IN VIVO ANTIPLASMODIAL ACTIVITY AND ACUTE TOXICITY OF STANDARDIZED EXTRACT OF EURYCOMA LONGIFOLIA JACK. ROOT TRADITIONALLY USED TO TREAT MALARIA

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ABSTRACT

A series of studies has been conducted to prove the Eurycoma longifolia Jack. root as an antimalarial. However, the in vivo antiplasmodial activity of E. longifolia Jack. root standardized extract and its lethal dose 50% (LD50) values is unknown. In vivo antiplasmodial activity was conducted on Plasmodium berghei infected Swiss mice as malaria model with 4-day suppression methods. Sixty mice were divided into 6 groups. Five groups as treatment groups received test material with 5 various doses and one group was given distilled water as control group. Parasite growth inhibition was calculated by comparing the parasitemia at treatment groups to control group. Effective dose that could inhibit parasite growth by 50% (ED50) was calculated by probit analysis based on the relationship between dose and the percentage of parasite growth inhibition. The results showed that E. longifolia Jack. root standardized extract have in vivo antiplasmodial activity in P. berghei infected Swiss mice with ED50 value of 28.78 mg kg\(^{-1}\). Acute toxicity testing was conducted on 60 mice, divided into 6 groups. Five groups received test materials with 5 various doses as a single dose orally. One other group was given distilled water as control group. Each animal was observed for the first 24 h and observation was continued for 14 days. The lethal dose 50% (LD50) was calculated by probit analysis based on the number of animal deaths that occurred within 24 h after the administration of the test material. The results showed that the LD50 value of E. longifolia Jack. root standardized extract was 6128.71 mg kg\(^{-1}\). Therapeutic Index was calculated as ratio of the LD50 and ED50 with results 212.95. It showed high therapeutic index which indicated that E. longifolia Jack. root standardized extract has low toxicity.

Keywords: Acute Toxicity, In Vivo Antiplasmodial Activity, Eurycoma Longifolia, Standardized Extract, Therapeutic Index

1. INTRODUCTION

There were estimated 219 m cases of malaria and 660,000 deaths in 2010 and an estimated 660,000 deaths. Africa is the most affected continent: About 90% of all malaria deaths occur there. Between 2000 and 2010, malaria mortality rates fell by 26% around the world. In the WHO African Region the decrease was 33%. During this period, an estimated 1.1 million malaria deaths were averted globally, primarily as a result of a scale-up of interventions. Antimalarial drug resistance is a major concern for the global effort to control malaria. Plasmodium falciparum resistance to artemisinins has been detected in four countries in South East Asia: In Cambodia, Myanmar, Thailand and Vietnam (WHO, 2012).

Traditional medicines have been used to treat malaria for thousands of years and are the source of the two main groups (artemisinin and quinine derivatives) of modern
antimalarial drugs. Over 1200 plant species from 160 families are used to treat malaria and fever. On average, a fifth of patients use traditional herbal remedies for malaria in endemic countries (Willcox and Bodeker, 2004).

The discovery and development of new antimalarial from natural materials, especially medicinal plants is mostly conducted by researchers in the world in recent decades. For example is the discovery of new antimalarial artemisinin and its derivatives from Artemisia annua that has been used in China for centuries traditionally. This proves that the medicinal plants is a natural source of new antimalarials that still need to be explored (Li and Wu, 1998). Other herbs that are proven to have antimalarial activities such as Nigella sativa (Abdulelah and Zainal-Abidin, 2007) Vernonia staehelinoides Harv. Clarkson et al. (2004), Acalypha fruticosa, Azadirachta indica and Dendrosicyos socotrana (Alshawsh et al., 2007), Arcangelisia flava (L.) Merr., Fibraurea tintoria Lou., Harrisonia perforata (Blanco) Merr., Irvingia malayana Oliv. Benn ex., Elaeocarpus kontumensis Gagn. and Anneslea fragrans Wall. (Nguyen-Pouplin et al., 2007), Andrographis paniculata, Hedyotis corymbosa (Mishra et al., 2009), Enicostemma littorale (Soni and Gupta, 2009) and Quassia amara L. (Houel et al., 2009).

Eurycoma longifolia Jack. has been used traditionally as an antimalarial in Indonesia. The research showed that water extract of E. longifolia Jack. has antiplasmodial activity both in vitro and in vivo (Qamariah, 2002). Active Ingredients of E. longifolia root growing in Southeast Asia have been isolated and proven for their pharmacological activity by some researchers. Jiwajinda et al. (2001) succeeded in isolating several quassionoids from Thailand that were longilactone, dehydrolongilactone, 11-dehydroclaineanone, 15 β-hydroxyclaineanone, 14.15 β-dehydroxyclaineanone and 15-O-acetyl-14-hydroxyclaineanon. These active compounds have proved for their cytotoxicity and antiplasmodial activity (Jiwajinda et al., 2002). Ang et al. (1995) succeeded in isolating eurycumanone and proving its antimalarial activity.

Some studies have proved antimalarial activity and some mechanism of action of the E. longifolia root. The active compound groups including quassinoid eurycumanone was known that it was one of the compounds responsible for the antiplasmodial activity of the E. longifolia roots (Kardon et al., 1991). However the LD50 and the effective dose of E. longifolia root standardized extract containing eurycumanone as an antimalarial in experimental animals were unknown. This study was conducted to know the antiplasmodial activity of E. longifolia Jack.

standardized root extract in mouse malaria model and its LD50 value through acute toxicity testing.

2. MATERIALS AND METHODS

2.1. Animals

A total of 60 male and 60 female Swiss mice, weighing 25±5 g were obtained from Animal House Faculty of Medicine Universitas Gadjah Mada Yogyakarta. They were maintained in the room with 12 h light/dark cycle, 70% humidity, temperature around 26°C, sufficient ventilation and housed five per cage based on their sex in cages covered with wire net. All animals were given access to food and water ad libitum. Experiments were performed to minimize animal suffering in accordance with the internationally accepted principles for laboratory use and care and approved by the Medical and Health Research Ethic Committee of Faculty of Medicine Universitas Gadjah Mada Yogyakarta.

2.2. Materials

Eurycoma longifolia standardized extracts containing 2% of eurycumanone was purchased from Javaplant, Karanganyar, Surakarta. Plasmodium berghei was obtained from the Department of Parasitology Faculty of Medicine Universitas Gadjah Mada. Other materials used in this study were methanol, Giemsa stain, chloroform, formalin and immersion oil from Merck and 70% of ethanol (Indofarma). RPMI 1640 was from Sigma.

2.3. In Vivo Antiplasmodial Activity Test

In vivo antiplasmodial activity was conducted on ANKA strain Plasmodium berghei infected Swiss mice as malaria model with 4-day suppression methods according to Peters (1975). Sixty mice were divided into 6 groups (5 male and 5 female for each group). Five groups as treatment groups were given E. longifolia root standardized extract with 5 various doses (6.25, 12.5, 25, 50, 100 mg kg\(^{-1}\)) and one group was given distilled water as control. The test material and control were given once per day for 4 days. During the study, all mice were put in cages covered with wire net. Each cage was occupied by five mice based on their sex in cages covered with wire net. The test material and control were given once per day for 4 days. During the study, all mice were put in cages covered with wire net. Each cage was occupied by five mice based on their sex. On the first day, all mice were infected intraperitoneally with 200 µL erythrocytes containing 1×10\(^7\) of P. berghei at erytrocytic stages obtained from donor mice. P. berghei infected mice with parasitemia of 20-30% was used as donor mice. The percentage of parasitaemia and the number of erythrocytes per mL was calculated from donor mice, then donor mice blood diluted in RPMI 1640 medium in order to get 5×10\(^7\)/mL density. Mice were given
standardized extract with a dose of 6.25; 12.5; 25; 50; 100 mg kg day\(^{-1}\) orally for 4 days for group I-V respectively with a maximum volume of 1 mL 2 h after infection. Group VI received distilled water 50 mL kg day\(^{-1}\) for 4 days as control. The test materials or control were given once daily for 4 days. Blood of each mouse was taken from the tail end and was made thin blood smear on day 5. Thin blood smear preparations was dried at room temperature and fixed in absolute methanol for 30 sec and stained with 5% Giemsa for 30 minutes. Parasitemia was calculated based on microscopic examination by counting the number of erythrocytes infected with \textit{P. berghei} from about 1000 erythrocytes. Parasite growth inhibition by test materials was calculated by comparing the parasitemia in control group. Effective dose that it could inhibit parasite growth by 50% (ED50) was calculated with probit analysis based on the relationship between dose and the percentage of parasite growth inhibition.

### 2.4. Acute Toxicity Test

Acute toxicity test was conducted to determine the range of Lethal Doses (LD50), according to the OECD (2008). Sixty mice weighing 20g ± 5g were divided into 6 groups (5 male and 5 female for each group). Each group of 5 groups was given single dose of standardized extracts at dose 24, 120, 600, 3000 and 15000 mg kg\(^{-1}\) orally. The highest dose is the highest dose that can be administered to mice technically, determined by preliminary study). The other group was given distilled water as control group. The test material was prepared in suspension for oral administration. Each animal was observed and recorded for poisoning symptoms that arose within the first 24 h and observation was continued for 14 days. The LD50 was calculated by probit analysis based on the percentage of animal deaths that occurred within 24 h after the test material administration.

### 3. RESULTS

#### 3.1. \textit{In Vivo} Antiplasmodial Activity of \textit{Eurycoma Longifolia} Root Standardized Extract on \textit{Plasmodium berghei} Infected Swiss Mice as Malaria Model

The percentage inhibition of parasite growth in each group was shown at Table 1. Effective dose 50% (ED50) was calculated based on the percentage inhibition of parasitemia Swiss mice. The ED50 value was 28.78 mg kg\(^{-1}\), which indicates that the \textit{E. longifolia} root standardized extract has \textit{in vivo} antiplasmodial activity in Swiss mice infected by \textit{P. berghei}.

| Group and dose (mg/kg/day) | The percentage inhibition (%) of parasitemia |
|---------------------------|-------------------------------------------|
| I (6.25)                  | 29.55                                     |
| II (12.5)                 | 30.77                                     |
| III (25)                  | 43.63                                     |
| IV (50)                   | 60.86                                     |
| V (100)                   | 57.30                                     |
| VI (control)              | 0.00                                      |

### 3.2. Acute Toxicity test of \textit{Eurycoma Longifolia} Root Standardized Extract

Observations of physical conditions, toxic symptoms for both treatment and control groups of mice were performed on the first 24 h and continued every day for 14 days. The result showed that sign of toxicity was seen in experimental animals that eventually died only, exaltation before death. Other experimental animals did not show any symptoms. The observation of experimental animals death was presented in Table 2. Until the end of the study on the 15th day, 15 mice died. One mouse of group III, 4 mice of group IV and 10 mice of group V. The LD50 value which calculated based on the percentage of animal deaths that occurred within 24 h after the test material administration was 6128.71mg kg\(^{-1}\). The Therapeutic Index (TI) was calculated as ratio of the LD50 and ED50 with results 212.95, indicating that \textit{E. longifolia} Jack. root standardized extract has low toxicity.

#### Table 2. The death of experimental animals in acute toxicity tests of \textit{Eurycoma longifolia} root standardized extract on Swiss mice in the first 24 h and in 14 days

| Group (n = 10) and dose (mg/kg/day) | Percentage (%) of died mice in the first 24 h | Percentage (%) of died mice in 14 days |
|-----------------------------------|---------------------------------------------|--------------------------------------|
| I (24)                            | 0                                           | 0                                    |
| II (120)                          | 0                                           | 0                                    |
| III (600)                         | 0                                           | 10                                   |
| IV (3000)                         | 40                                          | 40                                   |
| V (15000)                         | 70                                          | 100                                  |
| VI (control)                      | 0                                           | 0                                    |

### 4. DISCUSSION

Developing new compounds from natural products could be an important source of new antimalarials in the long term, it is also possible to develop standardized and validated phytomedicines more quickly and cheaply. Much of the development work has already been done on these: Their safety has been demonstrated and they seem
Effective Dose 50% (ED50) 1.18 mg kg \(^{-1}\) \(_{vivo}\) methanolic extract of insoluble fraction also more active on fraction of ethyl acetate had a better activity (IC50 value, 0.061±0.003 µg mL\(^{-1}\)).

In this study, the standardized extract of \(E. \) longifolia root containing 2% of eurycomanone showed the LD50 value was 6128.71 mg kg\(^{-1}\) and the ED50 was 28.78 mg kg\(^{-1}\), so that the Therapeutic Index (TI) was 212.95, indicating that \(E. \) longifolia Jack. root standardized extract has low toxicity. The standardized extract showed LD50 value of 6128.71 mg kg\(^{-1}\). This result showed that \(E. \) longifolia standardized extract showed practically non toxic in mice (Loomis and Hayes, 1996). It was supported by high therapeutic index which indicated that \(E. \) longifolia Jack. root standardized extract had low toxicity. It showed that \(E. \) longifolia root standardized extract had high potent to be developed as new antimalarial phytomedicine. Further investigation in clinical study was needed to develop it. The new technology in planting of the \(E. \) longifolia need investigation. It is also to provide continuous material.

5. CONCLUSION

The result showed that the LD50 value of \(E. \) longifolia Jack. root standardized extract was 6128.71 mg kg\(^{-1}\). Therapeutic Index was calculated as ratio of the LD50 and ED50 with results 212.95. These results showed that \(E. \) longifolia Jack. root standardized extract have \textit{in vivo} antiplasmodial activity with low toxicity.

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