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Research Article

Acute Toxicity of Non-Hexane Fraction of Ethanolic Extract of Ant-Plant (*Myrmecodia tuberose* (Jack) Bl.) Hypocotyls in Rats

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ABSTRACT

Ant-plant (*Myrmecodia tuberose* (Jack) Bl.) is a medicinal plant originating from Papua which have broad therapeutic uses that potential to be developed as phytomedicine. The purpose of this research was to estimate acute-toxic potency of ant-plant by a modified OECD 423 method approach. This research is a part of phytomedicine preclinical test for ant-plant. Non-hexane fraction of 95% ethanolic extract of an-plant hypocotyls (NHF) were administered via oral, single and daily doses in female *Wistar* rats. Animals divided in 300, 2000, and 5000 mg/kg BW doses groups. Every group used 6 animals and divided into 2 steps, except for 5000 mg/kg BW which was only 3 animals used in a step. There is no dose administered for control. Animals were observed 24 hours after dose administration of extract. Especially for 2000 mg/kg BW, observation was continued till 14 days. Result showed LD_{50} of NHF greater than 5000 mg/kg BW, thus the extract were included in category 5/unclassified according to GHS. Histopathology of animals sacrificed after 24 hours dose administration showed abnormalities in renal tubular (epithelial vacuolation), lungs (peribronkhiolitis and perivaskulitis), liver (hydropic degeneration) and stomach (gastritis). While animals after 14 days observation resulted focal inflammation occurred in the liver.

Keywords: *Myrmecodia tuberose*, NHF, acute toxicity, OECD Guideline 423

INTRODUCTION

Ant-plant (*Myrmecodia tuberose* (Jack) Bl.) hypocotyls is widely used as herbal remedies by Papuans for many medicinal purposes, such as pain caused by rheumatics, tuberculosis, immune disease, even cancer. This plant's active fraction contained alkaloid, phenols, and terpenoid [1,2]. This plant grow well as an epiphyte and bioactive constituents of this plant depend on where it grows and how old itself. Wild forest ant-plant containing more bioactive chemicals than cultivated ones [3]. Ant-plant hypocotyls often used as a health supplement for mother's recovery after childbirth and breast-feeding period through some immunomodulation mechanisms by its plant. This fact proven according to previous research [1,2], concluded that *Myrmecodia sp.* (*M. pendens* and *M. tuberosa*) were significantly increased lymphocytes proliferation and macrophage phagocytosis activity. Another report showed that *M. tuberose* have anti microbial bioactivity against *C. albicans, E. coli*, and *S. aureus* [4]. The non-hexane fraction of ethanolic extract *M. tuberose* concentration of 20 µg/mL exhibited the highest activityin vitro to increase macrophage phagocytosis in comparison to the other fractions. It was related with high total phenolic content of this fraction and also the absence of non-polar chemicals inhibiting macrophage phagocytosis activity. It was also discovered that the immunomodulatory activity comes by increasing of $TCD4^+$ cell proliferation[2]. According to its wide therapeutic uses, ant-plant is highly recommended to be developed as phytomedicine. Therefore, this study was designed to evaluate the safety of ant-plant by carrying out the acute toxicity study in rats. Toxicity evaluation is required to establish a part of preclinical test for phytomedicine [5].

MATERIAL AND METHODS

Materials

The main material used in this study is a non-hexane fraction of 95% ethanol extract of ant-plant (*M. tuberosa*) hypocotyls (NHF). Ant-plant hypocotyls was obtained from Babo, Bintuni, in West Papua and processed by maceration and fractionation in the Laboratory of Pharmaceutical Biology, Faculty of Pharmacy Universitas Gadjah Mada, Yogyakarta, Indonesia.

Materials used in this study were:10% Formalin (General Labora), aquadest (General Labora), 0,5% Na-CMC solution(Merck), 95% ethanol (General Labora), n-hexane (General Labora), 0,9% physiological NaCl (General Labora).

Extraction and Fractionation

Dry powder of ant-plant hypocotyls was macerated using ethanol 95% for 24 hours and then the

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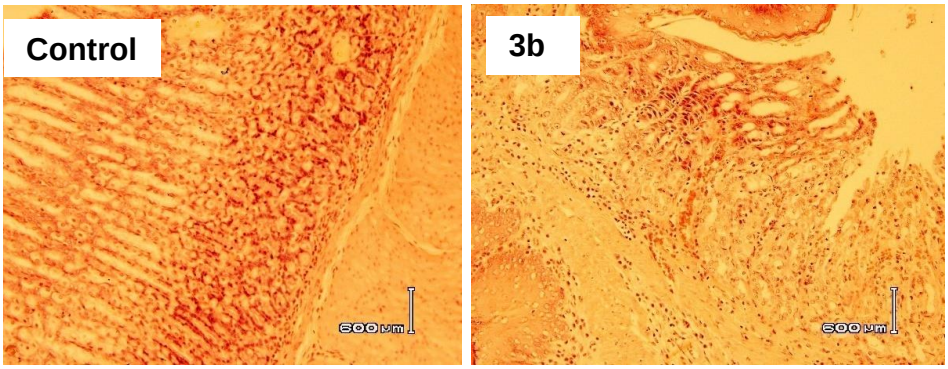

Figure 1. Acute toxicity effects non-hexane fraction of 95% ethanolic extract of Ant-plant (*M. tuberose*) hypocotyls in female *Wistar* rat stomach tissue

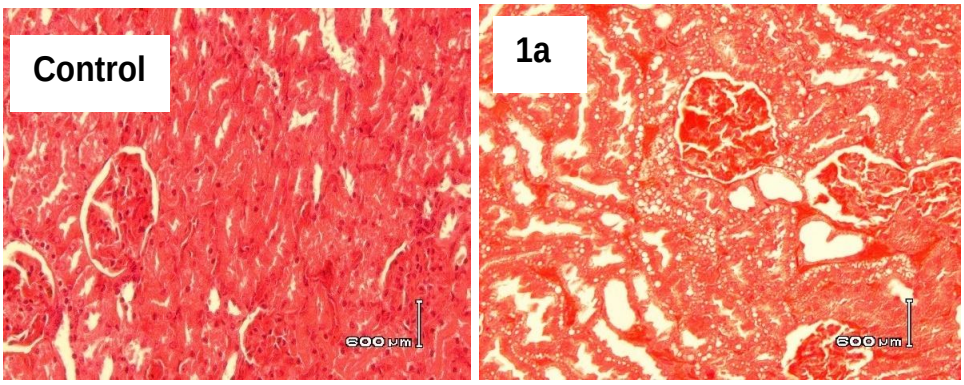

Figure 2. Acute toxicity effects NHF in female *Wistar* rat kidneys tissue

Table 1. Test and control animals mortality data

Dose (mg/kg BW)	Groups	Number of test animals	Number of death
Control	K	1	0
300	1a	3	0
	1b	3	0
2000	2a	3	0
	2b	3	0
5000	3a	1	0
	3b	2	0

result was evaporated using a rotary evaporator to obtain a viscous extract. Viscous extract dissolved in 90% methanol and then fractionated using n-hexane (1:1) for 15 minutes. The methanol layer (bottom) evaporated in water bath until a viscous extract was obtained and the smell of methanol disappeared. The fraction was dissolved in 0,5% Na-CMC solutions for administration
with maximum volume not exceed 5 mL/kg BW per animal.

Acute Toxicity Test (OECD 423)

Method used in this test was modified OECD Guidelines for Testing Chemicals 423 (OECD 423). Animals were divided into groups based on doses administered and non-administered control. Each group consists of 3 female *Wistar* rats. Starting dose of the test was 300 mg / kg BW, if animals die before 24 hour, surgery should be performed immediately to collect some vital organs: lungs, liver, stomach, spleen, and kidneys for histopathological preparation. But if animals survive within 24 hours, the surgery then performed in 24th hour immediately. To determine the delayed toxic effects after dose administration, observation followed up to 14 days for the first dose of 2000 mg/kg BW group (2a). Repeating, increasing or decreasing the dose using 3 different rats referred to the flow defined in OECD 423 to obtain results of LD_{50} cut-off (OECD, 2001).

Histopathology

Histopathological observations was evaluated to kidneys. The isolated organs were washed with 0,9% NaCl solution and observed by it's gross pathology with naked eye before weighed. Organs fixed with 10% formalin solution after drained of residual 0,9% NaCl solution. Microscopic observation of the organs were performed using Haematoxylin-Eosin (HE) staining technique.

RESULT AND DISCUSSION.

From several tests carried out starting from 300 mg/kg BW up to 5000 mg/kg BW, no animals died within a period of 24 hours after dose administration (Table 1) Based on animals mortality data (Table 1), LD50 of NHF is greater than 5000 mg/kg BW (> 5000 mg/kg BW). According to the Globally Harmonized System (GHS), it is included in the category 5 or unclassified (OECD, 2001). There was no difference in the observation of gross pathology between animal groups were sacrificed 14 days after dose 2000 mg/kg (2a) BW administration and animal group were sacrificed 24 hours after dose 2000 mg/kg BW (2b) with control (C), as well as between low dose (2000 mg/kg BW [2b]) and high dose (5000 mg/kg BW [3a and 3b]) with control animal (C). Organ weights data of all animals presented in Table 2. Based on animals mortality data

Table 2. The data of organ weights of animals test

Groups	Organs	Animal I (g)	Animal II (g)	Animal III (g)	Average of ±SD
C	Stomach	1,3	-	-	1,3
	Spleen	0,6	-	-	0,6
	Lungs	1,3	-	-	1,3
	Liver	5,5	-	-	5,5
	Kidneys	0,9	-	-	0,9
2a	Stomach	0,8	1,1	1,3	1,07±025
	Spleen	0,3	0,6	0,2	0,37±0,21
	Lungs	0,1	1,2	1,4	0,9±0,70
	Liver	5,9	5,5	5,5	5,63±0,23
	Kidneys	0,9	1,1	0,9	0,97±0,12
2b	Stomach	1,7	1,2	1,2	1,37±0,29
	Spleen	0,7	0,7	0,5	0,63±0,12
	Lungs	1,2	1,2	1,0	1,13±0,12
	Liver	5,6	5,4	5,0	5,33±0,31
	Kidneys	0,7	0,7	1,2	0,87±0,29
3a and 3b	Stomach	1,0	1,4	1,3	1,23±0,21
	Spleen	0,7	0,4	0,4	0,50±0,17
	Lungs	1,2	1,2	0,9	1,10±0,17
	Liver	5,6	6,7	5,0	5,77±0,86
	Kidneys	0,9	1,1	0,8	0,93±0,15

Note: C= Negative control; 2a= 2000 mg/kg BW (1); 2b= 2000 mg/kg BBw (2); 3a= 5000 mg/kg BW (1); 3b= 5000 mg/kg BW (2)

(Table 1), LD50 of NHF is greater than 5000 mg/kg BW. According to the Globally Harmonized System (GHS), it is included in the category 5 or unclassified (OECD, 2001). There was no difference in the observation of gross pathology between animal groups were sacrificed 14 days after dose 2000 mg/kg (2a) BW administration and animal group were sacrificed 24 hours after dose 2000 mg/kg BW (2b) with control (C), as well as between low dose (2000 mg/kg BW [2b]) and high dose (5000 mg/ kg BW [3a and3b]) with control animal (C). The data of organ weights of all animals test presented in Table 2. Histopathologic examination of the organ showed six kinds of cellular abnormalities in any organ except spleen. It was: Fatty degeneration of kidneys tubular epithelial (1 rat dose of 300 mg/kg BW), Focal inflammation of liver (1 rat dose of 2000 mg/kg BW) and 3 rats of 5000 mg/kg BW), Perivasculitis (1 rat dose of 2000 mg/kg BW and 2 rats of 5000 mg/kg BW), liver hydropic degeneration (3 rats dose of 5000 mg/kg BW), and gastritis (1 rat dose of 5000 mg/kg BW).High dose administration of NHF dose of 5000 mg/kg BW (3b) may result in gastritis in the stomach, evidenced by: erosion of gastric mucosal epithelial cells,unclear boundary between mucosal and submucosal layer, and bleeding (existence of red blood cells) and eosinophil-neutrophil infiltration (Figure 1).Figure 2 showed the swelled tubular epithelial cells, pressed cell nucleus to the edge, and the presence of fatty vacuolation. This abnormality is a sign of fatty degeneration [6]. It was occurs in a rat dose of 3000 mg/kg BW (1a). Dose administration of NHF may cause focal inflammation in one rat dose of 300 mg/kg BW (2a) and high-dose administration of 5000 mg/kg BW (3a and 3b) can cause hydropic degeneration in all three animals. Focal inflammation indicated by the presence of inflammatory cells around the central vein of the liver. Hydropic degeneration is characterized by an increased size of hepatocytes, blurred cytoplasm and less obvious bounded vacuolation (Figure 3).

Table 3. Histopathologic examination of organs

Groups	Rats	Sacrifice period	Results
K	1	14 days	-
1a	1	24 hour	-
	2	24 hour	FDTE
	3	24 hour	-
1b	1	14 days	-
	2	14 days	-
	3	14 days	-
2a	1	24 hour	-
	2	24 hour	-
	3	24 hour	FI
2b	1	24 hour	-
	2	24 hour	-
	3	24 hour	PB, PV
3a	1	24 hour	HD, PB
3b	1	24 hour	HD, PB, PV
	2	24 hour	HD, PB, PV, G

Explanation: (-) no abnormalities; FDTE: Fatty degeneration of tubular epithelial (kidneys); FI: Focalinflammation (liver); PB: Peribronchiolitis (lungs); PV: Perivasculitis (lungs); HD: Hydropic degeneration (liver); G: Gastritis (stomach)

There are 3 forms of cellular abnormalities in lungs tissues. It is interstitial pneumonia, peribronchiolitis, and

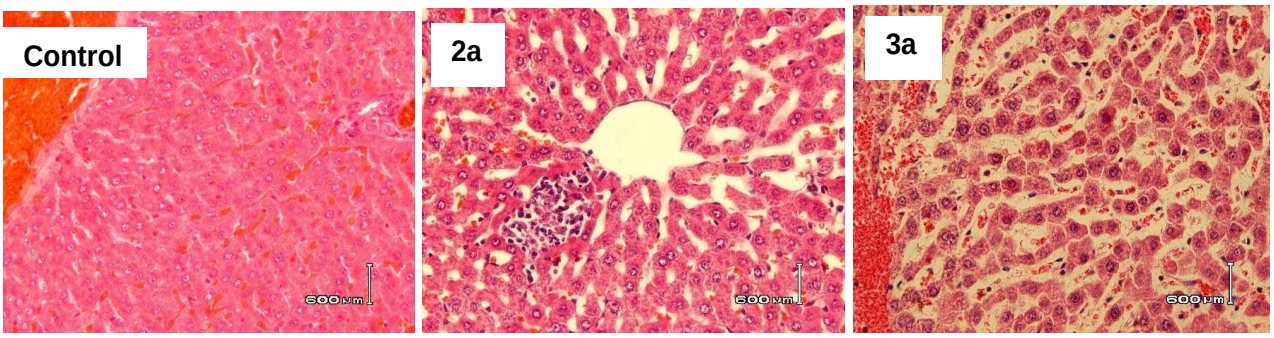

Figure 3. Acute toxicity effects of NHF in female *Wistar* rat liver tissue

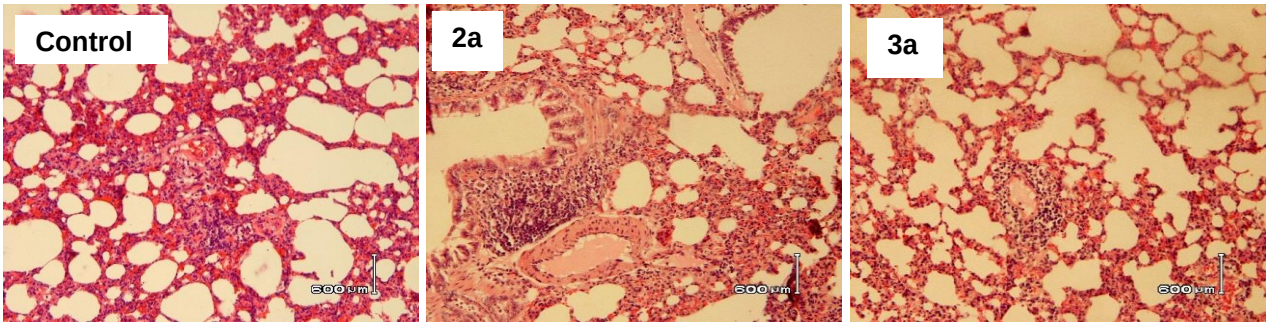

Figure 4. Acute toxicity effects of NHF in female *Wistar* rat lungs tissue

perivasculitis. While interstitial pneumonia is non-specific because of encountered in all test animals (Figure 4). Peribronchiolitis occurs in 1 rat in dose of 2000 mg / kg BW (2b) and all rats in dose of 5000 mg/kg BW (3a and 3b). While perivasculitis occurs in a rat dose of 2000 mg/kg BW (2b) and 2 rats dose of 5000 mg/kg BW (3b). It can be concluded that peribronchiolitis and perivasculitis may occur as a result of the administration of high doses of NHF although were not resulting in death.

CONCLUSION

LD_{50} cut-off value of NHF is greater than 5000 mg / kg BW (> 5000 mg / kg BW), thus it is included in category 5 or unclassified according to Globally Harmonized System (GHS). Acute toxicity observations in female *Wistar* rats showed that the non-hexane fraction can cause cellular abnormalities in the organs lungs, liver, kidney, and stomach, such as: gastritis in the stomach, fatty degeneration of the kidneys, focal inflammation and hydropic degeneration of the liver, peribronchiolitis and perivasculitis in the lungs. Further research is needed to find out the exact value of LD_{50} of NHF

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AUTHORS' STATEMENTS

Competing Interests

The authors declare no conflict of interest

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