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ACUTE TOXICITY OF METHANOL EXTRACT OF *ACORUS* SP. RHIZOMES ON THE FEMALE RATS WISTAR STRAIN

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ABSTRACT
Acorus sp. is a traditional medicinal plant that is empirically widely used by the Dayak inland communities in treating various diseases. Scientifically *Acorus* sp. has been proven to have various activities including anti-bacterial, anti-inflammatory, anti-pain, nephroprotector and hepatoprotector. This study aims to obtain a lethal dose 50 (LD50) from methanol extracts of *Acorus* sp. rhizomes and observe the effects on behavior, body weight and organ index. The acute toxicity test method was adapted from the OECD 425 guidelines using female wistar rats. The *Acorus* sp. rhizome was extracted by maceration method using methanol as a solvent. The results of the study indicate that the *Acorus* sp. rhizome contains flavonoids, phenols, tannins, triterpenoids, saponins and alkaloids. Testing the toxicity of methanolic extract of red *Acorus* sp. rhizome with a limit test of 2000 mg / kg and 5000 mg / kg, there is no death in experimental animals so that LD50 is greater than 5000 mg / kg. The conclusion of this research is the *Acorus* sp. rhizome methanol extract is in the category of relatively non-toxic.

KEYWORDS: OECD 425, *Acorus* sp. rhizome, acute toxicity.

INTRODUCTION
The use of traditional medicines in maintaining public health has long been known to us, even to this day according to estimates by the World Health Organization (WHO), 80% of the world's population is still dependent on traditional medicine (Iwuanyanwu et al, 2011). Traditional medicine is commonly known as herbal medicine which has several advantages compared to synthetic drugs. One of the traditional medicinal plants used empirically to treat various diseases by the West Kalimantan Dayak tribe is *Acorus* sp. (Purwaningsih, 2009). *Acorus* sp. rhizomes Contains alkaloids, essential oils, tannins, flavonoids, and saponins Helmi, 2010). *Acorus* sp. has properties as anti-infectious, anti-inflammatory, anti-toxic, heat-reducing, urine laxative, anti-leprosy, anti-syphilis, antioxidants, brain repair, nerve protection and hypertension as medicines for stomach aches and skin diseases (Saman, 2003).

Several studies on the *Acorus* sp. activity have been carried out including ethanol extracts of the *Acorus* sp. rhizome having an anti-inflammatory activity of 34.20 (Safrina, 2016). The ethanol extract of red jeringau has a better inhibition zone against the bacteria Staphylococcus aureus and Eschericia coli than antibiotics (Purwanti, 2016). Effective dose of red jeringau rhizome ethanol extract as nephroprotector 250 mg / kg BW (Yustria, 2018). Ethanol extract of red jeringau rhizome dose 75 mg / kg BW has analgesic effect comparable to paracetamol (Jannah, 2016) Ethanol extract of red jeringau rhizome has activity as a hepatoprotector with decreased levels of SGOT and SGPT (Susanti, 2017). However, scientific research is needed to provide proof of safety of these traditional medicines.

Drug toxicity tests in the preclinical stage include general toxicity tests with single (acute) and repeated doses. The OECD 425 method is one of the internationally accepted acute toxicity testing standards for testing product safety, including chemicals, pesticides, treatments and others. This method was chosen to test the safety of traditional medicinal products in estimating toxic doses (Setzer and Kimmel, 2003). At present there are no reports on the level of safety in the use of red jeringau rhizomes. Therefore, it is necessary to do research on the acute toxicity test of red jeringau rhizome by determining LD50 and the effect on behavior, body weight, and organ index in female rats.

MATERIALS AND METHODS
Plant materials
Acorus sp. rhizomes was obtained in the hamlet of sungai ambawang, West Kalimantan, Indonesia and determined in the laboratory of biology, Faculty of Mathematics and Natural Sciences, University of Tanjungpura, Indonesia. *Acorus* sp. rhizomes are dried in the oven, then blended until smooth and sieved.

Extract preparation
Samples of *Acorus* sp. rhizomes was extracted using maceration method with methanol. After macerated concentrated using a rotary evaporator and then dried over a water bath.

Phytochemical screening
To determine the chemical content, phytochemical screening qualitatively using a reagent like alkaloids *(Mayer, Wagner, and Dragendoff test)*, test gelatin ($FeCl_3$), Flavonoids, Terpenoids and Steroid *(Liebermann Burchard)*, tannins, phenols ($FeCl_3$), Saponin.

Animals
Female Wistar rats aged 2 months and weighing 150-250 grams were used to study the acute toxicity. Animals kept in standard cages and maintained under standard conditions of light, temperature, and relative humidity. They were fed with rat pellets and animal studies conducted under the Code Animals, Faculty of Medicine, University of Tanjungpura, Indonesia.

Preparation and administration of Test preparation
The test preparation is given in the form of methanol extract of *Acorus* sp. rhizomes that have been suspended. A suspending agent used is CMC-Na.

Acute Toxicity Test
Acute toxicity test conducted by the OECD guidelines 425: Acute Oral Toxicity Up and Down Procedure.

a. *Main Test*
Main test conducted with respect to dose levels where death occurs in a preliminary test. One test animals were given a dose. If after the 4 hour observation of the animals did not showed mortality, the dose for subsequent animal increased by a factor of 3.2 times increase in the initial dose. If it die, the dose for subsequent animal downhill developments same dose. The same dose in one animal test again. Each animal should be observed carefully for up to 48 hours before making a decision how many doses of animals used next. If the animal is given a test dose and no mortality, the dosing was stopped and all the test animals were observed for 14 days.

b. Limit Test
Limit test 5000 to see whether the LD_{50} samples are in the range of 2000-5000 mg/kgbw or are in the range above 5000 mg/kgbw. The testing procedure is performed similarly to the limit test 2000. Just that at 5000 when there is a limit test three test animals did not show the mortality, the dosing was stopped and the LD_{50} is above 5000 mg/kgbw. If there are three test animals showed the mortality, it will be the main test with the highest dose of 5000 mg/kgbw.

Observation of Acute Toxicity
Observations carried out for 14 days to test animals that did not show the mortality. The observations made qualitative and quantitative observations. Quantitative observations in the form of weight, the amount of consumption of food and drink consumption for 14 days. Quantitative observations in the form of signs of toxicity in multiple organ systems that leather and fur, mucous membranes, respiratory system, eyes, autonomous system, circulatory system, animal behavior test and some additional parameters like diarrhea, lethargy and salivation.

Analysis Data
Data obtained in the form of quantitative and qualitative data. Qualitative data is data of weight, test behavior, psychomotor activity and macro pathology. Psychomotor activity such as convulsions, salivation, diarrhea, weakness, sleep and coma. While macro pathology is done by observing the liver, kidneys, heart, lungs and spleen. Quantitative data such as the number of dead animals (analyzed using the software AOT425 to obtain LD_{50} values).

RESULTS
Research carried out has passed the ethical review with the letter number: 6623 / UN22.9 / TA.00.03 / 2019. The study was conducted using a true experimental method with a Posttest Control Group Design research design using a Wistar strain female rats.

Sample Processing
Simplicia used in research are *Acorus sp* determinasi tumbuhan evidenced by the results in the Science Faculty, University Tanjungpura, Pontianak, West Kalimantan, Indonesia. The extraction process rhizomes of *Acorus sp* with methanol yield as much as 17.66% yield. Results of phytochemical screening of *Acorus sp* can be seen in Table 1. Results of phytochemical screening *Acorus* sp. extract showed that the extract contains flavonoids, phenols, tanins, saponins, triterpenoid and alkaloids.

Table 1: Screening Phytochemicals.

No.	Examination	Rsult	Information
1.	Flavonoid	(+)	Formed red
2.	Phenol	(+)	Formed black
3.	Tanin	(+)	Formed precipitate white
4.	Saponin	(+)	Formed foam bubble
5.	Triterpenoid	(+)	Formed brown
6.	Alkaloid	(+) (-) (+)	Formed precipitate white *(Meyer)* Not formed precipitate chocolate *(Dragendroff)* Formed precipitate brownish/brick-red *(Wagner)*

The results of the examination characteristics of the methanol extract of *Acorus sp* rhizomes can be seen in Table 1. The aim is to standardize the examination so as to ensure that the final product (drug, extract, or extract product) has a constant value of certain parameters and the set especially ago. Recuirements the extract consisting of specific parameters and parameter nonspecific.

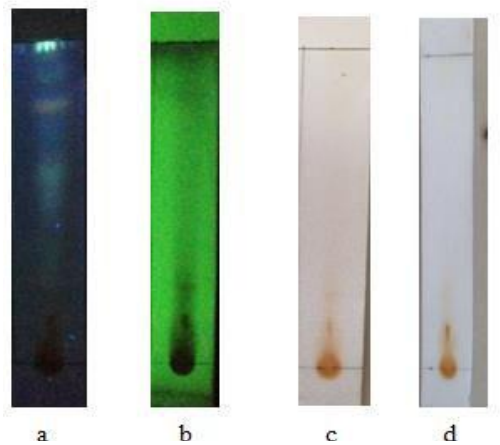

Figure 1: Patterns of chromatograms.

Description: (A) Plate under 366 nm UV lamp;
(B) plate under 254 nm UV lamp;
(C) Sprayed sight Plat before spotting
(D) Plate after spotting sight sprayed $AlCl_3$

Acute Toxicity Test

Test result acute toxicity of methanol extract of *Acorus* sp. rhizomes using female Wistar rats by using 8 test animals, which comprises 5 rats at a dose of 2000 mg/kgbw and 3 rats at a dose of 5000 mg/kgbw. Given the initial dose is 2000mg/kgbw and observed for 14 days no animal showed that mortality LD_{50} values> 2000 mg/kgbw. Continued dose of 5000 mg/kgbw in 3 rats and showed no mortality up to day 14, it can be concluded that the value of LD50> 5000mg/kgbw. This study also notice symptoms inflicted and toxic effect of a single dose of methanol extract of the *Acorus* sp. rhizomes on the function of the liver, kidney, heart, pancreas and lungs with the observation of the index index organ. Observation organs can be seen in **Figure 2.**

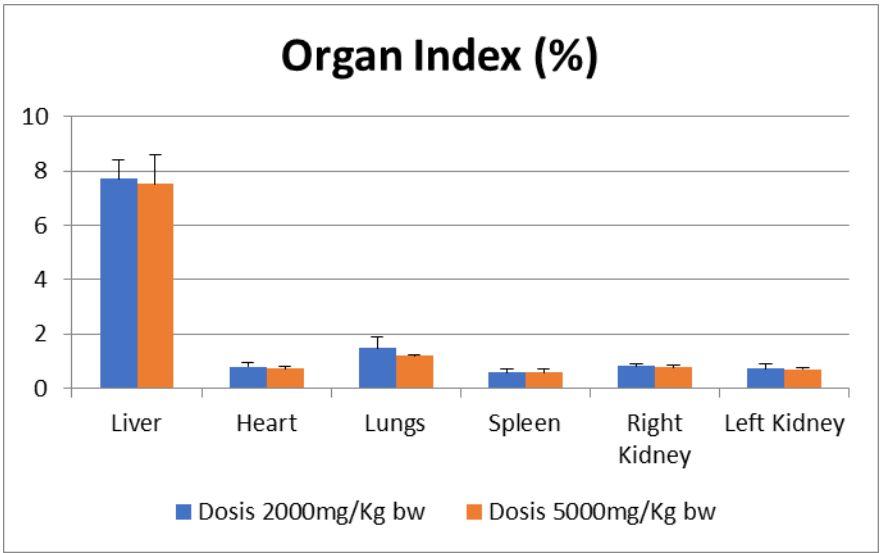

Figure 2: Observation indeks organ dosis 2000 mg/kgbw dan 5000 mg/kgbw.

DISCUSSION

Acute toxicity testing of *Acorus* sp. rhizome extract is done because *Acorus* sp. rhizome has been proven both empirically and scientifically to have a variety of activities but for testing its safety it has never been done. This study aims to determine a symptom as a result of

giving a substance and to determine the level of toxicity of the compound. Acute toxicity tests in this study using the OECD *(Organization for Economic Cooperation and Development)* 425, data were then processed using AOT425statPgm program, and observed signs of toxicity and weight change over 14 days. Test *Limit Test* 2000 mg/kgbw *Acorus* sp rhizomes can be seen in **Figure 3.**

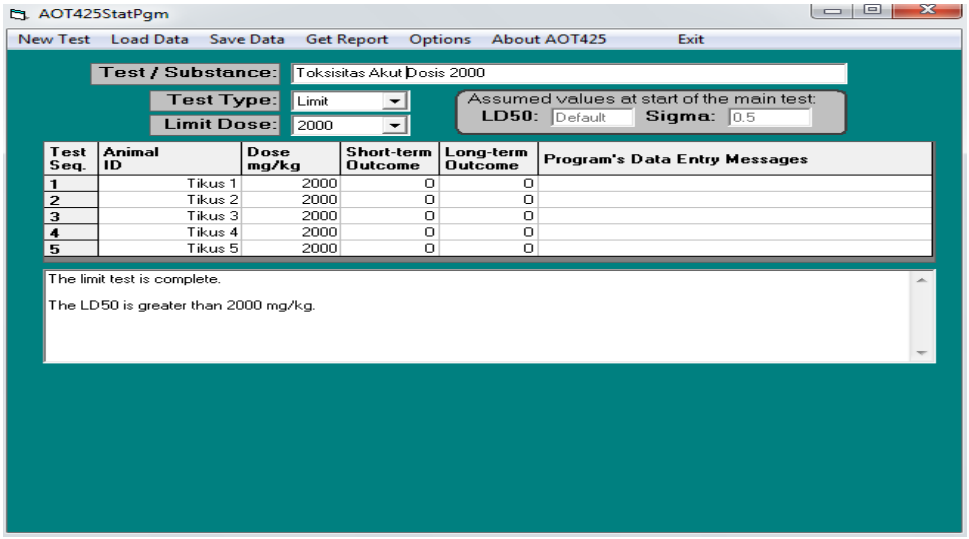

Figure 3: *Limit Test* 2000 mg/kgbw Methanol Extract *Acorus* sp. Rhizomes.

Furthermore, the test 5000 test procedure limit equal to the limit test 2000. However, the limit test 5000 if there are three test animals showed no mortality, the dosing was stopped and the LD_{50} is above 5000 mg/kgbw.

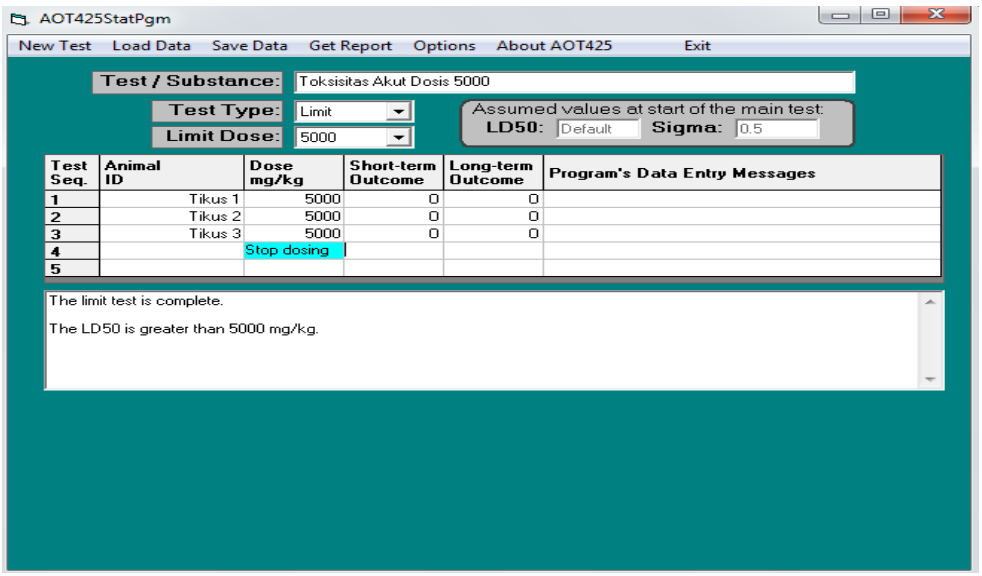

Figure 4: Limit Test 5000 mg/kgbw Methanol Extract *Acorus* sp. Rhizomes.

The data were then processed using AOT425statPgm program. *Limit Test*, Result 5000 mg / kgBB ekstrak methanol *Acorus* sp. can be seen in Figure 4. From these test results it can be concluded that the dose that can be used to test the pharmacological activity of methanol extract of *Acorus* sp. rhizomes is above 5000 mg/kgbw. Observation second subsequent weight changes test animals. Weight changes assessed by weighing the body weight of rats on day 1 to day 14 after a single dose orally. Body weight of rats before treatment and after treatment were analyzed using a test related, paired samples T test for the resulting data is parametric analysis.

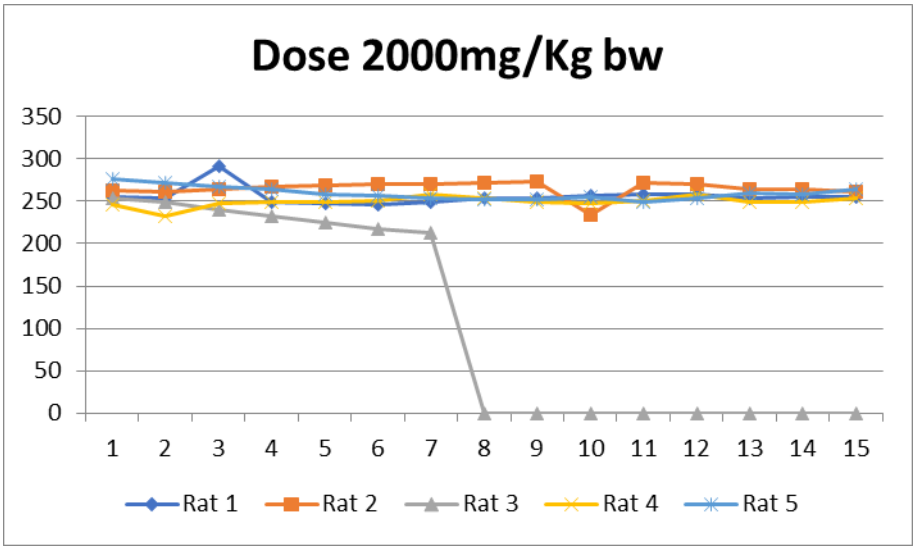

Figure 5: O**bservation of body weight of rats a dose of 2000 mg/kgbw.**

This shows that the *Acorus* sp. extract, has no toxicity to the growth of test animals. Observation of body weight of rats a dose 5000 mg/kgbw can be seen on the figure 6.

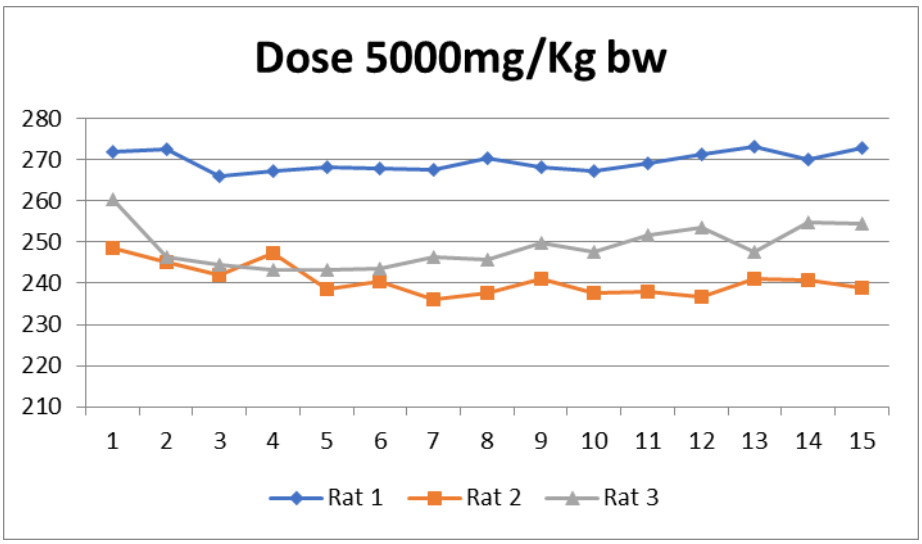

Figure 6: Observation body weight of rats a dose of 5000 mg/kgbw.

CONCLUSION

The methanol extract of rhizomes of *Acorus* sp. has a LD_{50} greater than 5000 mg/ kgbw. At the methanol extract of *Acorus* sp. rhizomes doses 2000 mg/kgbw and 5000mg/kgbw did not leave a change of behavior, psychomotor and indexes organs in rats so as not to give effect to the parameters of acute toxicity and effective dose of the methanol extract of rhizomes of *Acorus* sp.

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