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Assessment of dermal and ocular irritation potential of soap from extracts of leaves of *Morinda morindoides*

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Abstract

Dermal and ocular irritation potentials of soap from extracts of leaves of *Morinda morindoides* codified "Morinda" were assessed using *in vivo* acute irritation tests in albino rabbits. Irritation risk of this soap Morinda was performed according conventional test for assessing skin and eye irritation potentials of chemicals with Draize rabbit test. For skin irritation test, 0.5 g dose of soap Morinda was applied to intact and abraded skin approximately 2.5×2.5 cm square of six albino New-Zealand rabbits and left in place for 24 hrs. Acute eye irritation test was carried out in nine albino rabbits. Soap Morinda at concentration of 50 mg/mL amount 0.1 mL was once placed in conjunctival sac of left eye of each animal after gently pulling the lower lid way from eye ball. Soap Morinda was non-irritating on rabbit skin test with a primary irritation index of 0.125. For eye irritancy test, this soap was weakly irritating with index of acute ocular irritation index of 2.66. Results from the present study suggest that soap Morinda made from hexane extract of *M. morindoides* as antimicrobial agent have promising potential medicated soap that do not induce significant levels of skin and eye irritation.

Keywords: *Morinda morindoides*, antimicrobial soap, skin irritation, eye irritation, Draize rabbit test

Introduction

Medicinal plants have long been subject of investigations concerning their biological and pharmacological properties by scientists. Several virtues of these plants have been discovered such as antidiabetic, antidiarrheal, anti-hypertensive, anti-inflammatory, antiparasitic, laxative, antimicrobial, etc. [1-2-3]. However valorization of results of these research works is a major challenge for scientific world. In this perspective our research team is engaged in development of various products based on properties of studied plants. Thus the soap codified "Morinda" was formulated with hexane extract of *Morinda morindoides* as antimicrobial agent. This soap tested against pathogenic microbial involved in skin infections revealed an effective antimicrobial power [4-5]. Before used this product, evaluation of eye and skin irritation potential is essential to ensure the safety of humans in contact with a wide variety of substances [6]. The present study aimed to investigate the tendency of soap containing hexane extract of *M. morindoides*, soap Morinda to cause eye and skin irritation.

Materials and Methods**Collection of plant Material**

Leaves of *M. morindoides* (Rubiaceae) were collected from Daloa (central west region of Côte d'Ivoire). The plant was identified and authenticated with voucher specimen no. 17710 in herbarium of National Floristique Center of University Felix Houphouët-Boigny (Côte d'Ivoire).

Preparation of extract

Leaves of *M. morindoides* were cleaned of extraneous matter, air-dried at room temperature for 7 days and ground into a fine powder. For each extraction, 100 g of dry powdered plant material was extracted with 250 ml of hexane (Merck, Darmstadt, Germany) for 24 h using a Soxhlet extractor. Extract was filtered with Whatman filter paper no.1, and filtrate was evaporated under vacuum in a rotary evaporator (Buchi) at 55°C. A greenish paste obtained with a yield of 9.24±0.18% is hexane extract [7].

Preparation of soap

The soap codified "Morinda" was obtained with the cold method by adding 2 mixtures A and B. Mixture A was obtained by dissolving 16.135 g of sodium hydroxide crystals in 59.58 g of

distilled water. To sodium hydroxide solution obtained after 24 hours were added 1.5 g of sodium chloride and 1.2 g of sodium bicarbonate at the time of use. The mixture B which will be used as fat in preparation of soap consists of 50 g of coconut oil and 50 g of palm oil. Mixture A was gradually added with stirring to mixture B. The mixture (A + B) obtained was homogenized until a viscous mass (tracing) was formed. To obtain soap Morinda, 10 g of hexane extract of *M. morindoides* was added to 90 g of soap mass obtained (basic soap) and then homogenized. Homogeneous mass obtained was poured into the molds. After 24 hours, soap Morinda was obtained with greenish-colored [8-9].

Animal care and use

New-Zealand albino rabbits (weighing between 2 and 3 kg) were purchased from Bingerville (Côte d'Ivoire). Among these rabbits, six were used for skin irritancy test and nine over for eye irritancy test. Each animal was housed in a stainless steel cage during the acclimatization and experimental periods. The animals were kept under standard laboratory conditions, at 18-22°C and 52-55% relative humidity with a 12 h light/dark cycle. The animals were free access to a standard laboratory diet and water ad libitum. All animals were acclimated to the laboratory environment for at least one week prior to the commencement of the experiments [6-10].

Skin irritancy test

For the skin irritation test, the dorsal back sides of six rabbits were clipped free of fur at least 4 hr before application of the soap Morinda. Just prior to test soap application, each rabbit received three parallel epidermal abrasions with eyebrow razor while the opposite site remained intact. The 0.5 g dose of soap was applied to intact and abraded skin approximately 2.5×2.5 cm square of six rabbits and left in place for 24 hrs. Separately, 0.5 mL of physiological saline was applied for the control in the same way as the test soap. The entire trunk of animals was wrapped with non-irritant adhesive tape to protect the test and control sites. After a 24 hr exposure, the applied sites were wiped with the physiological saline to remove soap. The primary irritation index (P.I.I.) was calculated with the scores of erythema and edema (Table 1) obtained at 24 and 72 hr observation after soap application. If the P.I.I. score of greater than or equal to 5.0, the soap Morinda would be considered positive and a primary irritant to the skin [6-11].

Eye irritancy test

Healthy nine male rabbits were divided into two groups. First is rinsed eye group having three animals, then remain of six were non-rinsed eye group. The soap Morinda at concentration of 50 mg/mL was once (amount of application were 0.1 mL) placed in the conjunctival sac of left eye of each animal after gently pulling the lower lid way from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the test soap. The untreated eye was used as control. Three animals were rinsed with sterilized saline after the soap application.

The eyes were examined at 1, 2, 3, 4 and 7 days after soap application. The grades of ocular reaction were recorded at each examination according to the grades (Table 2) proposed by Draize *et al.* (1944) [13]. Based on the number of lesions observed in the conjunctiva, iris and cornea of each animal, an Individual Index of Ocular Irritation (I.I.O.I.) was calculated for each observation time. A mean score for each part of the

eye examined was also calculated; the sum of these scores equaled the Mean Index of Ocular Irritation (M.I.O.I.) for each observation time. The largest mean index over the 7 days observation period was considered the Index of Acute Ocular Irritation (I.A.O.I.); this was used to classify the test product in terms of irritant potential. If the I.A.O.I. value is greater than or equal to 30.0, the soap Morinda would be considered positive and irritant to the eye [6-12].

Results

The results of evaluation of skin irritancy risk of soap Morinda on rabbits were summered in Table 3 with scores of erythema and edema obtained at 24 and 72 hr observation after soap application. The primary irritation index (P.I.I.) calculated was found to be 0.125. This value was lower than 5.0, the soap Morinda was non-irritating on rabbit skin. For ocular irritancy, the Table 4 presented results of lesions observed in conjunctiva, iris and cornea after soap application. These ocular lesions were clearly reversible 7 days after removal of soap application. The Mean Index of Ocular Irritation (M.I.O.I.) for each observation time gave 2.66. The Index of Acute Ocular Irritation (I.A.O.I.) was also 2.66. This value is lower than 30, the soap Morinda was weakly irritating.

Table 1: Acute skin irritation study in rabbits with soap Morinda

	Intact	24 hr		72 hr	
		24 hr	72 hr	24 hr	72 hr
Skin reaction	Erythma	0	0	3	0
	Edema	0	0	0	0
Score average ^a		0.0	0.0	0.5	0.0
Sum of score average		0.5			
Primary irritation index ^b		0.125			
Response category		Negligible			

a. Score average, Sum of the scored reactions/6 rabbits

b. Primary irritation index (PII), Sum of score average/4.

Table 2: Acute eye irritation study in rabbits with soap Morinda

			Days (d) after application			
			2	3	4	7
		Redness (A)	0	0	0	0
Ocular lesions	Conjunctiva	Edema (B)	0	0	0	0
(Sum of the		Discharge (C)	0	0	0	0
scored lesions)	Iris	Reaction (D)	0	0	0	0
	Cornea	Degree of opacity (E)	0	0	0	0
		Diffuse areas of opacity (F)	1	1	1	1
M.I.O.I. (Mean index of ocular irritation)			0.0	0.0	0.0	0.0
I.A.O.I. (The index of ocular irritation) ^a			2.66			
Response category			Weakly irritating.			

Discussion

In this study *in vivo* skin and eye irritations of antimicrobial soap containing hexane extract of *M. morindoides* were evaluated on rabbits. Our investigations showed that the use of this soap Morinda did not presented any risk of skin and eye irritations. However the weak eye irritating could be explained by its slightly basic pH of 8 ± 0.1 . This weak basicity could be attributed to trace amounts of sodium hydroxide and carbonate in soap. In fact the skin and eye irritations studies conducted by Thes (2008) on soap containing extract of *Mitracarpus scaber* [15] and Akapo (2010) on body cream based on extract of *Terminalia catappa* [16] were also revealed that these products with pH close to neutrality did not irritated. Also the results of work of

Baranda *et al.* (2002) ^[17] demonstrated a significant correlation between the pH of the dermatological preparations (lotion, gels, cream and soap) and their irritant effect ^[17]. In view of the above, it is clear that soap *Morinda* is suitable for a better skin use.

Conclusion

The results from the present study suggest that the soap *Morinda* made from hexane extract of *M. morindoides* as antimicrobial agent have promising potential medicated soap that do not induce significant levels of skin and eye irritation. In perspective our research plan to test the effectiveness of soap *Morinda* against microbial skin infections.

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