RESEARCH ARTICLE

Acute Toxicity Assessment of Cream Ethanolic Extract of *Vitex pinnata* Bark from Central Kalimantan

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ABSTRACT

Vitex pinnata bark has the ability to scavenge free radicals and has antimicrobial activity, thus accelerating wound healing. The formulation of *V. pinnata* bark preparations has good organoleptic properties, homogenity, pH, dispersion, and absorption. We studied the acute toxicity of *V. pinnata* skin cream in Wistar rats according to OECD guidelines 402. Seven nulliparous, non-pregnant female Wistar rats were exposed 2000 mg/kg of body weight. There were no signs of toxicity in any behavioral, necropsy dan histopathological changes. The Globally Harmonized System classified *V. pinnata* cream extract as category 5 (unclassified) with LD_{50} more than two thousand mg/kg.

Keywords: Skin toxicity, Kalapapa bark, OECD guidelines.

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INTRODUCTION

The need for herbal medicines is rising day by day tremendously all over the world. It is a medicine or a kind of treatment that is based on the use of plants and used as a medicine to treat a wide range of diseases.[1,2] Indonesia is a densely populated country with rich medicinal plant resources. Indigenous Indonesians developed the "local medicine" that is still practiced today by using plants to cure disease and injuries in a challenging natural environment.[3,4] Kalimantan is a region of Indonesia, an island with a very high biodiversity due to the diversity of its ecosystem.[5]

Kalimantan is one of the islands in Indonesia which is famous for its knowledge of traditional healing using plants and this knowledge has been passed down from generation to generation.[6] The Dayak tribe living in Kalimantan use medicinal plants to treat various diseases because they are easy to obtain and process.[7] One of the plants that is often used by the people of the interior of Central Kalimantan is the *Vitex pinnata* plant.[6] *V. pinnata* wood is an endemic wood species originating from Kalimantan.[8] It is an Asian tropical plant widely used by the Dayak tribe as a traditional anti-inflammatory medicine.

V. pinnata is a tree, commonly known as "Kalapapa" in Central Kalimantan (Lamiaceae family). *V. pinnata* have a trifoliate leaves with brown bark that can reach a height of 20 m. It has whitish blue flowers with purple-black fruit. Its leaves consist of 3-5 elliptical strands measuring 3 to 25 cm long and 1.5 to 10 cm wide. Other characteristics include terminal flowers that are white and bluish and fruits that are 5 to 88 mm in diameter and turn black when ripe.[9,10]

A many pharmacological studies have used the *V. pinnata* tree's skin, stems, leaves, and fruit.[10] This plant is reported to have several ethnomedicinal uses. This plant has analgesic, anti inflammatory, antipyretic, antibacterial and stomach pain relief effect. Young leaf sprouts can treat fever and high blood pressure. The root can relieve back pain, body aches, and fatigue. The leaves can be used to treat fevers and wounds, scrape of the bark can be used to treat cramps, dysentery and stomach ailments. Bark extract used to treat jaundice.[9,11,12]

The improved rate of wound healing seen is due to the extracts from *V. pinnata* leave and barks' capacity to scavenge free radical and exhibit antibacterial activities.[13-17] Its leaves and stems have potential antioxidant properties due to their content of flavonoids, alkaloids and terpenoids. The formulation of Kalapapa bark preparations has good organoleptic properties, homogeneity, pH, dispersion, and absorption.[18]

In general, there is no harm in using Kalapapa herbal medicine. However, scientific evidence is required in pharmacotherapy,[19-22] so studies must be conducted that demonstrate its safety. Therefore, we studied the acute toxicity of *V. pinnata* skin cream in Wistar rats in accordance with

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Organization for Economic and Development guidelines (OECD). Creams are semi-solid topical formulations. Cream formulations have advantages such as the convenience of application, good spreadability, and release of active ingredients into the skin.[23]

MATERIALS AND METHODS

V. pinnata L was collected in February of 2021 in the town of Kapuas Central Kalimantan. The experiment was performed on seven nulliparous, non-pregnant female Wistar rats weighing between 170 and 206 g. The test for acute dermal toxicity was performed at the Laboratory of Pharmacology and Therapeutics, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. All animals included in the study received food and water ad libitum for life. They were kept in favorable environmental conditions with a temperature of 25°C and each rat has a cage 25 cm long x 20 cm wide x 10 cm high.

The study was conducted in accordance with OECD Test Guideline number 402. *V. pinnata* cream preparations are administered between 7 and 8 AM. *V. pinnata* cream was applied to skin that had been cleaned of hair 24 hours prior to treatment and then exposed to the test material for 24 hours. The starting dose is one thousand milligram/kilogram of body weight. If death does not occur at a dose of 1000 mg/kg during the first 24 hours of testing, the dose is continued up to 2000 mg/kg. The main test was conducted at a dose of 2000 mg/kg body weight if no deaths were noticed in the test animals at that dose within the first 24 hours.

Two observers checked on the animals' bodily weights and clinical symptoms once a day. Body weight was weighed multiple times, specifically prior to dosing, on the seventh day of dosing and on the 14 day of dosing before terminal and necropsy. After sacrifice, all survivors had their primary organ weights (heart, liver, lungs, pancreas, stomach, intestines, kidneys, brain, spleen, and ovaries) measured.

At the end of the experiment, the Wistar rats were euthanized with ether after blood collection. Postmortem examination of all organs was carried out on all sacrificial animals (skin, liver, glomerulus, tubulus) which were then fixed with 10% formalin buffer and stored individually for histopathological examination. Hematoxylin-eosin (H & E)-stained tissue sections from each group of experimental rats were dissected one at a time. Any histological changes were studied use a light microscope at 200 or 400 times magnification.

RESULTS AND DISCUSSION

Mortality and Clinical Signs

There was no signs of toxicity to skin, eyes, hair, mucous membranes, respiratory system, somatomotor activity, peripheral nervous system, central nervous system, behavior pattern during the acute toxicity studies. Further, no sign of tremor, shaking, abnormal salivation, abnormal urination, weakness, or coma on the main test with a dose of 2000 mg/kg of body weight after *V. pinnata* cream treatment (5 animals). Death did not occur within 24 hours of treatment in the main trial at a dose of 2000 mg/kg of body weight or up to 14 days after exposure.

Body Weight

There were no differences in final body weight between treatment and control groups. The result shown in Table 1.

Organ weights and necropsy

There were no significant changes in the bruto weight and in the microscopic observations of the organs of the experimental animals compared with the control animals as shown in Table 2.

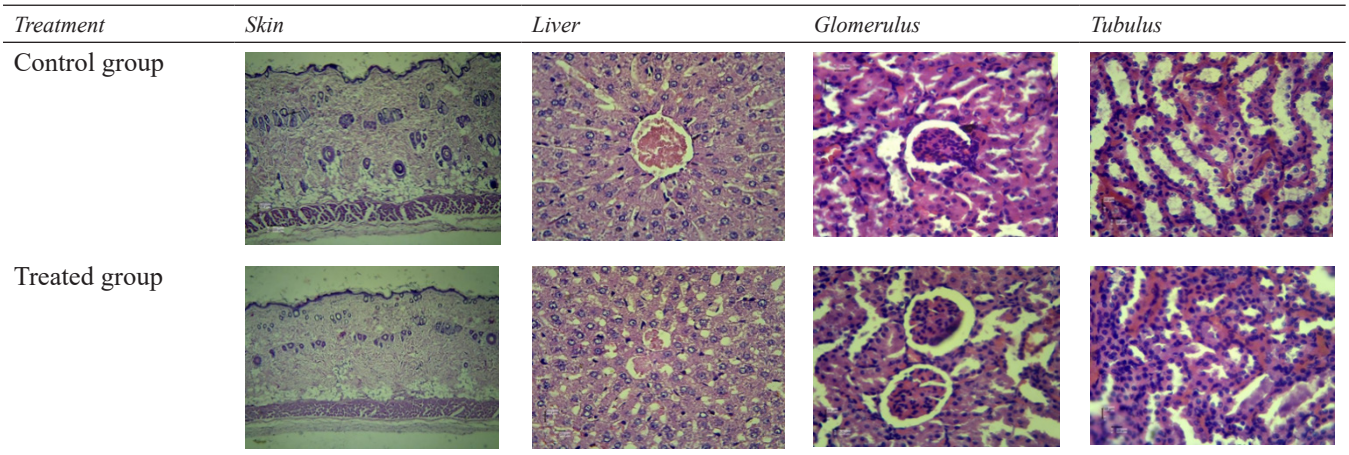
Table 1: Body weight treated rats treated *V. pinnata* cream dermal during 14 days experiment

Rats identity (Preliminary test)	*Dose (mg/kg) Body weight*	*Weight (g)*		
		Day 0	*Day 7*	*Day 14*
Control	1000	200	206	209
No. 1	1000	206	210	215
Rats identity (Main test)	Dose (mg/kg) Body weight	Weight (g) Day 0	Day 7	Day 14
Control 1	2000	172	185	190
Control 2	2000	182	189	194
Average		177	187	192
SD		7,07	2,83	2,83
No. 1	2000	180	189	193
No. 2	2000	186	188	211
No. 3	2000	180	189	217
No. 4	2000	170	186	203
No. 5	2000	170	170	187
Average		177.2	184.4	202.2
SD		7.01	8.14	12.38

Table 2: Organ weight treated rats treated *V. pinnata* cream dermal after 14 days experiment

Dose treatment doses	*Organ weight (gr)/ body weight (gr)*											
	Heart	*Liver*	*Lungs*	*Pancreas*	*Stomach*	*Intestines*	*Brain*	*Spleen*	*Kidneys*		*Ovaries*	
									Right	*Left*	*Right*	*Left*
2000 mg/ control	0.0041	0.0474	0.0072	0.0034	0.0085	0.0517	0.0095	0.0032	0.0045	0.0044	0.0007	0.0005
2000 mg/ control	0.0038	0.0405	0.0046	0.0043	0.0067	0.0501	0.0085	0.0026	0.0039	0.0040	0.0005	0.0004
Average	0.0040	0.0439	0.0059	0.0039	0.0076	0.0509	0.0090	0.0029	0.0042	0.0042	0.0006	0.0005
SD	0.0002	0.0048	0.0018	0.0006	0.0013	0.0012	0.0007	0.0004	0.0004	0.0003	0.0002	0.0001
2000 mg	0.0043	0.0528	0.0081	0.0025	0.0107	0.0442	0.0095	0.0032	0.0042	0.0042	0.0004	0.0005
2000 mg	0.0033	0.0482	0.0077	0.0021	0.0073	0.0364	0.0077	0.0024	0.0032	0.0036	0.0004	0.0005
2000 mg	0.0035	0.0524	0.0053	0.0031	0.0065	0.0195	0.0085	0.0028	0.0043	0.0041	0.0006	0.0006
2000 mg	0.0036	0.0478	0.0067	0.0023	0.0086	0.0318	0.0073	0.0030	0.0036	0.0036	0.0004	0.0005
2000 mg	0.0039	0.0418	0.0050	0.0043	0.0066	0.0516	0.0086	0.0025	0.0039	0.0038	0.0005	0.0004
Average	0.0037	0.0486	0.0065	0.0029	0.0079	0.0367	0.0083	0.0028	0.0038	0.0039	0.0005	0.0005
SD	0.0004	0.0045	0.0014	0.0009	0.0017	0.0122	0.0008	0.0003	0.0005	0.0003	0.0001	0.0001

Figure 1: Histopathology of rats exposed 2000 mg/kg BW of V. pinnata cream dermal



Histopathology

Histopathological examination of several organs showed no abnormalities or changes compared with controls group. Skin, liver, and kidney (glomerulus and tubule) showed no difference between the treatment group and control groups as shown in Figure 1.

There is a need of development of new drug delivery system from herbal source because of the effectiveness of phytoconstituent in management of various diseases.[24] *V. pinnata* L is known to contain a number of secondary metabolites that have pharmacological effects in humans.[12,21,25,26] The secondary metabolite are flavonoids, steroids, saponins, tannins and phenolic compounds.[27-29] *V. pinnata* Linn stem bark extract can decrease serum malondialdehyde (MDA) levels and increase plasma superoxide dismutase (SOD) activity in rats exposed to oxidative stress.[30]

The acute dermal toxicity test method gives information about the possible hazard for health because of short-term skin exposure to the test chemical. The hazards of the compound of the cream extract can be classified according to the Globally Harmonized System of Chemical Classification and Labeling (GHS). According to the data, the LD_{50} of *V. pinnata* L is 2000 mg/kg of body weight with no death, which means that it has been included in GHS classification and classified as a Category 5 chemical (non-toxic). It has been proven there is no harmful effect for the dermal application of *V. pinnata* bark.

CONCLUSION

From our findings it can be concluded that *V. pinnata* has no toxic potential even at a dose of 2000 mg/kg of body weight when its applied topical on skin. Because the dose used is several orders of magnitude higher than the dose normally used in a clinical condition, the changes at therapeutic dose levels may not be observed. Therefore, due to its dermal toxicity level, *V. pinnata* cream is classified according to the GHS classified as a Category 5 chemical (unclassified).

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