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Acute toxicity of the intranasal administration of *Anredera cordifolia* extract in Wistar Rats

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ABSTRACT

Anredera cordifolia (AC) known as the binahong plant in Indonesia has been commonly used for traditional medicine since the ancients. AC contains secondary metabolites such as flavonoids that have anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties. Many flavonoids compound that has anti-inflammatory activity have the potential for the treatment of nasal inflammatory such as allergic rhinitis. This study aims to evaluate the preclinical safety of acute intranasal administration of AC extract in Wistar rats with mortality, clinical changes, blood laboratory, and body weight evaluation. Acute toxicity using the intranasal irrigation administration of AC extract was evaluated on 30 female Wistar rats, divided into five rats for control, and each five of doses 5%, 10%, 25%, 50%, and 75%. We perform blood laboratory after administration and observation for 14 days for the incidence of mortality and signs of toxicity such as tremors, hypoactivity, irregular respiration, and bodyweight loss. The AC extract intranasal administration doses at 5, 10, 25, 50, and 75 did not show mortality, or treatment-related adverse events and did not show significantly changes in blood profile. Based on the study the AC extract was found safe until 75% for nasal administration in Wistar rats.

Keywords: *Anredera cordifolia*, toxicity, nasal administration, anti-inflammatory

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INTRODUCTION

Anredera cordifolia (Ten.) Steenis (AC) is a plant that comes from the Basellaceae family which used for medical purposes since ancient Indonesia, a country rife with biodiversity (Rofida, 2010). Phytochemical content found in AC were terpenoids, steroids, glycocide, flavonoids, saponin, and alkaloids had pharmacology activities (Alba et al., 2020; Kočevar et al., 2013).

Some scientific research has reported the pharmacological activity of AC as antibacterial (Leliqia et al., 2017; Sari et al., 2020), antifungal, antivirus, antidiabetes, antihypertensive, vasodilator, hypolipidemic, antioxidant (Rajathi & Suja, 2017), gastroprotective, hepatoprotective, cytotoxic, antiinflammatory (Sutrisno et al., 2016), analgetic and wound healing (Hanafiah et al., 2019).

Inflammation is an important biological response in various diseases including allergic rhinitis. *Anredera cordifolia* is reported to contain bioactive compounds of flavonoids for anti-inflammatory activities. It has been reported that AC posses the anti-inflammatory by inhibits inflammatory mediators including TNF-α, IL-1β, IL-6, and nitric oxide (Laksmitawati et al., 2017).

The longtime treatment for allergic rhinitis control is challenging from several aspects such as the first pass metabolism, unwanted side effects, patient inconvenience, and high cost (Hossenbaccus et al., 2020; Licari et al., 2019). The intranasal drug is one of the options to resolve the problems and has higher bioavailability (Laffleur & Bauer, 2021; Keller et al., 2022). Nowadays intranasal drug administration is commonly used for the treatment of local inflammatory diseases including allergic rhinitis. The advantages of nasal drug application were rapid absorption, quick local effect, prevention of first-pass metabolism, reduced systemic side effects, avoid drug damage in the Gastrointestinal Track (GIT), and better compliance or comfort for the patient (Keller et al., 2022).

This study aims to evaluate the preclinical safety of acute intranasal administration of AC extract in laboratory Wistar rats.

MATERIALS AND METHOD

Materials

Thirty adult female Wistar rats nulliparous and nonpregnant (120-200 gram) were fed adaptively for acclimatized a week at laboratory before the experiment. All rats were placed in 5 divided cages, in a temperature-controlled, humidity-controlled, and light controlled experimental environment. Rats were fed on pelleted feed and provided pure potable water with steel sipper tubes. The whole research process was conducted at the experimental animal research laboratory of Laboratorium Penelitian dan Pengujian Terpadu (LPPT) Gadjah Mada University. The study protocol was approved by the Ethics Committee of Universitas Muhammadiyah Yogyakarta no. 071/EC-KEPK FKIK UMY/XII/2021.

Binahong leaves used in this study came from the Hargobinangun Pakem Sleman Yogyakarta area, which was determined by CV Merapi Farma Herbal. AC leaf extract is done by maceration method using 96% ethanol. The maceration process is carried out 5 times, and the results filtered and evaporated using a rotary vacuum evaporator. The concentration of extract used was 5 %, 10%, 25%, 50%, and 75% with sterile aquadest solvent.

Methods

This procedure was modification of OECD Acute Inhalation Toxicity Testing, evidence toxicity is a general term describing clear signs of toxicity following the administration of a test chemical. The animals are exposed to the test chemical in the nose only single exposure method and this approach not only using death/moribundity as endpoint but also evident clinical signs of toxicity at one of a series of fixed dose levels. The acute toxicity study of AC extract was performed with the nasal administration. The doses of 5, 10, 25, 50, and 75% AC extract were instilled intranasal in each group of five Wistar rats and control group with saline isotonic. The rats were observed for 2 weeks for any incident of mortality or signs of toxicity.

Before and one day after intranasal administration the rat's olfactory function is tested by measuring the time to find food under a layer of bedding, its procedure according to the buried food test relies on the animal's natural tendency the ability to smell odors for foraging (Thakurdesai, 2021). We also collected blood samples for laboratory evaluations and measured the body weight.

RESULT AND DISCUSSION

The rats nervus olfactorius function test relies on the animal's natural ability to find the food palette hidden under a layer of bedding. The effect of extract binahong on the olfactory system was carried out before initiation of treatment and one day after the procedure, the recorded result of time to find the food was 241.49 seconds and 316.92 seconds, there was an increasing time to find the food palette but the was none rat anosmia so the AC extract not toxic for the olfactory nerves. More details can be seen in Table 1.

Table 1. Results of olfactory function test data before and after intranasal administration of *Anredera cordifolia* extract

	Before treatment (seconds) Mean ± SD	After treatment (seconds) Mean ± SD
Control group NaCl 0.9%	298.44 ± 26.01	46,45 ± 2,35
Group of 5% AC extract	167.97 ± 28.01	508,98 ± 425,69
Group of 10% AC extract	222.90 ± 65.93	666,56 ± 130,98
Group of 15% AC extract	189.52 ± 118.76	53,41 ± 16,21
Group of 50% AC extract	353.25 ± 391.28	231,56 ± 179,80
Group of 75% AC extract	216.89 ± 90.70	394,54 ± 481,07

The acute study at intranasal administration dose until 75% extract AC did not reveal any mortality or abnormal clinical signs in female Wistar rats see Table 2.

Table 2. The acute toxicity symptoms and death of rats after intranasal administration of *Anredera cordifolia* extract

Nasal Irigation	Sample Number	Toxic Symptom	Death
Control NaCl 0.9%	5	0	0
5% extract AC	5	0	0
10% extract AC	5	0	0
25% extract AC	5	0	0
50% extract AC	5	0	0
75% extract AC	5	0	0

No significant body weight loss was observed during the 14-day observation post dosing period, and in the observation of body weight in rats, there was no significant weight gain compared to the control group and there was no dose response increasing see Figure 1.

The blood profile associated with a human health status, the effects of AC extract were also evaluated in Wistar rat's blood profile after nasal administration as showed in Table 3.

The results of the hematological measurement of Wistar rats were analyzed statistically using one way ANOVA method did not show any significant differences between the control group and all dose groups, with each significant value for WBC p=0.612, RBC p=0.262, PLT p=0.267, HB p=0.072, HCT p=0.186, MCV p=0.366, MCH p=0.243, MCHC p=0.227.

Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa, caused by a hypersensitivity reaction mediated by immunoglobulin E after repeated allergen exposure (Small et al., 2018). Symptoms

of AR include runny nose, sneezing, nasal congestion, itchy nose, and sometimes accompanied by complaints of itchy, red eyes and tears. Allergic Rhinitis can cause symptoms of respiratory tract obstruction during sleep and become a risk factor for other diseases including chronic rhinosinusitis, asthma, and otitis media (Bousquet et al., 2020). Allergic Rhinitis is the most common chronic respiratory disease, with prevalence varying between regions and environmental conditions ranging from 10-40% of the total population (Savouré et al., 2022). Symptoms of AR are generally harmless but cause a decrease in quality of life, sleep disturbances, work/school performance and the treatment costs a lot (Bjermer et al., 2019).

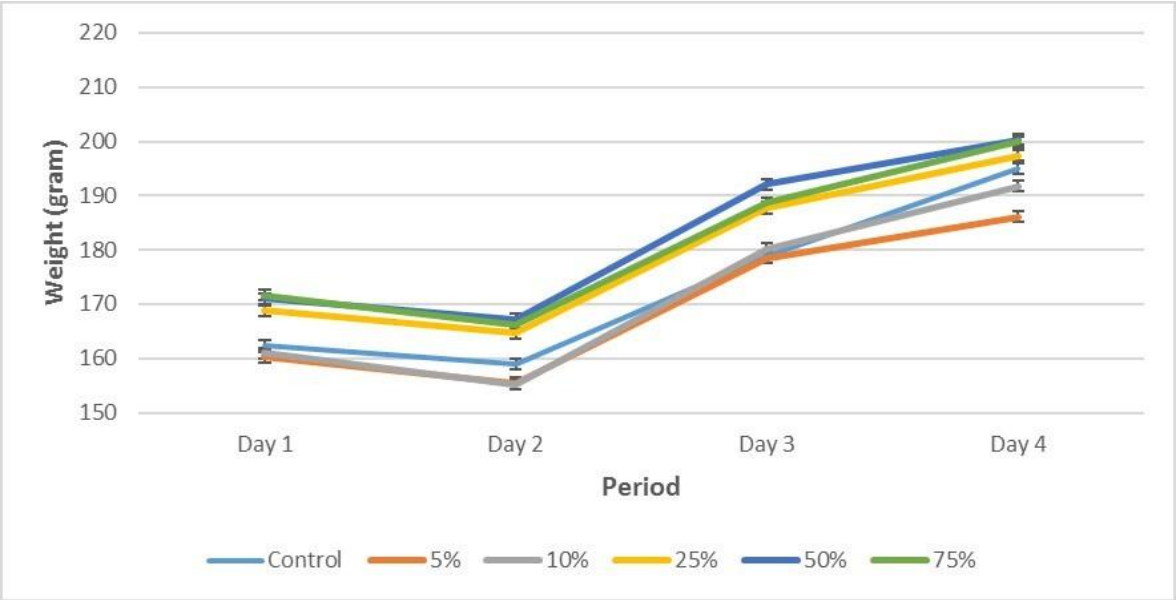

Figure 1. The comparisons of body weight in rats control and intranasal irrigation with extract AC

Table. 3. Blood Profile measurement after *Anredera cordifolia* extract nasal administration

	Control (*mean* ± SD)	**Dose 5% (*mean* ± SD)**	**Dose 10% (*mean* ± SD)**	**Dose 25% (*mean* ± SD)**	**Dose 50% (*mean* ± SD)**	**Dose 75% (*mean* ± SD)**
WBC (10^3)	10.18 ± 1.55	10.42 ± 2.19	9.38 ± 1.68	8.70 ± 1,54	10.14 ± 1.46	9.72 ± 1.50
RBC (10^6)	6.39 ± 0.41	6.83 ± 0.54	6.33 ± 0.29	6.26 ± 0.37	6.52 ± 0.47	6.11 ± 0.64
PLT (10^3)	1070.6 ± 93.91	950.8 ± 77.89	983.8 ± 79.79	975.0 ± 65.04	969.4 ± 59.64	963.8 ± 106.74
HB	13.32 ± 0.61	14.98 ± 1.41	13.64 ± 0.66	13.78 ± 0,74	14.30 ± 1,04	13.28 ± 1.03
HCT (%)	41.04 ± 1.92	44.38 ± 3.56	41.32 ± 1.77	40.84 ± 2.06	42.04 ± 2.85	39.66 ± 3.77
MCV (fL)	-64.30 ± 1.19	-64.98 ± 0.44	-65.32 ± 0.73	-65.24 ± 0.57	-64.40 ± 1.20	-64.94 ± 0.91
MCH	-20.90 ± 0.77	-21.94 ± 0.77	-21.56 ± 0.36	-22.0 ± 0.77	-21.94 ± 1.07	-21.80 ± 0.74
MCHC	32.46 ± 0.94	33.76 ± 1.30	33.0 ± 0.72	33.76 ± 1.03	34.04 ± 1.46	33.54 ± 0.79

The current management of RA includes education, allergen avoidance, pharmacology, immunotherapy, biology, and probiotics (Zhang et al., 2021). The clinical practice therapy guide was developed by Allergic Rhinitis and its Impact on Asthma (ARIA) by means of integrated services

utilizing social media, mobile health, and multidisciplinary services (Linton et al., 2021). Intranasal steroids are recommended as the first choice for controlling the symptoms of moderate-severe persistent allergic rhinitis, but there were a few incidences of adverse effects such as epistaxis, headache, taste disorder, and pharyngitis. There the potential of AC medical used as an anti-inflammatory agent for allergic rhinitis needs safety evaluation using international guidelines before administration in humans (Sasmito et al., 2017).

Preliminary safety evaluation or acute toxicity test of AC extract after nasal administration in this study showed no death, no abnormal clinical signs, no decreasing body weight, and blood profile significant changes of all concentrations compared to the control group. The results are necessary for the development of AC extract in order to be phytomedicine for nasal administration. The result showed nasal irrigation with 75% extract binahong safe and relatively not hazardous, it is consistent with Salasanti et al. (2014) that reported *Anredera cordifolia* leaf extract ethanol LD50 was greater than 15 g/kg body weight. Repeated oral treatment of ethanol extract of A. cordifolia leaves for 90 days at doses of 0.1, 0.4, and 1 g/kg body weight did not result in any deaths, changed the behavior of the rats, affected their blood profile or biochemistry parameters, and was not hazardous to their organs (Salasanti et al., 2014).

Similar to the research of Ramadhan et al. (2023), AC extraction, formulated with transdermal binahong leaf extract patches using a formula that includes hydroxypropylmethylcellulose, (polymer), glycerol (plasticizer), oleic acid (enhancer) and tween 80 (enhancer), is safe and does not cause death in mice. The transdermal patches of AC 30% and 47% had the effect of reducing blood glucose levels in rats that had been induced by insulin but were not significantly different. Another evidence for the safety of AC also in topical application, which reported that ethanol extract preparation of 70 % AC leaf on Wistar rat did not cause a death with observation for 14 days. So AC has met the standard of safety according to Indonesian Herbal Phamacopoeia (Samirana et al., 2018)

CONCLUSION

This present study reported the safety of intranasal acute administration of extract AC without any effect on nervus olfactory function and blood profile in laboratory female Wistar rats. The maximum dose of 75% concentration did not find mortality or sign of toxicity or practically non-toxic.

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