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ETHNO PHARMACOLOGY STUDY, ACUTE AND ANALGESIC BAMBOO LEAVES EXTRACT ETHANOL *(BAMBUSA VULGARIS)* ENDEMIC PLANT WEST KALIMANTAN

Annafiatuzakiah
Pharmacy Department, Faculty of Medicine, Tanjungpura University
E-mail Correspondence: annafiatu_zakiah@yahoo.com

Inarah Fajriaty
Pharmacy Department, Faculty of Medicine, Tanjungpura University

Rafika Sari
Pharmacy Department, Faculty of Medicine, Tanjungpura University

Pratiwi Apridamayanti
Pharmacy Department, Faculty of Medicine, Tanjungpura University

Abstract

Objectives: The purpose of this study was to determine the benefits of bamboo leaves as empirically analgesic drugs is safe to eat. **Methods:** Ethno pharmacology study was see the benefits of bamboo leaves use empirically in the community and continued test of pharmacology, where bamboo leaves were extracted by maceration using 96% ethanol. The results of the extraction continued with pharmacological trials method toxicity test to determine the level of safety of a drug and continued with analgesic test to determine the effective dose in bamboo leaves. Rat wistar were using orally for toxicity tests consist of a dose of 2000mg/kgbwbw and dose of 500mg/kgbw to see the LD_{50} and continue with test of analgesics use mice strains Swiss consisting the positive control group (Paracetamol 500mg/kgbw), negative control (CMC-Na) and 1 dose (300mg/kgbw), second dose (600mg/kgbw), the third dose (1200mg/kgbw). Research on analgesic test using stretching to give a 0.6% acetic acid intraperitoneally and observed every 5 minutes for 60 minutes, the next is done with the data analysis. **Results:** Study Ethno pharmacology bamboo leaves empirically used as a pain medication, but clinical use of traditional medicine is not recognized, if not scientifically proven the test of pharmacology to determine efficacy as an analgesic, because not secure, study was conducted determine the lethal dose (LD_{50}) ethanol extract of bamboo leaves use Wistar rats. In the acute toxicity results obtained LD_{50}>5000mg/kgbw and organ index see no effect between the dose of 2000mg/kgbw and 5000mg/kgbw. Further research on analgesic test to determine whether there is the analgesic effect of the ethanol extract of the leaves of bamboo *(Bambusa vulgaris)* using mice Swiss strain. This study is divided into 5 in the control group positive control (Paracetamol 500mg / kgbw), Negative control (CMC-Na) and Dose 1 (300 mg/ kgbw), Dose II (600mg/kgbw), Dose III (1200 mg/kgbw), Testing the analgesic effect is done by giving chemical stimulation in the form of a 0.6% acetic acid intraperitoneally. The

response of mice were observed amount of stretching, observations were made every 5 minutes for 60 minutes. Data were evaluated with SPSS to compare the results from each dose group exhibited significantly with the controls at a rate of 0.05%. **Conclusions:** The utilization of bamboo leaves empirically showed that bamboo leaves are consumed safely and effectively as a pain medication. The results of this study are supported on the acute toxicity test LD50> 5000mg/kgbw. The next in analgesic trials showed that the ethanol extract of bamboo leaf has efficacy as an analgesic percent protection stretching on the dose I: 71.08%, dose II: 42.85%, dose III: 28.23%.
Keywords : Analgesics, Bamboo Leaf, Ethno pharmacology, Toxicity acute.

INTRODUCTION

Biodiversity is are spread in the forests of Kalimantan is very large and has many benefits as well as untapped fullest. Untapped potential of which is the potential of plants that have medicinal properties[1]. The use of plants used in medicine has long been done by people in Indonesia with a diversity of tribes and inherited[2]. Natural materials will be developed into a standardized herbal medicine in health must be eligible for and the usual dose is safe for use[4]. Traditional medicine is commonly known as a medicinal herb that has several advantages over synthetic drugs[5]. Alternative options can be expected in dealing with this is the use of traditional medicinal plants. Bamboo leaves *(Bambusa vulgaris)* is one of the traditional plants of Indonesia. Scientific classification of bamboo leaves that division Magnoliophyta, class Liliopsida, Poales order, family Poaceae, Bambusa genus, and species Bambusa vulgaris Schrad. ex J.C[5]. Bamboo leaves are often used for generations by the community as a pain relief medication to one as the gout medication[11]. Based on the class of secondary metabolites known that the leaves of bamboo has the potential to be used as an analgesic, anti-inflammatory and antipyretic[6.7]. Compounds such as flavonoids, triterpenoids and volatile oil can be used as an analgesic[8]. Terpenoids is a chemical compound that has a pharmacological effect and the toxic effects[9]. Flavonoids act as an analgesic activity by blocking the action of the enzyme cyclooxygenase which is a pain mediators like prostaglandins[10,11]. Based on the results of the phytochemical screening that has been done in the research, the need for pharmacological tests on acute toxicity test to determine the safety and analgesic continued test to determine the effective dose of the ethanol extract of bamboo leaves.

MATERIALS AND METHODS

Plant materials

Bamboo leaves was obtained in the hamlet of Sekajang, West Kalimantan, Indonesia and determined in the laboratory of biology, Faculty of Mathematics and Natural Sciences, University of Tanjungpura, Indonesia. Bamboo leaves are dried in the oven, then blended until smooth and sieved using a 40 mesh.

Extract preparation

Samples of bamboo leaf powder was extracted using maceration method with 96% ethanol. 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 and the Swiss strain male mice 2 months old and weighing 12-25 grams. 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 ethanol extract of bamboo leaves 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).

Preliminary test Analgesics

Testing the analgesic effect of bamboo leaf extract, previously conducted a preliminary test beforehand. The purpose of a preliminary test to establish something that will be done in the actual testing, in order to obtain results more valid and accurate. In a preliminary test used rats, the results did not show pain response. Further dose determination acetic acid using chemical induction method, in which the animals were given intraperitoneal acetic acid. The purpose of the orientation of acetic acid is to determine the effective dose of acetic acid capable of causing stretching which does little nor too much. Based on preliminary test results that has been done then using 0.6% acetic acid as an inducer of pain. The test material that give the paracetamol at a dose of 500mg/kgbw body weight for comparison purposes analgesic activity.

Analgesic Test

The test animals were randomly divided into five groups, each group of 5 animals test, then the test animals were fasted. Each treatment group was given orally at dose levels were determined.

Group I: negative control Na CMC
Group II: positive control paracetamol suspension
Group III: ethanol extract of bamboo leaf suspension 300 mg/kgbw
Group IV: ethanol extract of bamboo leaf suspension 600 mg/kgbw
Group V: suspension of the ethanol extract of bamboo leaves 1200 mg/kgbw

After mice were treated according the treatment group, 15 minutes later the mice induced pain stimuli that 0.6% acetic acid by intra peritoneal. Stretching mice occurs observed every 5 minutes for 1 hour.

Statistical analysis

Data are expressed as mean ± standard deviation for each group of animals at the number in figures. Statistical analysis was performed with one-way analysis of variance (ANOVA). All analysis and comparison were evaluated at 5 % ($P< 0.05$) level was considered statistically significant.

RESULTS

Studies Etnofarmakologi

The design of this study is a study etnofarmakologi that uses descriptive method with techniques used to collect the results of the interviews and the results of observations on society in Sekajang hamlet, village Tell Tembawang, sub Entikong, Sanggau district, West Kalimantan Province. According to empirical data on the activities acquired Ristoja 2015, there are eight species of plants that are known to have benefits for analgesic as a traditional medicine ingredient one of the plants of bamboo leaves. Medicinal plants used in ways consumed 3 times a day within 1 week. Adapaun way of making the herb is taken as two handheld leaf crop. Inserted in one container then crushed all the ingredients until smooth then added boiling water to taste and filtered. The herb is consumed in about 7 days with one day taken 3 times a day and the waste from these plants may be applied or transdermal on the area.

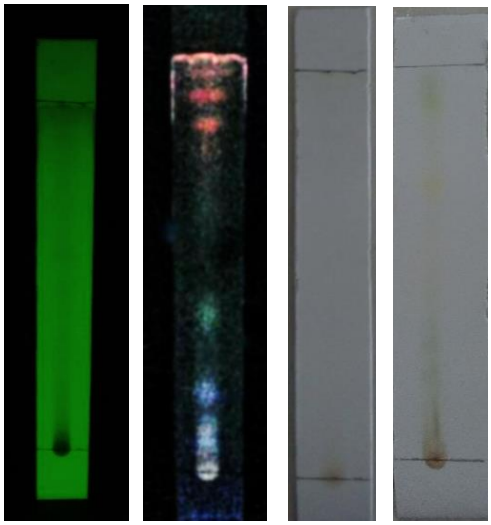
Sample Processing

Simplicia used in research are bamboo leaves *(Bambusa vulgaris)* determinasitumbuhan evidenced by the results in the Science Faculty, University Tanjungpura, Pontianak, West Kalimantan, Indonesia. The extraction process leaves of bamboo *(Bambusa vulgaris)* with ethanol 96% yield as much as 13.75% yield. Results of phytochemical screening of bamboo leaves (Bambusa vulgaris) can be seen in Table 1. Results of phytochemical screening bamboo leaf extract showed that the extract contains flavonoids, phenols, saponins and triterpenoid.

Table 1. Screening Phytochemicals

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

The results of the examination characteristics of the ethanol extract of bamboo leaves *(Bambusa vulgaris)* 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.



A B C D

Figure 1. Patterns of chromatograms

Description: (A) Plate under 254 nm UV lamp; (B) plate under 366 nm UV lamp;
(C) Sprayed sight Plat before spotting
(D) Plate after spotting sight sprayed H_2SO_4 10% in methanol

Acute Toxicity Test

Test result acute toxicity of ethanol extract of leaves of bamboo *(Bambusa vulgaris)* using female Wistar rats by using 10 test animals, which comprises 5 rats at a dose of 2000 mg/kgbw and 5 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 5 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 ethanol extract of the leaves bamboo *(Bambusa vulgaris)* 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.**

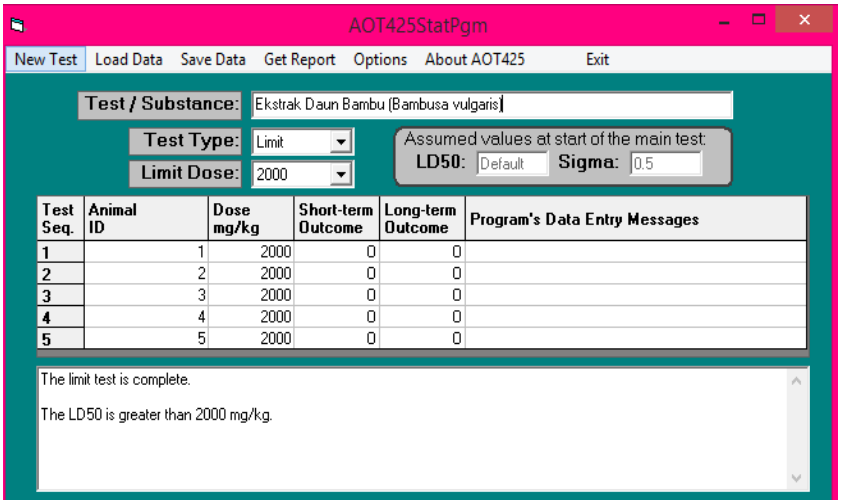


Figure 2. Observation indeks organ dosis 2000 mg/kgbw dan 5000 mg/kgbw

Test Analgesic

Test result analgesic test of ethanol extract of leaves of bamboo *(Bambusa vulgaris)* using Swiss with strain of male mice using 30 test animals with a 5 groups of pain dosis. Stimulus provided in the form of acetic acid intraperitoneally and observed the amount of wriggling every 5 minutes for 1 hour. The results of testing analgesic effect that the effective dose of 300mg/kgbw dose whichcomparable to the positive control paracetamol 500 mg/kgbw. The average amount of stretching can be mice in each test group are shown in Table 3. Furthermore done with data analysis using SPSS. Based on the results show, the test showed that the analgesic effect of stretching the number of mice at doses of 1.2 and 3 groups increased compared with positive group and decreased compared to the negative group. It can be seen that the amount of stretching will increase until the 15 minute to the 25 minute after minute 25 the amount of stretching will decrease.

DISCUSSION

Description that leaves Bamboo *(Bambusa vulgaris)* in Village Sekajang can be used as an herbal remedy to overcome the pain. So that the people eating lots of bamboo leaves as traditional medicine is effective as a pain medication. However, the use of herbal medicine is given to know the safety and effectiveness as an analgesic that is necessary to study the acute toxicity and continued on the analgesic activity test. Acute ujitoksisitas research aims to see the bamboo leaves toxic effects that occur in a short time, through a single oral administration or with repeated doses within 24 hours. 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 hari. Test *Limit Test* 2000 mg/kgbw extract ethanol bamboo leaves can be seen in **Figure 3.**

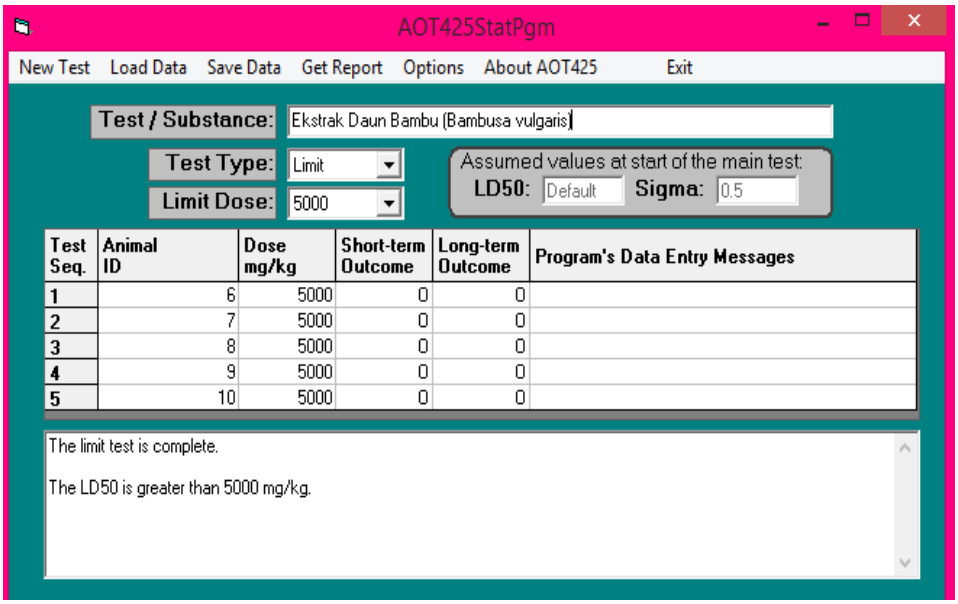


Figure 3. *Limit Test* 2000 mg/kgbw Ethanol Extract Bamboo Leaf.

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

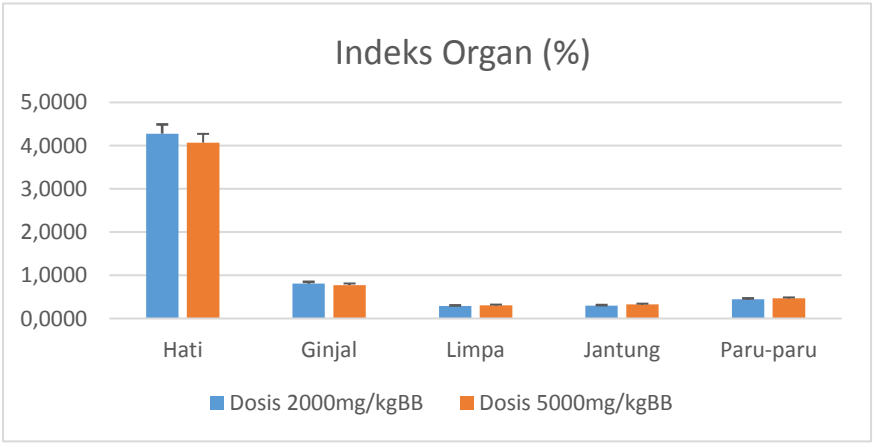


Figure 4. Limit Test 5000 mg/kgbw Ethanol Extract Bamboo Leaf.

The data were then processed using AOT425statPgm program. *Limit Test*, Result 5000 mg / kgBB ekstrak ethanol bamboo leaves 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 ethanol extract of bamboo leaves 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.

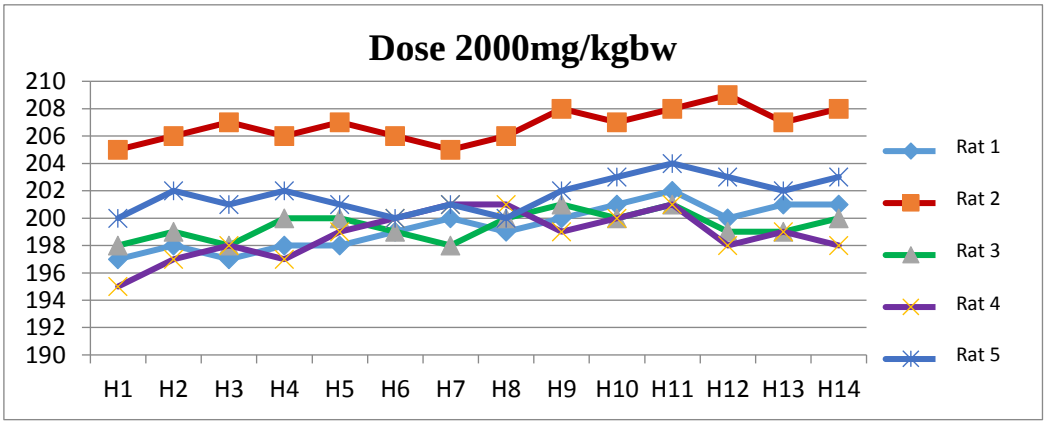


Figure 5. O**bservation of body weight of rats a dose of 2000 mg/kgbw**

This shows that the bamboo leaf 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.

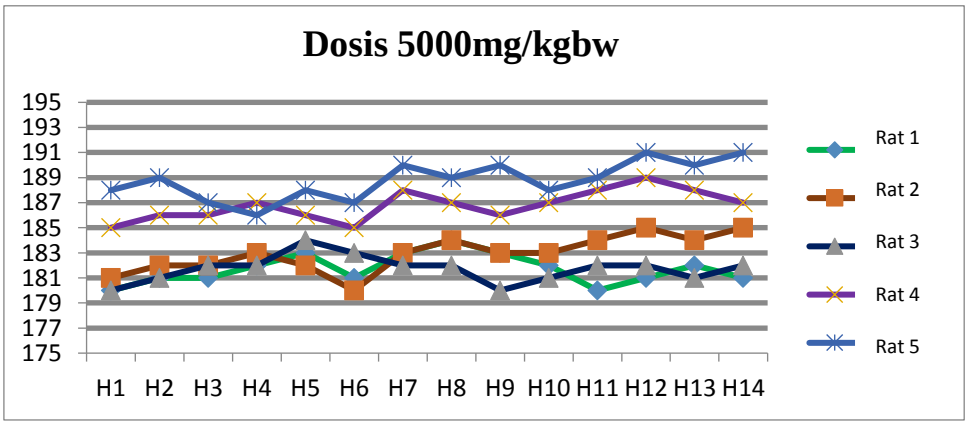


Figure 6.Observation body weight of rats a dose of 5000 mg/kgbw

Furthermore analgesic test, the method used in this study is a method of stretching a modified based on a preliminary test. Induction used in this study is acetic acid. Stretching method which uses acetic acid is a sensitive method to determine the

effects analgesik. Concluded that the highest average amount of stretching at minute 15 to minute 25 and the highest amount of stretching at minute 15 to minute 25 means it can be said that acetic acid will lead to maximum effect in the 15th minute to the 25th minute. The average cumulative amount of stretching can be seen in Figure 7.

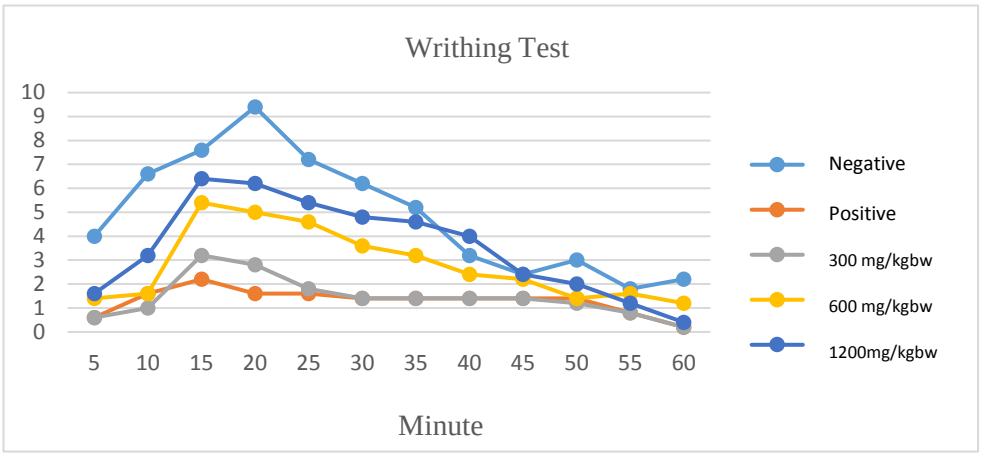


Figure 7. The average amount of stretching for 1 hour with 5 groups

The average cumulative amount of stretching decreases with lack of preparation dose of ethanol extract of bamboo leaves that successive 300, 600, 1200 mg/kgbw but the amount of stretching at least going to treatment with the positive control that paracetamol doses of 500mg/kgbw. Preparations test that indicates the number of stretching means having a power greater analgesic getting smaller, because the analgesic able to reduce pain in mice induced by acetic acid so that the movement of the perceived pain will be reduced.

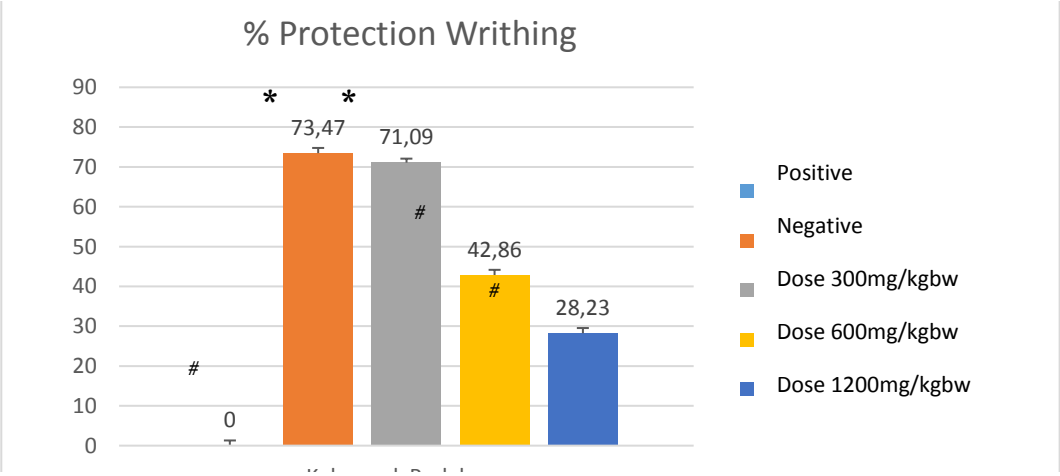


Figure 8. Inhibition of total writhing mice

Information: * : Different meaningful and control negative
: Different meaningful and control positive

The percentage of inhibition was obtained by comparing the average number of writhing group of test material to the negative control group, the percentage protection obtained writhing group of paracetamol 500mg/kgbw have a percentage of 73.46% protection stretching, followed by the first dose of the extract 300 mg/kgbw body weight of 71.08 %, extract II dose 600 mg/kgbw body weight of 42.85% and a dose of extract III 1200 mg/kgbw body weight of 28.23%. Percent analgesic power function to determine whether there is an analgesic effect on the ethanol extract of bamboo leaves at a dose of 300 mg/kgbw, 600 mg/kgbw, and 1200 mg/kgbw. Based on the results that have been done, test the analgesic effect shows that the number of mice writhing test substance dosage groups I, II, and III increased compared to the negative control group. This shows that the bamboo leaf extract can reduce the incidence of writhing in mice as a response to pain induced by intraperitoneal administration of acetic acid. Allegedly flavanoid compounds act as analgesic mechanism of action inhibits the action of the enzyme cyclooxygenase and will reduce the production of prostaglandins by arachidonic acid, thereby reducing pain[12].

CONCLUSION

The ethanol extract of leaves of bamboo *(Bambusa vulgaris)* has a LD_{50} greater than 5000 mg/ kgbw. At the ethanol extract of leaves bambu 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 ethanol extract of leaves of Bamboo *(Bambusa vulgaris)* is a dose of 300 mg/kgbw body weight to provide the analgesic effect comparable to a positive control which is paracetamol 500mg/kgbw.

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CONFLICT OF INTEREST

None to declare.

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