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Toxicity test of cream from an extract mixture of turmeric and tamarind leaves as antiaging

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Abstract

Cream from a mixture of turmeric and tamarind leaves extract needs to be tested for its toxicity to really make sure it doesn't have side effects. The purpose of this study was to test the toxicity of a mixture of turmeric and tamarind leaves extract cream using Wistar rats. This study used rats as experimental animals which were carried out in 2 stages, namely preliminary tests using 5 groups of experimental animals. After adaptation the experimental animals were given cream doses of 50, 200, 1000, 2000 mg/kg bw. The second stage was carried out after the results of the first stage showed symptoms of toxicity and death. The second stage used two groups of rats (3 each), namely the treatment group using the largest dose of cream that did not cause death and the control group which was a placebo. In addition, observations of collagen content were also carried out. The results showed that the turmeric and tamarind extract cream did not have toxic properties. The skin of rats that were given turmeric and tamarind extract cream produced higher collagen content and was different from the other groups.

Keywords: Turmeric extract; tamarind leaves extract; cream; toxicity; Antiaging

1. Introduction

Currently the development of antiaging creams has led to the use of natural ingredients, because the use of chemicals tends to have side effects. One of the creams being developed as anti-aging is a cream made from an extract mixture of turmeric and tamarind leaves [1]. Mulyani et al [2] showed that a cream was made from an extract mixture of turmeric and tamarind leaves has the ability as an anticollagenase and antioxidant, with a vitamin C content of 3.5 mg/100 g and phenolics up to 0.12 mg GAE/ml. Phenolic compounds with a range of 0.05-0.26 mg GAE/ml, have great potential as antiaging [3].

The use of cosmetic products including creams really needs to be tested for toxicity, even though they are made from natural ingredients. This is very important because every skin that is smeared with cream has a different sensitivity. Therefore, the cream was made from an extract mixture of turmeric and tamarind leaves needs to be tested for its toxicity to make sure it doesn't have side effects. The purpose of this study was to test the toxicity of an extract mixture cream of turmeric and tamarind leaves using rats.

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2. Material and methods

2.1. Material

Research materials include turmeric, tamarind leaves, ethanol, stearic acid, triethanolamine (TEA), Virgin Coconut Oil (VCO), mineral oil, cetyl alcohol, methyl paraben, propyl paraben, sodium metabisulfite, EDTA, aquadest, moisture conditioner, Wistar rats aged 3-4 months weighing 200-250 grams, animal feed, placebo cream (base cream). The equipment used includes complete mouse cage.

2.2. Method

2.2.1. Making turmeric and tamarind leaves extract cream

Cream is prepared by mixing the components according to the formula in Table 1 and stirring them homogeneously at 65 °C.

Table 1 Turmeric and tamarind leaf extract cream formula

Material	Weight (g)
Stearic Acid	10.9
VCO	3.64
Mineral oil	2.27
Cetyl Alcohol	0.91
Span 80	2.80
Tween 80	2.20
Moisturizer conditioner	10
Turmeric and tamarind leaf extract	0.2
Water addition up to	100

2.2.2. Toxicity testing of turmeric and tamarind leaves extract cream

This study used experimental animals of female white rats of the Wistar strain. Rats are in good health, aged 3-4 months weighing 200-250 grams, nulliparous and not pregnant. The animals were acclimatized for one week in an experimental room in an individual cage, with a base area of 300 cm^2 and a height of 18 cm that was given husk base with wire cover. The room temperature ranges from 25°C, humidity 60-70 %, and given a lighting cycle of 12 hours dark/light. The animals are given laboratory-standard commercial pellet feed as much as 20 grams / each / day and provided with ad libitum drinking water, as well as a minimum stress environment. All cages and experimental animals were numbered and randomized before the treatment began. These conditions have met the welfare of the experimental animals according to the National Research Council [4].

One day prior to the test, the hair was shaved on the dorsal/flank area of 6x8 cm (at least 10 % of the body surface area) for the place of exposure of the test material. In the process of shearing the experimental animals, anesthesia is given Ket-A-Xyl® 0,1 ml/each, and done carefully so as not to cause damage / injury to the dermis. Only animals with healthy, intact skin were used in the study [5].

The phase of dermal acute toxicity test begins with preliminary tests using 5 groups of experimental animals. After adaptation, experimental animals were given cream doses of 50, 200, 1000, 2000 mg / kg bw. Dermal test procedure based on the Globally Harmonized System [6]. The cream is applied to the specified skin, covered with sterile gauze, and bandaged with a non-irritating plaster. This is to ensure that the test material remains in contact with the skin for 24 hours and there is no chance of the test material being eaten by the animals. At the end of the exposure period, the plaster is opened and the remaining test material is rinsed using aquadest or another neutral solvent. Observations include; clinical assessment, irritation, pathology observation as well as animal mortality on the 0, 6^{th} , 12^{th} , 24^{th} , 36^{th} , 48^{th}, and 72^{th} hours after exposure to the test cream. If the tested animal experiences death or severe toxicity symptoms, it is necropsy and sacrificed using chloroform, then continued with testing using the second stage. The second stage is

carried out after the results of the first stage show symptoms of toxicity and death. The second stage used two groups of rats (3 each), namely the treatment group (TG) using the largest dose of cream that did not cause death and the control group (CG) which was a placebo. The cream is applied daily and observed at 12th, 24th , 36th hours then day 2 to day 14.

Determination of LD50 test preparations based on the Globally Harmonized System of Classification and Labelling of Chemicals [6]. Assessment and determination of irritation index based on Amended Draize Test. The scoring using an arbitrary scale i.e., no erythema: 0, very mild erythema: 1, clearly visible erythema: 2, moderate to severe erythema: 3 and severe erythema: 4. No edema: 0, very mild edema: 1, edema clearly visible: 2, moderate edema: 3 and severe edema: 4. Primary irritant index classification i.e. Non-irritating: <0.5; Mild irritation (slightly irritating): 0.5-2.0; Moderate irritation (Moderately irritating): 2.0-5.0; and Highly irritating: 5.0-8.0 [7]. In addition, observations of collagen content were also carried out.

3. Results and discussion

3.1. The toxicity of the turmeric and tamarind leaves extract cream

The results of toxicity testing of turmeric extract and tamarind leaves cream in the first phase showed that up to 2000 mg/kg bw application showed no signs of poisoning and allergy to rat skin until the 24th hours as shown in Table 2. Similarly, the results of toxicity testing with the application of turmeric extract and tamarind leaves cream at a dose of 2000 mg/kg bw every day for 14 days the rats skin did not experience allergies, this is the same as placebo treatment, as shown in Table 3.

The absence of symptoms of poisoning in experimental animals is likely due to all the ingredients forming turmeric extract and tamarind leaves cream are natural ingredients that do not have toxic properties. This is according to the results of research by Utami et al [8] which shows that *nipah* leaves ethanol extract cream contains flavonoids, tannins, saponins and steroids / triterpenoids which have an LC_{50} value of 1140 ppm which means that *nipah* leaves extract cream has no toxic effects.

Table 2 The results of the first phase of toxicity testing

Dose (mg/kg bw)	Hour 0	Hour 6	Hour 12	Hour 18	Hour 24
0	ud	ud	ud	ud	ud
300	ud	ud	ud	ud	ud
800	ud	ud	ud	ud	ud
1000	ud	ud	ud	ud	ud
2000	ud	ud	ud	ud	ud

ud: undetectable

Table 3 The results of the second stage of toxicity testing

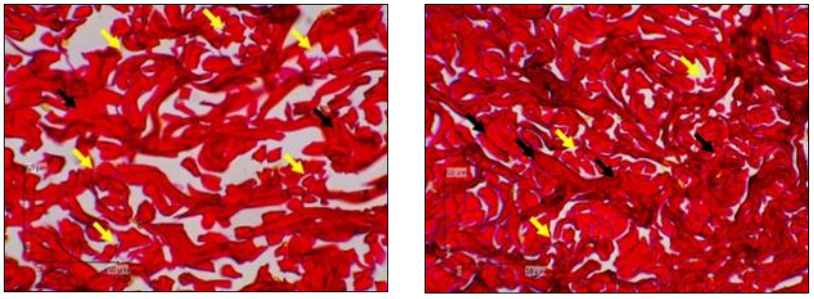
Group	Hour 12	Hour 24	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Placebo	ud	ud	ud	ud	ud	ud	ud	ud	ud
Treatment	ud	ud	ud	ud	ud	ud	ud	ud	ud

ud: undetectable

3.2. Collagen content

The results of this study showed that the skin of rats that were given turmeric and tamarind extract cream produced higher collagen values and was different from the other groups. The amount of collagen in the dermis of rats is presented in Figure 1. Figure 1b shows that the collagen of the rat dermis has a thin red fiber structure. Meanwhile, those that

were smeared with a mixture of turmeric and tamarind leaf extract cream had collagen with a thick fiber structure indicated by black arrows in Figure 1b.



a b

Figure 1 Skin collagen content of rats (a) control, (b) smeared with cream from an extract mixture of turmeric and tamarind leaves

According to Savitri and Efendi [9], the presence of antioxidant compounds in creams causes inhibition of collagenase enzyme activity, and prevents free radicals so that collagen damage is inhibited and actually increases collagen content. According to Bissett et al. [10], creams containing antioxidants such as vitamins, polyphenols and flavonoids can reduce collagen degradation by reducing the concentration of FR in tissues. Meanwhile, the presence of cell regulators can improve collagen metabolism so collagen production increases [11].

4. Conclusion

The results showed that the turmeric and tamarind extract cream did not have toxic properties. The skin of rats that were given turmeric and tamarind extract cream produced higher collagen content and was different from the other groups

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest between the authors and any other party in this publication

Statement of ethical approval

The authors state that this study has received approval from the ethical commission.

Statement of informed consent

The authors state that this study does not require informed consent, because it uses experimental animals

References

[1] Mulyani S. Antioxidant synergism of turmeric and tamarind leaves (*Curcuma domestica* Val. - *Tamarindus indica* L.) as active ingredients in cream. Dissertation. Doctoral Study Program of Agricultural Sciences, Faculty of Agriculture, Udayana University. Denpasar. 2017

[2] Mulyani S, Harsojuwono BA, Antara NS, Kencana IN. 2016. An assessment of antioxidant characteristics from different ratios of turmeric and tamarind (*Curcuma domestica* Val.-*Tamarindus indica* L.) leaf extracts. Australian Journal of Basic and Applied Sciences, 2016; 10(14):347-353. www.ajbasweb.com/old/ajbas/2016/September/347-353.pdf

[3] Thring TSA, Hili P and Naughton DP. Anticollagenase, anti-elastase and anti- oxidant activities of extracts from 21 plants. BMC Complementary and Alternative Medicine. 2009; 9:27. http://www.biomedcentral.com/1472-6882/9/27

[4] NRC. Nutrient requirements of fish and shrimp. National Academies Press, Washington DC, USA. 2011

[5] OECD. Guidelines for the testing of chemicals, acute dermal irritation/corrosion. Publication EHaS, Paris. 2015.

[6] UN. Globally harmonized system for the classification and labeling of chemicals. Fourth ed. Unece.org. New York. 2011.

[7] Baldisserotto A, Buso P, Radice M, Dissette V, Lampronti I, Gambari R, Manfredini S and Vertuani S. Moringa oleifera leaf extracts as multifunctional ingredients for “natural and organic” sunscreens and photoprotective preparations. Molecules. 2018; 23(664):1-16. doi:10.3390/molecules23030664

[8] Utami TFY, Setia DA, Nurfatimah R. Evaluation of cream preparations and acute toxicity test of nipah leaf extract (*Nypa fruticans* Wurmb) Cilacap Region with VCO oil phase as an antibacterial candidate. Indonesian Journal of Pharmacy and Natural Products. 2022; 5(1): 82-90. http://jurnal.unw.ac.id/index.php/ijpnp

[9] Savitri ES and Efendi A. Antioxidant activity and inhibition of the silver nanoparticle collagenase enzyme using red algae (*Gracilaria verrucosa*). Proceedings of SEMNAS BIO 2021, Padang State University. ISBN : 2809-8447

[10] Bissett DL, Miyamoto K, Sun P, Li J, Berge CA. Topical niacinamide reduces yellowing, wrinkling, red blotchiness, and hyperpigmented spots in aging facial skin. International Journal of Cosmetic Science. 2004; 26:231-8. http://dx.doi.org/10.1111/j.1467-2494.2004.00228.x

[11] Ganceviciene R, Liakou AI, Theodoridis A, Makrantonaki E and Zouboulis CC. Skin anti-aging strategies. Dermato-Endocrinology. 2012; 4(3):308–319. http://dx.doi.org/10.4161/derm.22804