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Research Article

THE ACUTE TOXICITY OF METHANOLIC EXTRACT OF *EURYCOMA LONGIFOLIA* JACK ROOTS AND HISTOPATHOLOGIC CHANGES OF RAT VITAL ORGANS

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Abstract: The acute toxicity of methanolic extract of *Eurycoma longifolia* Jack roots have been studied on rats. The study was based on increasing used of *E. longifolia* Jack roots for maintaining health and treatment by the people. This experimental study used "completely randomized design" and 30 rats were divided in 5 groups (n = 6 each group) for treated and control group. The rats were given tested extract with a single dose 0; 25; 75; 225; 625 mg/kg BW/day orally. The observed parameters were the fifty percent of lethal dose, its influences on toxicological profile, the development of body weight and mortality. The microscopic observation of vital organ (kidney, stomach, and liver) have been done on fifteenth day. The results of acute toxicity test after giving the single dose extract to males and females rats showed that the fifty percent of lethal dose (LD_{50}) is 7498.94 mg/kg BW, orally. The increasing of body weight was shown on dose 75 mg/ml and 225 mg/kg BW. Hematoxylin-eosin stain was used to evaluate the histopathologic changes of rat vital organs (kidney, liver , heart, stomatch) and showed the apparent change on dose 675 mg//kg BW like necrosis. The symptom of toxicity were shown on dose 225 and 675 mg//kg BW such as depression, shallow breath, convulsion, coma and then died.

Key**words:** Acute toxicity, LD_{50}, *Eurycoma longifolia* Jack..

INTRODUCTION

The increasing of drug prices and the limited purchasing power of people, making traditional medicine as an alternative for the purpose of maintaining their own health and treatment[1]. Although the use of traditional medicine is widespread and entrenched, but its use has not been supported by scientific study as well as modern drugs. The truth efficacy and safety of a drug, including traditional medicines have been proven through a scientific study and the test is performed to prove the safety of a drug, known as toxicity tests. Toxicity test not only for modern drugs but also important for traditional medicine, especially for traditional medicine most commonly consumed by the people.

One of the most popular traditional medicine is *Eurycoma longifolia* Jack, known as "tongkat ali" or "pasak bumi" plant in Indonesia. *Eurycoma longifolia* Jack is the most widely used traditionally to treat a variety of diseases such as malaria, inflammation, aphrodisiac, cancer and as a tonic drug[2]. In Malaysia, the roots of this plant is best known as an aphrodisiac to address male sexual dysfunction[3,4].

The roots of this plant contain compounds such as quassinoids, xanthinon alkaloids, β carboline alkaloids, triterpene trycullane, squalene and biphenolneolignane derivatives[5]. The main compounds are a class of quasinoid with skleton structure composed of C_{18}, C_{19}, and C_{20}. Extraction is performed on the root of *E.longifolia* Jack standardized contains quassinoid with three main types namely, eurycomanone, 13 β, 21 α-dihydroeurycomanone and 13 21-epoxyeurycomanone[6] have reported that quassinoid include eurycomanone as antimalarial, euryomanol and 13 - (β -epoxy) eurycomanone efficacious as antiulcer.

The scientific studies of the roots, contain quassinoid derivatives that active against *Plasmodium falciparum* in vitro[6]. The other studies have suggested that the roots of *E. longifolia* Jack which grown in Thailand contain quasinoid that have antimalarial activity and cytotoxic effects[7]. Its antiplasmodial activity was tested in vitro and eurycomanone is the most potent active compound and have cytotoxic effects against nasopharyngeal epidermal cells carcinoma[8].

Beside that, the extract can raise blood levels of testosterone[9]. Based on pharmacological studies obtained information that compounds in this plants can inhibit cancer cell growth, whereas quasinoid compound also serves as a potential anti-leukemia and anti-HIV[10].

The chemical compounds contained within the *E. longifolia*, Jack has attracted the attention of many researchers due to a fairly broad biological activity[9]. Traditional medicinal products containing the roots of this plant attracted many people in the world, not only because it can cure malaria, cancer, aphrodisiac, but this natural ingredient is also the most widely used as

an ingredient in the drug-tonic$_{10}$. Its use as a tonic is very popular not only consumed by men but also used by women[11].

In addition to in vivo studies in mice infected *Plasmodium berghei*, were obtained ED_{50} values between 11.2 mg / kgBW[12]. But in the study of acute toxicity tests of ethanolic extract orally in mice, LD_{50} values has obtained 2.6 g / kg BW, and the symptoms of toxicity were found such as depression, shallow breathing and convulsions, in that study 95% of mice died at a dose of 0.43 g / kg[13].

Acute toxicity test of methanolic extract of *E. longifolia* Jack roots through oral administration in rats has not been done. The widespread use of traditional medicines containing *E.longifolia* Jack roots in the community, it is felt necessary by the researchers to assess the acute toxicity on rats that can be known the safety limits. It is hoped that the results of this study can be useful as an initial information to the public to prevent side effects or toxic effects resulting from the use of traditional medicines containing the roots of *E. longifolia* uncontrolled[14].

Based on the things mentioned above, this study was conducted to determine the median lethal dose (LD_{50}) of methanolic extract of *E. longifolia* Jack roots on rats, the symptoms of toxicity, the profile change of vital organs microscopically after oral administration[15].

MATERIALS AND METHODS

Materials:

The roots of *E. longofolia* Jack were collected from 4 years old plants that grown in Study Forest Park, Faculty of Forestry University of Lambung Mangkurat, Banjar Baru, South Kalimantan. The root have been deposited and identified by a specialist (Prof Dr.Wahyono,SU) from Departement of Biology Pharmacy, Faculty of Pharmacy University of Gadjah Mada. A voucher speciment No BF/123/Ident/Det/III/2010. In this study the methanolic extract of *E. longifolia* Jack roots is used as test drug that made by standard method.

Preparation of White Rats (*Rattus norvegicus*)

Experimental animals (rats) were acclimatized for 1 week before the study is done in a way kept in cages measuring 50 x 30 x 50 cm with each cage containing 6 individuals (male and female, five groups, n=6). Cages placed in a room kept clean, with a 12-hour cycle of light and 12 hours of dark light. Prior to testing, animal were weighed and then fasted for 12 hours but the water is still given, then taken to a laboratory for adaptation to the environment. Food animal given back 6 hours after administration of the test drug[16]. The study protocol was appoved by Animal Ethics Comittee, Faculty of Medicine University of Syiah Kuala

Acute Toxicity Test Procedure and Parameters

The serial test dose given only once, on day 1 after acclimatization. Serial test dose of the extract administered orally (25 mg/ml, 75 mg/ml, 225 mg/ml, 675 mg/ml) using a metal sonde to experimental animals (1 ml/200g BW). As a negative control is used distilled water.

The observation of toxicity symptoms in the form of behavioral changes conducted 0.5 - 1, 1.5 – 2, 3-6 hours after administration of the test dose. Development of the body weight of rats was measured three (3) times during 1 week, that is on day 1; 3; 5 and day 7 after administration of the test drug and the number of deaths was calculated for 24 hours on day 1.

Subsequently the animals were still alive and observations continued 2 times a day for 14 days. On day 15 the autopsy was performed by microscopic examination of the kidney, liver, heart and stomach. The development of body weight and the profile changes of vital organ were measured and compared with the control group. Furthermore the number of the rats death in the treatment group used to calculate lethal dose 50 (LD_{50}) by the method of Weil CS (1952).

Histological Assessment

Rats were sacrificed by cervical dislocation and subsequently kidney, liver, heart and stomach collected from each rat and fixed with 10 % buffered formalin. Paraffin blocks were prepared and sections of 5μm were cut on a microtome and stained with hematoxylin and eosin. The tissue sections were examined and compared with negative controls.

Data analysis

All data were collected and processed statistically, the lethal dose 50 (LD_{50}) was calculated by the method Weil CS (1952). The body weight development between treatment groups were examined using Analysis of Variance (ANOVA) at the level of significance 0.05 and the results were expressed as mean ± SD. The symptoms of toxicity or changes in animal behavior qualitatively assessed by Ngatidjan guide (2006).

RESULT AND DISCUSSION

The result of maceration process of *E. longifolia* Jack powdered roots yielded 50 gram of dark brown extract. The number of animals death were 3 of tested group with dose 225 mg/kg BW and 6 with dose 675 mg/kg BW and the observation performed for 24 hours after oral administration of the extract. Animal mortality data were used to calculate lethal 50 dose (LD_{50}) using the method of Weil CS manually and obtained LD_{50} value 7498.94 mg/kg BW.

The LD_{50} is defined as "a single dose of a compound that is statistically expected to kill 50% of test animals". Other researchers obtained LD_{50} value of the methanolic extract in mice of 6.180 mg / kg[13], whereas the LD_{50} value of the ethanolic extract in mice at 2600 mg / kg orally.

The difference in the LD_{50} value of the extract can be affected by various factors, such as the concentrations of compounds present in the extract, species, age, weight, sex of animal used, nutritional, environmental temperature, humidity, and air circulation. Besides that the LD_{50} value is also influenced by health factors, animal stomach contents, route of administration and dosage form drug testing and how the implementation of the acute toxicity test.

The observations on the development of animal body weight (rats) were performed for 7 days after administration of a single dose of the extract. Weighing were performed before treatment on day 1; 3; 5 and 7 after oral administration of the extract shown in Table 1. The result showed that on day 1 there was no significant difference between all treatment groups. The same analysis conducted on the development of body weight on day 3, the results showed that there was no significant difference between the control groups vs. group 25 mg /kg BW, as well as between group 75 mg / kg BW vs group 225 mg /kg BW. However, a significant difference (*) was seen between the control groups vs 75 and 225 mg/kg BW. On day 5 there is a significant difference (*) between all treatment groups, except between group 75 and 225 mg/kg BW. Whereas on day 7 there are significant differences (*) between all dose groups, except group 75 and 225 mg/kg BW.

The weight development in 675 mg/kg BW group can be observed only on day 1, because all experimental animals in this group died within 24 hours after being treated with the extract. The decrease or increase in body weight in animals due to treatment is also influenced by various factors such as: nutrition, environmental temperature, the potential toxicity of the compound contained in the extract.

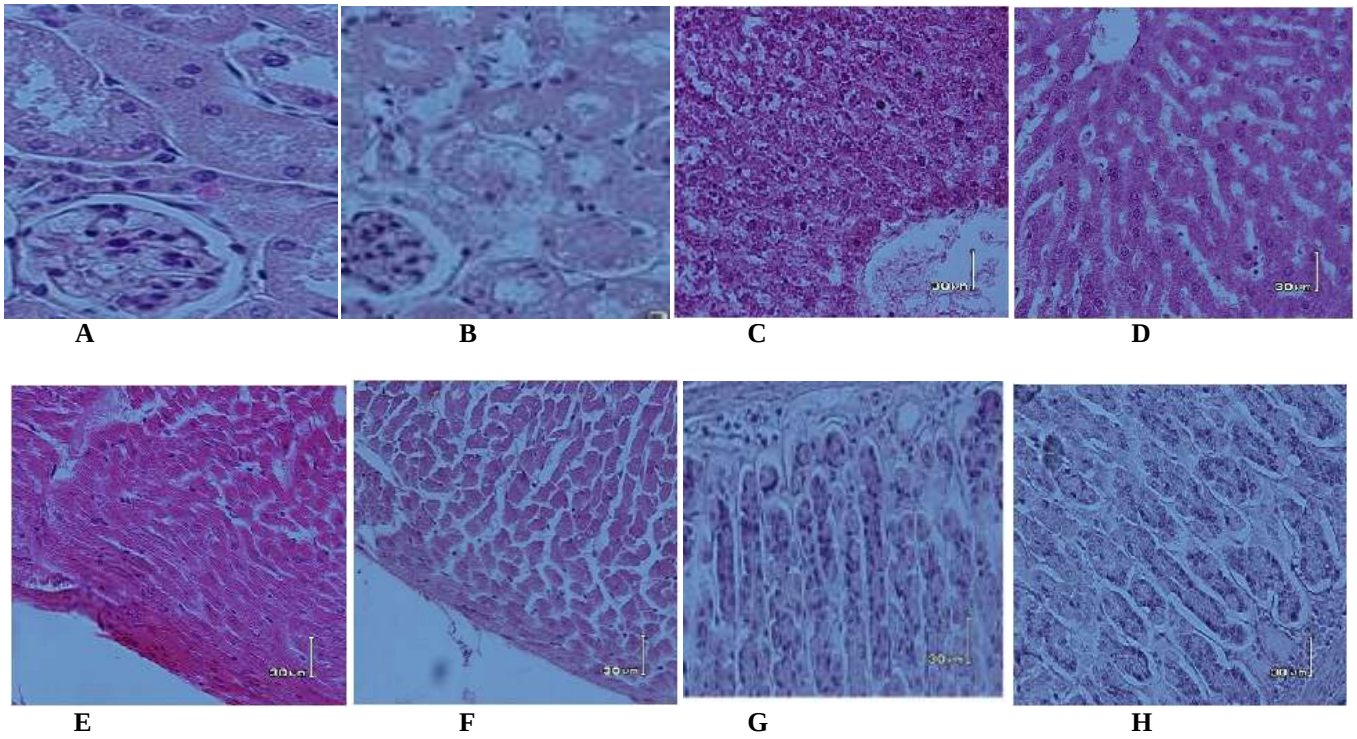
Symptoms of toxicity animals were observed before and after administration of single dose and the behavioral observation covers locomotoric activity, central nervous and autonomic nervous system, defication and urination (Thompson, 1985). Oral single dose administration of the extract didn't affect the behavior of male and female rats compared to controls group during the intensive observation in 0,5 -1 hour. However, after 1.5 - 2 hour administering a single dose of the extract showed an increase in locomotor activity behavior and aggressiveness. Sensitivity to pain slightly increased in group dose 225 and 675 when compared with the control group. Reflexes and awareness increased with increase in dose while the respiratory rate showed more increased in those groups.

On behavioral observations after 3-6 hours of the extract in group 225 to group 675 showed significant differences in behavior compared to the control group. The acute toxicity test results after administration of the extract with single dose 675 mg /kg BW orally seen a decrease in locomotor activity, aggressiveness, reflexes, awareness and tremor when compared to the control group. While symptoms of toxicity begin to occur in toxic doses 225 - 675 mg / kg BW in the form of depression, shallow breathing, convulsions, coma and death.

Table 1. The Development of Rats Body Weight on Acute Toxicity of The Extract *Eurycoma longifolia* Jack Roots

Level doses (mg/kg BW)	Rats Body Weight			
	Day 1*	Day 3**	Day 5**	Day 7**
0	158,95 ± 5,21	134,52 ± 5,49	169,38 ± 7,75	155,47 ± 6,03
25	159,28 ± 7,16	133,15 ± 8,03	151,33 ± 9,88	162,50 ± 9,84
75	177,78 ± 12,49	153,73 ± 11,01	191,32 ± 14,07	191,23 ± 14,68
225	181,22 ± 7,18	152,40 ± 2,16	187,20 ± 9,01	199,20 ± 14,50
675	185,87 ± 10,30	-	-	-

***** before given the extract of *E.longifolia* Jack roots
****** after given the extract of *E.longifolia* Jack roots



Histologic specimens of rats tissues were collected after 16 days of treatment. Tissue samples were stained with hematoxylin and eosin. The histological pictures were taken at following magnifications at 400x (A: Kidney; C: Liver; E: Heart; G: Stomatch of control grroup) and (B: Kidney; D: Liver; F: Heart; H: Stomatch of treated grroup in dose 675 mg/kg BW).

Conclusions

The lethal 50 dose (LD_{50}) of methanolic extract of *Eurycoma longifolia* Jack roots using the method of Weil CS manually were obtained LD_{50} value 7498.94 mg/kg BW, orally. The administration of a single dose of the extract with doses up to 225 mg / kg BW affect on the development of animal body weight (rats) during the 7 days of observation. The extract in oral administration on rats affect behavior primarily locomotor activity, aggressiveness, reflexes, awareness, sensitivity to pain and respiratory system. While symptoms of toxicity begin to occur in toxic doses 225 - 675 mg / kg BW in the form of depression, shallow breathing, convulsions, coma and died.

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