village, Langkat Regency (North Sumatra, Indonesia), albino rabbits, aquades, NaCl and FCA (Freunds complete adjuvant), Tests on test animals were carried out at the Pharmacology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara.

Preparation of test

Preparation of test was made aseptically.

The sample is half solid

- Sheet: the test preparation is cut to a size of 2.5 x 2.5 cm with a thickness of no more than 0.5 cm. For control can be used non-irritant sterile gauze.
- Solid (solid): the test preparation is made of powder, then moistened to form a paste with water or a suitable non-irritant solvent.
- Liquid sample: the preparation does not need to be diluted, but if necessary, the solid sample is diluted with the appropriate non-irritant solvent.

Test Dose

The test dose used was divided into 4 doses, namely 1.3%, 2.6%, 3.9%, and 5.2%. As for the challenge test, the highest dose should not cause irritation. The right dose for the test was determined by a preliminary test using FCA (Freunds complete adjuvant) in 3 animals.

Test Animals

The animals used for the test were young and healthy adult albino rabbits, weighing 300 - 500 g, male and female, and if using female test animals, they must be nulliparous and not pregnant. The number of animals was 15 animals, consisting of 4 treatment groups, each group with 3 test animals and

3 control groups.

Preparation of Test Animals

Before the test begins, the test animals was acclimatized in the experiment room for approximately 5 days and the animals was randomly grouped. Animals (rabbits) was shaved 24 hours before testing begins, for intradermal and topical induction, in the intrascapular region $\pm 4 \times 6 \text{ cm}^2$ and for the challenged test shaved on the back area (flank) $\pm 5 \times 5 \text{ cm}^2$. Hair removal can also use thresher chemicals, but must be maintained so as not to cause cuts or abrasions on the skin.

Preliminary Test

The preliminary test aims to determine the test dose that will be used in the main test. The preliminary test uses 2-3 animals. Intradermal Induction (day 0): A total of 0.1 ml of 50% FCA mixture was injected into the nape of each rabbit in areas A, B, C. Injections A and B were close to the head, while area C was close to the tail.

Main Test

a. Intradermal Induction Phase (day 0)

Intradermal induction: the following ingredients are injected in the nape of each rabbit

- Region A: 0.1 ml *FCA 50% (FCA / NaCl 1: 1 v/v)

- Region B: 0.1 ml of test preparation with concentration according to the results of the preliminary test

- Region C: 0.1 ml of the test preparation: FCA 50% (1: 1, v: v)

** FCA is dissolved with sterile physiological NaCl. If the test preparation was not water soluble, then the FCA is diluted with the appropriate solvent.

** The dose of the test preparation in area C is the same as the dose in area B for the same control and for negative control the sample was replaced with a solvent.

b. Topical Induction Phase (day 7)

The topical induction phase was carried out 7 days after the intradermal induction phase. 24 hours before treatment the nape of the rabbit was shaved again. On the trial day, 0.5 g was applied to the paste preparation and 0.5 ml of the liquid preparation with the dose obtained from the preliminary test results on filter paper size 8 cm^2 ($2 \times 4 \text{ cm}^2$), then the paper was affixed to the nape of each rabbit so that covering the intradermal injection site and covering it with occlusive dressing was then wrapped with elastic bandage, after 48 hours was opened and observed. If it did not show

irritation, then topical was repeated and 24 hours before the nape of the neck was smeared with 0.5 ml dodecylsulfate sodium 10% and covered with occlusive dressing then coated with elastic bandage, after 48 hours has been opened and observed. The same was done for the control, but the sample was replaced with a solvent.

c. Challenge Test (day 21)

Challenge tests were carried out 14 days after topical induction of the entire test group, which was shaved 24 hours earlier on the back. The exposure of the test preparation should not be on the part of the topical (nape) induction, the test preparation was presented topically in the C area on the shaved rabbit's back, then covered with occlusive dressing and wrapped with elastic bandage. The same was done on the controls. Challenge tests need to be carried out in the control group to ensure that the reactions that occur were correct sensitization reactions were not an irritant reaction. After 24 hours, occlusive dressing and elastic bandage were opened, observed and recorded for edema and erythema at 24, 48 and 72 hours.

Observation

Skin reactions were described and categorized against erythema and edema according to the Magnusson and Kligman scales. Additional notes can be made if unusual responses were found. The irritation observation score can be seen in Table 1 below.

Table 1: Magnusson and Kligman scales

Topical reaction	Score
Invisible Changes	0
Mild erythema	1
Moderate erythema	2
Severe erythema and edema	3

Results

Test Results on Preliminary Skin Tests

The results of the preliminary test for 7 days did not reveal any toxic symptoms and death after exposure to the test dose. The test group and the control group showed that the skin had not changed. Thus, the dose of 1.3%, 2.6%, 3.2% and 5.2% can be continued to the main test.

Intradermal Induction Phase (day 0)

In the intradermal induction test 0.1 ml the test preparation was mixed with 50% FCA, with a ratio

of 1: 1 then injected into the nape of each rabbit in areas A, B, C. Injections of area A and B close to the head while area C near with a tail. Observations were made after 7 days and continued with topical induction.

Topical Induction

In topical induction various concentrations of the sample were applied to the nape of the rabbit, the test material was made into a semi-solid sample and a liquid sample. A half-solid sample was made from the test material of agarwood leaf extract which was made into a paste form with a mixture of water and applied to rabbits by sticking to the skin using sterile gauze. While the liquid sample of agarwood extract material made into a solution with the help of water was applied by using a sterile gauze that had been dripped with the test material and then attached to the test animal. This induction observation was done after 24 hours.

From the results of testing in the intradermal induction phase it was found that there were no changes in all test animals with different doses. This shows that there was no effect of agarwood leaves extract on intradermal induction testing. Food that can be consumed by the public must be in the criteria of being healthy and safe according to Republic of Indonesia Law No. 7 of 1996 concerning Food, namely the conditions and efforts needed to prevent food from the possibility of biological, chemical, and other objects that can interfere with, harm and endanger human health [6].

By carrying out the injection of Freund's Complete Adjuvant (FCA) in the test animals it is hoped that an inflammatory reaction will occur on the skin of the rabbit before proceeding to topical induction. Injection is done subcutaneously, namely injection under the skin so that the antigen is injected indirectly into the bloodstream. Adjuvants also cause the antigen to be released slowly into the bloodstream because antigens are trapped in adjuvant emulsions so that the immune response takes longer.

Topical Induction Phase (day 7)

The topical induction phase was carried out 7 days after intradermal induction. In this experiment, 0.5 g was applied for the paste preparation and 0.5 ml for the liquid preparation at a dose of 1.3% 2.6% 3.9% 5.2% on filter paper size 8 cm² (2 x 4 cm²). For the liquid preparation 0.5 ml was taken from each dose and then smeared on filter paper and for pasta paste preparation the paper was attached to the nape of each rabbit so that it covered the intradermal injection site and closed it with occlusive dressing wrapped in elastic bandage.

Test results from doses of 1.3%, 2.6%, 3.9%, 5.2% showed that there were no changes and

symptoms of irritation in rabbit skin. Likewise, after administration of a paste preparation also does not show any irritation to the skin of the rabbit. After the topical induction phase with the test preparation with a time of 48 hours it was found that the induction did not show any skin changes in the area exposed by the test preparation, the results of the 48-hour topical induction phase can be seen in Table 2.

Table 2: Data on topical induction phase with a test preparation for 48 hours

Group	Test Animals	Score	Information
Dose 1,3	Rabbit 1	0	Invisible Change
	Rabbit 2	0	Invisible Change
	Rabbit 3	0	Invisible Change
Dose 2,6	Rabbit 1	0	Invisible Change
	Rabbit 2	0	Invisible Change
	Rabbit 3	0	Invisible Change
Dose 3,9	Rabbit 1	0	Invisible Change
	Rabbit 2	0	Invisible Change
	Rabbit 3	0	Invisible Change
Dose 5,2	Rabbit 1	0	Invisible Change
	Rabbit 2	0	Invisible Change
	Rabbit 3	0	Invisible Change
Control	Rabbit 1	0	Invisible Change
	Rabbit 2	0	Invisible Change
	Rabbit 3	0	Invisible Change

Note: 0 = Invisible Changes; 1 = Mild erythema; 2 = Moderate erythema; 3 = Severe erythema and edema.

Challenge Test (day 21)

Challenge tests were carried out 14 days after topical induction of all test groups, exposure to topical tests was presented in area C so that a comparison between irritated skin and skin that had no changes was seen, there was no irritation in the topical test.

In this test, using 12 rabbits where each level of dose given was 0.65 g/50 ml, 1.3 g/50 ml, 1.95 g/50 ml and 2.6 g/50 ml in the form of percent, namely 1.3%, 2.6%, 3.9% and 5.2%. The results of topical induction of direct exposure to rabbit skin do not irritate rabbit skin.

The results of observations can be seen in Table 3. Observations made for 3 consecutive days. This value indicates that after administration of the test application of agarwood leaves tea on the type of albino rabbit (*Oryctolagus cuniculus*) with four different doses, namely 1.3%, 2.6%, 3.9%, and 5.2%, where there was no sensitization on the skin.

Table 3: Data on Skin Sensitization Observation Results

Dose	Rabbit	Day 1	Day 2	Day 3	Score
		Invisible Change	Invisible Change	Invisible Change	
1,3 %	Rabbit I	Invisible Change	Invisible Change	Invisible Change	0
	Rabbit II	Invisible Change	Invisible Change	Invisible Change	0
	Rabbit III	Invisible Change	Invisible Change	Invisible Change	0
2,6 %	Rabbit I	Invisible Change	Invisible Change	Invisible Change	0
	Rabbit II	Invisible Change	Invisible Change	Invisible Change	0
	Rabbit III	Invisible Change	Invisible Change	Invisible Change	0
3,9 %	Rabbit I	Invisible Change	Invisible Change	Invisible Change	0
	Rabbit II	Invisible Change	Invisible Change	Invisible Change	0
	Rabbit III	Invisible Change	Invisible Change	Invisible Change	0
5,2 %	Rabbit I	Invisible Change	Invisible Change	Invisible Change	0
	Rabbit II	Invisible Change	Invisible Change	Invisible Change	0
	Rabbit III	Invisible Change	Invisible Change	Invisible Change	0
Control	Rabbit I	Invisible Change	Invisible Change	Invisible Change	0
	Rabbit II	Invisible Change	Invisible Change	Invisible Change	0
	Rabbit III	Invisible Change	Invisible Change	Invisible Change	0

Ket: 0 = Invisible Changes; 1 = Mild erythema; 2 = Moderate erythema; 3 = Severe erythema and edema.

Discussion

The results of the observation showed that there was no slight change in the rabbit's skin after being treated with the intradermal induction phase until the challenge test. This test proves that agarwood tea was safe for consumption, because it does not give any effect on the skin topically.

From these results it can be said that agarwood leaves tea did not release chemicals contained in the test preparation that are irritating to the skin or in other words the leaves of agarwood (*Aquilaria malaccensis* Lamk.) were not irritating to the skin of the rabbit's back. Skin sensitisation remains a key endpoint in the toxicological evaluation of chemicals [7], including natural chemicals. Recent changes in regulatory requirements and social views on animal testing have accelerated the development of reliable alternative tests for predicting skin sensitizing potential of chemicals. Develop a new in vitro assay that could predict the skin sensitizing potential of chemicals by measuring ROS production in THP-1 (human monocytic leukemia cell line) cells [8], the local lymph node assay (LLNA) [9]. Accordingly, the test methods used to identify these effects in terms of hazard and risk remain an important focus for toxicologists.

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