

**STUDY ON ANTIMALARIAL ACTIVITY OF CYPERUSROTUNDUS LINN (MYET-MOUN-NYIN) BY PLASMODIUM BERGHEI MOUSE MODEL**Myint Myint Win\*<sup>1</sup>, Zaw Lin<sup>2</sup> and Mar Too Nyi Bu<sup>3</sup><sup>1</sup>Department of Biotechnology, Technological University (Kyaukse), Myanmar.<sup>2,3</sup>Department of Biotechnology, Yangon Technological University, Myanmar.

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**ABSTRACT**

Malaria is a common, most lethal parasitic and life-threatening disease in many tropical and subtropical areas. Attack of the disease can be very severe and can even lead to death if they remain untreated. The commonly used standard antimalarial drugs were becoming increasingly resistant by *Plasmodium falciparum* (*P. faliparioum*), the aetiologic agent of severe type of clinical malaria. This study as attempted as part of the search for new antimalarial drugs from herbal plant resources. In this study, crude extract from *Cyperusrotundus* Linn (Myet-Moun-Nyin) was investigated for antimalarial activity by *in vivo Plasmodium berghei* mouse model system and found to have supprepressive action on malarial parasite. A compound,  $\alpha$ -Cyperone, extracted from *Cyperusrotundus* Linn (Myet-Moun-Nyin) was investigated for antimalarial activity by the same method. The data indicated that this compound has potential to be used as antimalarial drug. ED<sub>50</sub> was studied on  $\alpha$ -Cyperone and was observed to be 13.11mg per kg per day.

**KEYWORDS:** Malaria, Antimalarial Herbal Plant,  $\alpha$ -Cyperone, *Cyperusrotundus* Linn, *Plasmodium berghei*.**INTRODUCTION**

As our country, Myanmar, is one of the developing countries, we need more and more facilities for our health services and we are trying our best to fulfill the needs of the people. Among the health problems, malaria is one of the most serious illness affecting thousands of individuals. Africa, South America and South East Asia including Myanmar are the well-known hyperendemics area of malaria in the world (Myint 1978).

Malaria is caused by a small organism called a parasite of genus, Plasmodium. These not only infect man but also apes, monkeys, bats, birds, reptiles and other vertebrates' hosts. Human malaria is caused by four species of Plasmodium namely, *P. falciparum*, *P. malariae*, *P. vivax* and *P. ovale* (Bruce 1980). By far, the most important species is *P. falciparum*. This species can cause cerebral malaria which is often lethal (Mareton 1999). Malaria is caused by the bite of a vector, female *Anopheles* mosquito, an infected with human parasite *Plasmodium* species, the parasite develops in the gut of the mosquito (Ko1994, Mare 1999). Accordingly research on traditional medicine from plant extracts leads to help effective treatment against malaria (Nawe 2002).

The use of herbal medicines against malarial fevers has a long history. Herbal medicines have been utilized for the malaria for many years (Perry 1980). In 1998, W.H.O.

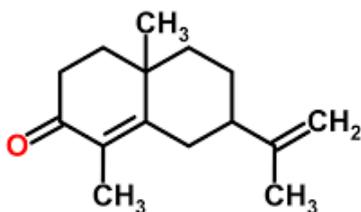
reviewed that medicinal plants are important for pharmacological research and drug development, not only their constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds (WH.O 1998). *Cyperusrotundus*, a species of sedge (Cyperaceae) is a perennial plant, named "nut grass" and "nut sedge" that somewhat resemble nuts, although botanically they have nothing to do with nuts. Figure 1 shows *Cyperusrotundus* (Myet-Moun-Nyin) and Figure 2 shows the tubers of *Cyperusrotundus*.

**Figure 1: *Cyperusrotundus* (Myet-Moun-Nyin).**



**Figure 2: Tubers of *Cyperus rotundus* (Myet-Moun-Nyin).**

Several pharmacologically active compounds have been identified from *C. rotundus* such as  $\alpha$ -cyperone,  $\beta$ -selinene, cyperene, patchoulone, sugeonol, kobusone, and isokobusone (Smith 2004). Figure 3 indicates the structure of  $\alpha$ -Cyperone compound.



**Figure 3: Structure of  $\alpha$ -Cyperone Compound.**

In Myanmar, malaria has re-emerged with substantial challenges in many forms and complexity such as border malaria, multi-drug resistant malaria, population migration and urban malaria etc. The highest mortality rate was seen in Kachin, Kayah and Shan States (Saw 2000). Drug resistance in *P. falciparum* is of such greater importance regarding frequency, degree and geographical distribution, but also because in term of significant mortality associated with falciparum malaria. The geographic distribution of chloroquine resistance has now become identical with that of autochthonous *P. falciparum* except in America and Southwestern Asia (Hnin 2000). Resistance to quinine has also become a problem in Thai/Cambodia and Thai/Myanmar border areas (Werns 1994, Sa Bai 2000). In Myanmar, *P. falciparum* resistant to chloroquine, Sulphadoxine-pyrimethamine and even to quinine has been reported. (Ye 1992).

Quinine obtained from *Cinchona* species and artemisinin obtained from *Artemisia annua* are the good examples of proven by the antimalarial potential of compounds derived from medicinal plants (Bhat, 2001). The selection of plants to be screened for antimalarial activity is done on the basis of traditional reputation of particular species for efficacy in the treatment of malaria (Phyu

2000). Vasicine compound isolated from *Adhatodavasica* Nees (Mu-yar-gyi) and admando-grapholide compound isolated from *Andrographispanniculata* Nees (Say-kha-gyi) were found to have antimalarial activity. LD<sub>50</sub> and ED<sub>50</sub> studies were done previously on andrographolide compound and were observed that this compound had high therapeutic index (Nawe 2002). The aim of the present study is to determine comparison of the antimalarial susceptibility of crude extract of herbal plant and standard antimalarial drug (Chloroquine) by using mouse model.

In this study, crude extract from *Cyperus rotundus* and also  $\alpha$ -Cyperone extracted from *Cyperus rotundus* were tested for suppressive effect on malaria parasite by *in vivo Plasmodium berghei* mouse model system.

## MATERIALS AND METHODS

### Materials

Crude Extract and  $\alpha$ -Cyperone compound were isolated from *Cyperus rotundus* (Myet-Moun-Nyin) in Pharmaceutical Research Department, MSTRD as collaborated research with Biotechnology Department.

### *In vivo* Technique and Microscopic Examination

*Plasmodium berghei* parasitized stabilate stored in liquid nitrogen was removed and mixed with phosphate-buffered saline (PBS) pH 7.2 and injected intraperitoneally into four mice using an inoculum of 0.2ml each. The parasitaemia was checked on alternate days until 5-10 percent was reached. The blood was withdrawn from the mice into a syringe containing 4 units of heparin by cardiac puncture. The bloods were pooled and parasitaemia was noted. The infected blood was diluted with pH 7.2 PBS until the required amount of parasite to be injected into the mice was adjusted to  $1 \times 10^7$  parasites/0.2ml by diluting the infected blood with PBS. When the parasitaemia reached 5-10 percent, blood was collected from the heart into a syringe containing 4 units of heparin. The blood was pooled and mixed with glycerol to a final concentration of 7.5%. Two to three thin blood films were made from the tail of mice on glass slide. And then, the blood films were dried quickly, fixed with methanol and stained with 10% solution of Giemsa. Next, the slide were washed and dried. Microscopic examination of thin blood smears, Giemsa's stain allowed for the differentiation of *Plasmodium* species responsible for malaria infection. The examination of thin blood smear, fixed and Giemsa-stained permits better species differentiation and provides information on hematological parameters but is much less sensitive and may miss low-grade infection.

### Drug Testing

The mice were intraperitoneally inoculated with  $1 \times 10^7$  infected erythrocytes in 0.2ml PBS of *P. berghei* and were then grouped randomly into 5 groups of mice each. One group was kept as untreated control group. The second control group was treated with Chloroquine. The others were treated with three different doses of the test

drug daily for 6 consecutive days. When the parasitaemia level reached between two to five percent, blood films were made daily starting from day zero ( $D_0$ ) and the activity of the drug was assessed. Doses of 10, 40 and 160mg/kg/day were given to individual groups. After 6 days of applying test compound, the experimental mice were observed for another 6 days parasitaemia counts were done and mean parasitaemia level was calculated for each group and assessed. Percent suppression was calculated for each dose daily. The drug having 25 percent suppression of parasitaemia or more in the test group compared to that of the untreated control was taken as having an antimalarial activity.

#### Determination of $ED_{50}$ Assay

Same concentration of parasites was inoculated into all the test mice. When parasitaemia level reached 2 to 5 percent, 6 mice were grouped into one test group. Mice in each tested group were given same concentration of compound ( $\alpha$ -Cyperone). Doses of 10, 40, 160mg/kg/day were given to individual groups. To plot the standard graph to determine  $ED_{50}$ , different percent suppression values were placed in Y axis and different doses values were placed in X axis  $ED_{50}$  value was determined from this group.

### RESULTS AND DISCUSSION

*In vivo* model was used in this study. Different doses were studied to have the information whether effectiveness was dose related. Peanut oil was used as a solvent in this study and oral route was selected for administration. The *in vivo* drug testing was conducted using *Plasmodium berghei* and laboratory reared DDY mice. Screening on different variable doses of crude extract and  $\alpha$ -Cyperone compound extracted from *Cyperusrotundus* (Myet-Moun-Nyin) for antimalarial activity and  $ED_{50}$  was investigated. The experimental results were expressed in terms of percent parasitaemia suppression. Percent suppression of parasite means percentage (%) of parasitaemia level reduced in treated group of mice due to tested drug than untreated control group of mice. The antimalarial effect of crude extract and  $\alpha$ -Cyperone compound were compared with standard drug Chloroquine as positive control and untreated control. Crude control was found to have effective suppression on parasite. The therapeutic effect of  $\alpha$ -Cyperone on *Plasmodium berghei* infection in mice is shown in Figure 4. Experimental results showed 66.17% suppression for dose of 10mg/kg/day. Chloroquine control group had 67.17% suppression in parasitaemia level as compared to untreated controls.

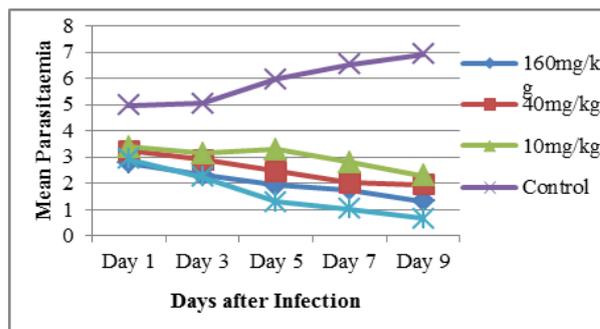


Figure 4: Therapeutic Effect of  $\alpha$ -Cyperone on *Plasmodium berghei* Infection in Mice.

*In vivo* testing experiment of  $\alpha$ -Cyperone compound, the varying dose from 10mg/kg $\times$ 2 times per day to 160mg/kg $\times$ 2 times per day were given to each group of mice showed the therapeutic effect of  $\alpha$ -Cyperone on *P. berghei* in mice. Figure 5 indicates the percent suppression by  $\alpha$ -Cyperone on *Plasmodium berghei* infection in mice.

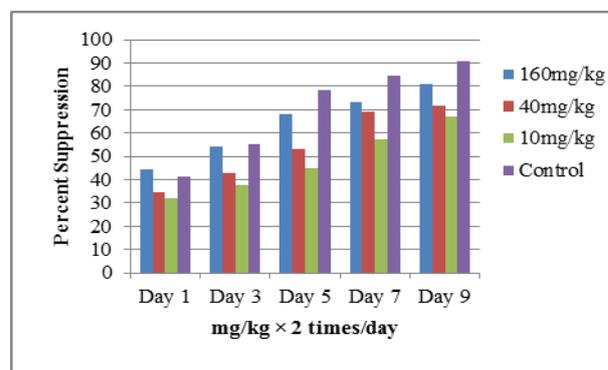


Figure 5: Percent Suppression by  $\alpha$ -Cyperone on *Plasmodium berghei* Infection in Mice.

Figure 6 shows the relationship between dosage (mg/kg/day) and percent suppression is positively related. The regression line was  $y = 0.1018x + 48.237$ ,  $R^2 = 0.9541$ . As a result, medium effective dose  $ED_{50}$  for  $\alpha$ -Cyperone compound was worked out as 13.11mg/kg/day. This study indicated that the mice treated with three varying doses of both crude extract and  $\alpha$ -Cyperone compound respectively showed effective antimalarial activity.

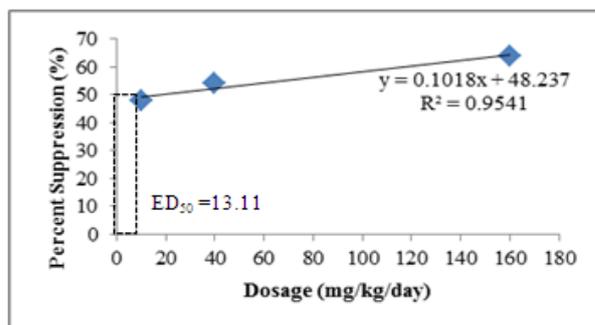


Figure 6: Plot showing Administration Medium Effective Dose of  $\alpha$ -Cyperone Compound Orally.

In Myanmar, *Plasmodium falciparum* resistant to antimalarial drugs has been observed since 1969 with gradually increasing degree of resistance level. The hospital based 28-days *in vivo* studies in Tharyarwadyand Mingaladon showed *P. falciparum* was 72% to 100% resistant to 4 amino-quinolines (chloroquine and amodiaquine), 16% resistant to quinine, 63% to 65% resistant to mefloquinesulfadoxine-pyrimethamine combination (MyintLwin 1997).

The consumption of medicinal plants has almost doubled in Western Europe, and sales of pharmaceutical drugs so called phytomedicines are rapidly increasing. According to the WHO report, a large proportion of the population in many developing countries relies heavily on traditional practitioners and medicinal plants to meet primary health care needs (WHO 1993). To replace the antimalarial drugs, which are expensive and increasingly resistant by *P. falciparum*, screening of extracts from naturally grown medicinal plants in Myanmar was done for the antimalarial activity *In vitro P. falciparum* red blood cell culture and *in vivo P. berghei* mouse model system were used to screen the extracts. Vasicine compound isolated from *Adhatodavastica* (Mu-yar-gyi) and andrographolide compound isolated from *Andrographispaniculata* (Say-khar-gyi) were tested for antimalarial activity. Both were found to have suppressive effect on malaria parasite. These compounds were administered orally to the experimental animal. LD<sub>50</sub> and ED<sub>50</sub> studies were done on Vasicine and andrographolide (Zaw2001, Nawe 2002). Injection form of andrographolide compound was studied for antimalarial activity and found to be as effective as Chloroquine. Extract (Myet-Moun-Nyin) was found to be effective against malaria parasite *in vitro* (Nawe 2002).

In this study, extract of Myet-Moun-Nyin was investigated for its antimalarial activity. The data agreed with *in vitro* test result. Using extract for treatment of microbial diseases are dangerous because some ingredients of that extract can have toxic effects on humans. It is a good practice to isolate different compounds from the extract and study the antimalarial effect of each compound singly. This  $\alpha$ -Cyperone was observed to have using *P. falciparum* clinical strains as challenging parasite.  $\alpha$ -Cyperone, a compound extracted from Myet-Moun-Nyin, was studied for its antimalarial activity in this research work. The data from this research suggested that the antimalarial effect of  $\alpha$ -Cyperone was highest with the highest dosage and vice versa. The effect was found to be comparable to Chloroquine which as a standard malaria drug with long history of effectiveness before emergence of resistant strain. During the first 3 days of administration of the compound, the suppressive effect was quite approached to the level achieved by the standard drug Chloroquine. However, after 3 days of administration, the suppressive level was low when compared to that by Chloroquine. This data indicated that the duration of action was shorter

than Chloroquine. From these data, ED<sub>50</sub> was worked out and observed to have effectiveness at low dosage.

#### ACKNOELEDGEMENTS

In the other study, LD<sub>50</sub> was observed to have lethal effect on oral administration up to 2560mg/kg/day (Nawe 2002). Thus as a conclusion this oral  $\alpha$ -Cyperone compound was assumed to be have high chemotherapeutic index.

#### CONCLUSION

Previous *in vitro* study indicated that crude extract of Myet-Moun-Nyin was found to have antimalarial activity. In the present study, the fact was confirmed by *in vivo* method and the data indicated that the crude extrat had antimalarial activity. The  $\alpha$ -Cyperone, a compound isolated from Myet-moun-nyin was studied for antimalarial activity. This compound was observed to have suppression on *P. berghei* in experimental animals. Different doses of oral  $\alpha$ -Cyperone were administered and ED<sub>50</sub> was worked out from the data. ED<sub>50</sub> was estimated to be 13.11mg/kg/day. To become a complete *in vivo* study, it is essential to be proceeded to investigation of chronic toxicity and pharmacokinetics in the experimental animals.

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