

# Antimutagenic activity of nanoparticles of *Rhaphidophora pinnata* leaves in mice using micronucleus assay

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## ABSTRACT

Cancer is one of the deadliest diseases in the world. Cancer may occur due to gene mutation. *Rhaphidophora pinnata* is a plant that has many benefits, especially in the leaves which have been used traditionally to treat cancer. The aim of this research is to test the antimutagenic activity of nanoparticles *R. pinnata* using the micronucleus method. The mice were induced with cyclophosphamide and then followed with the administration of nanoparticles of *R. pinnata* at the doses of 50, 100, 200 mg/kg for 7 days. The antimutagenic activity was evaluated at the decrease in the number of micronucleus in 200 polychromatic erythrocytes (PCE) cells of mice bone marrow. The result showed that the reduction of amount of micronucleus in PCE of a negative control group, treatment groups, and normal group is 22.65%, 60.3%, 79.6%, 93.8%, and 100%. These results indicate that the antimutagenic activity of nanoparticle of *R. pinnata* increases proportionally as the doses were increased. It can be concluded that nanoparticles *R. pinnata* at the doses of 50, 100, and 200 mg/kg have antimutagenic activity.

**Key words:** Antimutagenic, micronucleus, *Rhaphidophora pinnata*

## INTRODUCTION

Mutation is a change that occurs in a gene so that it can cause changes to the products encoded by the gene. Mutations can be associated with the emergence of various disorders, including obesity, diabetes, cardiovascular disease, cancer, and also neurodegenerative diseases mainly Alzheimer's

disease and Parkinson's disease.<sup>[1-3]</sup> Micronucleus is one indicator of mutation and it has become the most prevalent method to assess genotoxicity of different chemical and physical factors.<sup>[1,4]</sup>

The micronucleus is a small nucleus outside the main nucleus in the cytoplasm. This is an abnormal nucleus due to the cleavage of chromosomes caused by mutagenic compounds. The micronucleus is easily observed in polychromatic erythrocytes (PCE). The number of micronucleus in PCE indicates the degree of genetic damage to the erythropoietic system of living things.<sup>[1]</sup>

The antimutagenic test was conducted to determine the possibility of compounds having antimutagenic properties.

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One of the test methods is cytogenetic antimutagenic tested by *in vivo* is the micronucleus test method.<sup>[5]</sup> One of the plants that is promising as antimutagenic is *Rhaphidophora pinnata* (L.f.) Schott. This plant is included in the Araceae family and its leaves have been used traditionally both in Indonesia and Singapore to treat cancer. The anticancer activity of this plant has also been tested against MCF-7 cells and T47D cells.<sup>[6-8]</sup> However, research on its antimutagenic activity has never been carried out. Therefore, this study is to determine the antimutagenic activity of nanoparticle of *R. pinnata* leaves in mice (*Mus musculus* L.) using the micronucleus test method.

## MATERIALS AND METHODS

### Materials

The leaves of *R. pinnata* were collected from Medan, North Sumatera, Indonesia, in July 2008. The plant authentication was carried out by Dr. Eko Baroto Walujo, APU from Research Center for Biology, Indonesian Institute of Sciences, Indonesia with the result is *R. pinnata* (L.f.) Schott, family Araceae (the reference number is 1052/IPH.1.02/If. 8/2008).

The leaves were dried and mashed in PT. Nanotech Herbal Indonesia, Bogor, Indonesia. The leaves were mashed by top-down method using high-speed milling until gained the particle with size  $616.0 \pm 128.0$  nm. The determination of particle size using Beckman Coulter Delsa nanoparticle analyzer which measured in temperature of 25°C with water as the solvent of the particle.

The chemical used were methanol (Merck, Germany), Giemsa solution (Sigma-Aldrich, United States), immersion oil, 0.9% NaCl (PT. Widatra Bhakti, Indonesia), bovine blood serum, and cyclophosphamide (Cyclovid®, PT Novell Pharmaceutical Laboratories).

The apparatus used in this study were surgical apparatus (Wells Spencer), microscope (Boeco, BM-180,

halogen lamp), centrifuge (Dynamica, velocity 18R), microtube, and digital camera.

### Animals

The animals used in this research are mice (*Mus musculus* of Swiss Webster strain) obtained from the Animal House of Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. The animal is 3-months-old and has never given birth with bodyweight about 20–30 g. The protocol of this research has been approved by the Ethic Committee of Animal Research of Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. The approval number is 0606/KEPH-FMIPA/2019.

### Testing of antimutagenic effects in mice

The mice were divided into five groups and each group consists of 5 mice. The experimental design is shown in Table 1.

On the 8<sup>th</sup> day, the animal was sacrificed then the femur was taken and cleaned. The end of the proximal bone was cut and then the femoral bone marrow was taken using a syringe containing 0.1 ml of bovine serum and phosphate buffer (1:1 v/v). The syringe was inserted into the open bone marrow channel to aspirate the marrow fluid so that it mixes with serum bovine and phosphate buffer. After that, the mixture was put in a microtube.<sup>[9-11]</sup>

### Preparation of femoral bone marrow smears

The mixture of bone marrow and serum bovine-buffer phosphate (1:1 v/v) in a microtube was centrifuged at 2000 rpm for 5 min, then the supernatant was discarded. The precipitate was resuspended with two drops of serum bovine-buffer phosphate (1:1 v/v). Then one drop of cell suspension was taken and placed on the slide until formed a thin layer. Then, the slide was dried and fixed with methanol for 3 min subsequently stained with May-Gruenwald dye. The staining was carried out for 3 min. After that, the sample was soaked again in May-Gruenwald dye which had been diluted with aqua dest in a ratio of 1:1 for 2 min, then

**Table 1: Experimental design**

Group	Treatment
I (negative control)	Animals induced by cyclophosphamide solution dose of 50 mg/kg bw on the first day, then after 30 h given a suspension of CMC Na 0.5% orally every day until the 7 <sup>th</sup> day.
II (dose of 50 mg/kg)	In the test group, the animals were induced by cyclophosphamide solution at a dose of 50 mg/kg bw on the first day, then after 30 h were given a nanoparticle suspension <i>R. pinnata</i> dose 50 mg/kg bw orally every day until the 7 <sup>th</sup> day
III (dose of 100 mg/kg)	In the test group, the animals were induced by cyclophosphamide solution at a dose of 50 mg/kg bw on the first day, then after 30 h were given a nanoparticle suspension <i>R. pinnata</i> dose 100 mg/kg bw orally every day until the 7 <sup>th</sup> day
IV (dose of 200 mg/kg)	In the test group, the animals were induced by cyclophosphamide solution at a dose of 50 mg/kg bw on the first day, then after 30 hours were given a nanoparticle suspension <i>R. pinnata</i> dose 200 mg/kg bw orally every day until the 7 <sup>th</sup> day
V (normal control)	Normal control, animals were given a solution of 0.5% CMC Na for 7 days orally

CMC: Carboxymethyl cellulose, *R. Pinnata*: *Rhaphidophora pinnata*

washed with aqua dest as much as two times. The next step, the samples were immersed in a glass filled with Giemsa dye solution mixed with phosphate buffer in a ratio of 1:23 for 10 min. The samples were washed with aqua dest and then dried. Furthermore, the samples were dipped in a glass containing a xylol solution for 10 min then washed with aqua dest. After drying, it was covered with a deck glass and observed under a microscope with a magnification of  $10 \times 100$  with the addition of oil immersion. The number of micronucleus cells in 200 PCE was counted.<sup>[12,13]</sup>

### Data analysis

Data were analyzed using the Statistical Product and Service Solutions (SPSS) 18 software. To determine the difference in antimutagenic activity in each treatment was carried out using one-way analysis of variance test.

## RESULTS

The result of amount of micronucleus existed in the erythrocyte femur is shown in Figure 1 and Table 2.

Based on the result in Figure 1 and Table 2, the percentage reduction in the number of micronucleus per 200 PCE cells in the negative control, doses of 50, 100, and 200 mg/kg, as well as normal control respectively, is 22.65%, 60.3%, 79.6%, 93.8%, and 100%. These results indicate that the increase of the dose of nanoparticle of *R. pinnata* is directly followed by the decrease in the number of micronucleus.

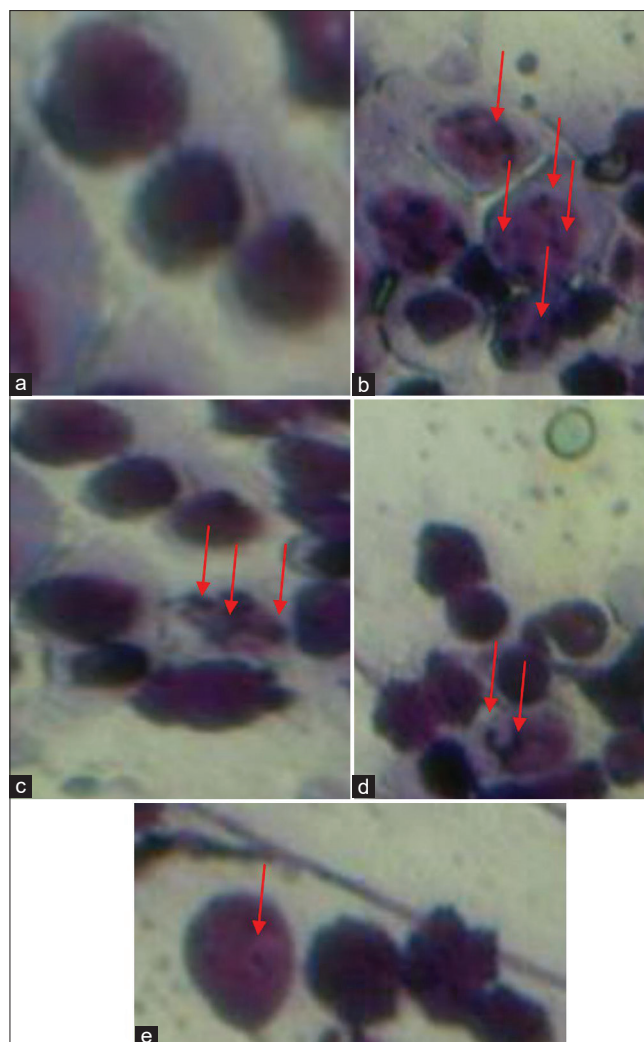
## DISCUSSION

Cyclophosphamide is mostly used to treat various malignancy and nonmalignancy disorder, however, it has a wide spectrum of toxicity.<sup>[14]</sup> Cyclophosphamide can cause toxicity in the bone marrow and produce micronucleus.<sup>[15]</sup>

Micronucleus is one indicator of mutation. Micronucleus is the result of mutations from intact chromosomes that are broken and then appear as a small nucleus in a cell. Micronucleus is easily observed in erythrocyte polychromatic cells in the bone marrow. The number of micronucleus PCE indicates the level of genetic damage in the erythropoietic system of a living creature.<sup>[11,16]</sup>

Theoretically, the prevention of carcinogenesis/mutagenesis can occur through inhibition of promotion until the progression phase. The initiation process can be inhibited by compounds that decrease the metabolic activation of carcinogens, increase the detoxification of carcinogens, or prevent the bonding between carcinogens and cellular targets.<sup>[17]</sup>

The antimutagenic mechanism of *R. pinnata* needs further research, but so far it is suspected related to its



**Figure 1:** The cells observed in the smear of femoral bone marrow of mice. (a) Normal control; (b) Negative control; (c) 50 mg/kg; (d) 100 mg/kg; (e) 200 mg/kg

**Table 2: The number of micronucleus per 200 peritoneal exudate cells in the bone marrow of mice for 7 days**

Kelompok	Number of micronuclei/1000 cells $\pm$ SEM	Percentage reduction micronucleus
I (negative control)	154.8 $\pm$ 3.397 <sup>a</sup>	22.65
II (dose of 50 mg/kg)	79.4 $\pm$ 3.458 <sup>a</sup>	60.3
III (dose of 100 mg/kg)	40.8 $\pm$ 2.596 <sup>a</sup>	79.6
IV (dose of 200 mg/kg)	12.4 $\pm$ 1.363 <sup>a</sup>	93.8
V (normal control)	0 $\pm$ 0	100

<sup>a</sup>Significantly different from negative control. SEM: Standard error of mean

antioxidant activity.<sup>[18]</sup> Previous research showed that the antioxidant activity of *R. pinnata* had a value of 74.2413  $\mu$ g/ml that is classified as a strong antioxidant. The antimutagenic activity shown by *R. pinnata* nanoparticles was stronger than the ethanol extract of *R. pinnata* leaves.<sup>[19]</sup> This result showed that by reducing the particle

size, it is thought that absorption in the intestine will be better because there is an increase in solubility, an increase in the permeability of the enterocyte membrane, and the opening of the paracellular tight junctions between enterocytes.<sup>[20]</sup>

## CONCLUSIONS

Nanoparticles of *R. pinnata* at the doses of 50, 100, and 200 mg/kg have antimutagenic activity by reducing the amount of micronucleus in PCE. Increasing the dose of nanoparticles of *R. pinnata* increases antimutagenic activity.

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## Conflicts of interest

There are no conflicts of interest.

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