THE ANTI-MALARIAL ACTIVITY OF MOMORDICA CHARANTIA IN COMBINATION WITH THE STANDARD ANTI-MALARIAL DRUG CHLOROQUINE

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ABSTRACT:

Malaria remains one of the most widespread global health problems which are caused by Plasmodium sp. and health threat because of the high mortality associated with its resistance to common anti-malarial drugs. Thus, development of new therapeutic strategies against malaria is urgently needed. One strategy involves the potential use of traditional medicinal plants to address this resistance. M. charantia is a medicinal plant which has been reportedly previously to exhibit many pharmacological activities. The aim of this study is to evaluate M. charantia fruit aqueous extract in combination with the standard anti-malarial drug chloroquine (CQ) using a murine model of malaria (Plasmodium berghei NK65). The administration of the M. charantia extract at doses 50, 75 and 100 mg/kg bw in combination with CQ at dose 3 mg/kg bw into ICR mice infected with P. berghei for four consecutive days resulted in significantly anti-malarial activity (dose-dependent manner) exceeding 60%. In addition, the combination treatment also prolonged median survival time of infected mice. Findings from the present study indicate that the combination treatment with chloroquine enhanced the anti-malarial activity of M. charantia as a medicinal plant suggesting the potential use of M. charantia in adjunctive treatment for malaria.

Keywords: Malaria, M. Charantia, Combination Treatment, Chloroquine

I. INTRODUCTION

Malaria remains one of the leading cause of morbidity and mortality in sub-Saharan Africa, Asia, and developing countries. This dilemma is partially due to the rapid spread and development of Plasmodium parasites resistant to standard drugs (malaria report, 2019). Hence, alternative strategies for malaria elimination are urgently needed. In tropical countries such as Southeast Asia countries, malaria is a significant cause of death. The anti-malarial drugs have become standard and fundamental tool in helping to eliminate malarial disease (Klein 2013). New therapeutic steps in treating the parasites are continuously researched as more cases of developed resistance to the conventional drugs have been demonstrated. WHO suggested the Artemisinin-based combination therapy (ACT), as a new therapy for controlling malaria and reducing the resistance of parasites to drugs, increase the effectiveness of anti-malarial drugs, and provide a safe, low cost, and available therapy. Therefore, WHO used combination therapy as the first-line of malaria treatment. Chloroquine (CQ) was used as the primary drugs for the treatment of malarial infection (Hobbs and Duffy, 2011). When the CQ resistance had spread, the treatment of P. falciparum by combination therapy (ACT) was proposed. CQ may be combined with tigecycline antibiotic or cephaparum compound to reduce the resistance and develop the combination therapy on P. berghei NK65 murine model (Desgrousas et al., 2014; Sahu et al., 2014). Current pharmacological studies focus on the use of traditional medicinal plants to treat malaria or combination with anti-
malarial drugs to develop new anti-malarials (Adebayo and Krettli, 2011). Many studies have used traditional medicinal plants as potential anti-malarials in Africa and Asia. Adebayo et al. (2014) used four traditional medicinal plants extracts and combined them with CQ in treating malaria as a study in Africa. In Asia, (Hafid et al., 2015) also reported the extract of *Andrographis paniculata* combined with CQ. Both studies indicated that combination therapy with CQ were able to increase the anti-malarial effectiveness by inhibition of parasite growth to treat malaria *in vivo* using the murine model of infection. *Momordica charantia* (MC) or bitter melon is a plant from the Cucurbitaceae family, which grows in tropical areas of Asia, South America, India and Africa, is used traditionally both as food and medicine. The plant, identified as a traditional remedy for many diseases (Ahmad et al., 2016), is known to contain various phytochemicals such as steroids, flavonoids and phenolic compounds with medicinal properties. Pharmacological activities associated with *M. charantia* extracts include anti-diabetic, anti-microbial, anti-oxidant, anti-inflammatory and immunomodulatory effects (Saeed et al., 2018). For the present study, *M. charantia* was selected based on the reported anti-inflammatory and immunomodulatory activities to be anti-malarials. In malarial infection, the use of *M. charantia* in combination therapy has yet to be investigated. In this study, *M. charantia* extract was assessed in combination with the standard anti-malarial drug CQ to enhance the potential anti-malarial effect against the development of parasitaemia during infection.

II. MATERIAL AND METHODS

2.1. Plant Material

*M. charantia* fruits were obtained from the Agriculture Department of Lekir, Perak, Malaysia. Authentication of the plant as *Momordica charantia* was done by botanists at Faculty of Science and Technology (FST), Universiti of Kebangsaan Malaysia (UKM). A voucher specimen (UKMB 40346) was deposited.

2.2. Preparation of *M. charantia* aqueous Fruit Extract

Fresh *M. charantia* fruit was washed with water to remove dust and other materials on the fruit surface, then rinsed with distilled water and cut from the middle to remove seeds, chopped into thin slices and then dried in an oven (Memmert, Germany) at 60°C for 24 hours. The dried fruit was ground using an electric grinder (Panasonic Model, Malaysia) and stored at 4°C until extraction. The extract was prepared with modifications according to Abas et al. (2014). The reflux extraction was used to prepare the extract with distilled water. The mixture was centrifuged and filtered using Whatman filter paper no.1 (Whatman, UK). The crude extract was stored at -80°C to prevent fungi contamination overnight before freeze-drying (Labconco, USA). The freeze-dried extract was then stored at -20°C in a labelled amber glass until use.

2.3. Experimental Design

Male ICR mice (6-7) weeks old, weighing approximately 25 ± 5 g were obtained from the Animal House Complex at FST, UKM. The mice adapted for a week before the experiments. They were fed with rat chow (Ridley Agri Products, Australia) and distilled water *ad libitum*. Permission to conduct animal studies from the UKM Animal Ethics Committee (UKMAEC) was obtained.

The animals were randomly divided into groups in two experiments, first experiment for standard anti-malarial drug CQ and second experiment for CQ- *M. charantia* combination treatment.

2.4. Standard Anti-malarial Drug Test

The test was preceded based on Peters (1975) to test different doses of CQ (Sigma, USA), in repressing parasitaemia development. Mice divided into grouped (n=6). Mice were then injected intraperitoneally (i.p) with 0.2 mL blood of *P. berghei*-infected erythrocytes inoculum of (2 x 10^7* parasitised erythrocytes/mL) on day 0. The rodent malarial parasite *P. berghei* NK65 (chloroquine-sensitive) strain was originally obtained from (MR4), USA. After three hours of infection, the animals were either treated (i.p) with different doses of CQ (1.5, 3 or 6 mg / kg bw) or injected with saline (0.85%) as a control group for four consecutive days. Thin blood smears were prepared, and then observed under light microscope (100x magnification). Furthermore, survivability of the experimental mice was recorded until day 30 post-infection. The average of parasitemia percentage was calculated on day 4 post-infection and the chemosuppression percentages were calculated as in the following formula:


\[ \text{Chemosuppression(\%)} = \frac{\text{Average of parasitaemia in control group} - \text{Average of parasitaemia in test sample}}{\text{Average of parasitaemia in control group}} \times 100 \]

2.5. Combination Treatment Experiment

The experiment was proceeded to evaluate the anti-malarial activity of \textit{M. charantia} aqueous extract in combination with CQ. Briefly, the animals were randomly divided into many groups (n=6) after adapted for a week, injected intraperitoneaely (i.p) with 0.2 mL blood of \textit{P. berghei}-infected erythrocytes on day 0. Three hours post-infection, mice were treated with different doses of \textit{M. charantia} aqueous extract (50, 75 or 100 mg / kg bw) and CQ. Another groups of mice treated either with doses of \textit{M. charantia} extract or with CQ dose. The control group of mice was injected with saline (0.85%) for four consecutive days. The chemo suppression percentages were calculated as well as the survivability of mice was recorded until day 30.

2.6. Statistical Analysis

The results were expressed as means ± standard deviation (SD) and conducted the rank test (Kaplan-Meier analysis) using Graph Pad Prism 5 (Graph Pad Software, California) and One-Way Analysis of Variance (ANOVA) with Tukey post-hoc test to determine the significance of data (p<0.05) between experimental groups and control.

III. RESULTS

3.1. Anti-malarial Activity of CQ

Three doses (1.5, 3 and 6 mg/kg bw) of CQ were tested on \textit{P. berghei} NK65- infected mice for 4 consecutive days to detect the level of parasitaemia. The treatment with CQ showed the CQ at doses 1.5, 3 and 6 mg/kg bw significantly inhibited the growth of parasite by 39.75 ± 3.40%, 63.30 ± 2.73% and 91.09 ± 0.34% respectively (Table 1), compared with that of the control group on day 4 post-infection (p<0.05). This suggested that CQ represented the dose-dependent chemosuppression of parasitaemia development and CQ at dose 3 mg/kg bw 63.30%, showed a good anti-malarial activity based on Rasoanaivo & Oketch-Rabah (1998) classification. The median survival time of the CQ treated groups was longer than that of the infected control group with a recorded median survival time of 15.5 days (Table 1, Fig. 1). The median survival time of the mice treated with CQ at 1.5 mg/ kg bw dose was 18.5 days, whereas the survivability of the infected mice treated with 3 mg/kg bw dose significantly improved the median survival time of the mice by 23.5 days. Furthermore, CQ at 6 mg /kg bw dose survived the mice within the 30 days observation period.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Drug} & \textbf{Doses (mg/kg/bw )} & \textbf{Parasitaemia suppression on day 4(\%)} & \textbf{Median survival time (days)} \\
\hline
CQ (Anti-malarial drug) & 1.5 & 39.75 ± 3.40* & 18.5 \\
& 3 & 63.30 ± 2.73* & 23.5 \\
& 6 & 91.09 ± 0.34* & >30 \\
0.85% saline (control) & 0.2 mL & - & 15.5 \\
\hline
\end{tabular}
\caption{In vivo anti-malarial activity of CQ against \textit{P. berghei} NK65 infected mice. The data revealed chemosuppressive ± SD (n=6).}
\end{table}

(*Significant difference with control, p<0.05).
3.2. M. charantia-CQ aqueous Extract Combination Treatment

Combination treatments of *M. charantia* aqueous extract at doses 50, 75 and 100 mg/kg bw and CQ at dose 3 mg/kg bw significantly suppressed the development of parasitaemia by 72.46 ± 1.85%, 80.56 ±1.60% and 89.23 ± 1.20% respectively (Table 2), compared with that of the single doses of *M. charantia* aqueous extract and CQ. The single doses also significantly suppressed the percentage of parasitaemia by 56.59 ± 2.81%, 61.78 ± 2.45% and 70.12 ± 1.90% for the *M. charantia* aqueous extract and 64.29 ± 2.16% for CQ (p <0.05), compared with those of the control group.

The median survival time of mice is summarised in Table 2 and shown in Fig 2. All the groups of combination and single treatments prolonged the survival time of the mice compared with that of the control group. The combination treatment of *M. charantia* aqueous extract at 50, 75 and 100 mg/kg bw and CQ at 3 mg/kg bw significantly improved the median survival time of the mice by 21, 26 and 28 days, respectively. The mice administered with combined doses of 75 and 100 mg/kg of *M. charantia* aqueous extract with 3 mg/kg bw CQ survived until Day 30. The median survival time of the infected mice in the control group was shorter by 16 days. Single doses *M. charantia* extract also prolonged the median survival time of the mice by 17.5, 21 and 21.5 days respectively. The doses of 75 and 100 mg/kg bw significantly prolonged the median survival time of the mice compared with that of the control group. In addition, the single dose of CQ significantly improved the median survival time by 24 days compared with that of the control group.

Table 2. *In vivo* anti-malarial activity of *M. charantia* aqueous extract in combination with CQ against *P. berghei* NK65 infected mice. The data revealed chemosuppressive ± SD (n=6)

<table>
<thead>
<tr>
<th>Drug/Extract</th>
<th>Doses (mg/kg/bw)</th>
<th>Parasitaemia suppression on day 4(%)</th>
<th>Median survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. Charantia</em> Extract</td>
<td>50</td>
<td>56.59 ± 2.81</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>61.78 ± 2.45*</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>70.12 ± 1.90*</td>
<td>21.5</td>
</tr>
<tr>
<td>CQ (Anti-malarial drug)</td>
<td>3</td>
<td>64.29 ± 2.16*</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>MC50+CQ</td>
<td>72.46 ± 1.85*</td>
<td>21</td>
</tr>
<tr>
<td>Combination Treatment</td>
<td>MC75+CQ</td>
<td>80.56 ± 1.60*</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>MC100+CQ</td>
<td>89.23 ± 1.20*</td>
<td>28</td>
</tr>
<tr>
<td>0.85% saline (control)</td>
<td>0.2 mL</td>
<td>-</td>
<td>16</td>
</tr>
</tbody>
</table>
(* Significant difference with control, p<0.05).

Fig. 2. Kaplan-Meier survival curve of infected mice treated with *M. charantia* aqueous extract in combination with CQ within observation period (30th D).

IV. DISCUSSION

Plants have been known since thousands of years as a source of traditional medicinal systems and potential pharmaceutical drugs. Despite the availability of numerous anti-malaria drugs, malaria still appears as a public health menace most specifically in tropical and sub-tropical regions of the world. Strategies on controlling malarial infection are increasingly been threatened by the continual emergence of drug-resistant strains of the
malaria parasite. There is therefore an urgent need for newer and efficacious sources of anti-malarial drugs that may be affordable and easily available which could alleviate malaria burden and traditional herbal remedies with novel modes of action are highly potential (Bapela et al., 2014; Kamaraj et al., 2012). Medicinal plants are availability, low cost, effectiveness and rich sources of many phytochemicals, such as terpenes, alkaloids, peptides and flavonoids which may exhibit anti-malarial, anti-plasmodial and other activities (Nogueira and Lopes, 2011). Medicinal or herbal plants constitute possible source of novel anti-malarial therapeutics, in view of the fact that they contain substantial amount of metabolites with huge structural diversity as well as pharmacological activities. It has been known that traditional concoctions (extracts) have been the primary source for malaria therapy in the continents where the disease is endemic (Ntie-Kang et al., 2014). This study evaluated the anti-malarial activity of the M. charantia aqueous fruit extract against P. berghei NK65 in monotherapy and combination therapy. In monotherapy, the potential anti-malarial activity of M. charantia aqueous fruit extract significantly increased as the doses increased. Hence, M. charantia suppressed parasitic growth and improved the median survival time of the mice on day 4 post-infection in a dose-dependent manner compared with that of the control group. This anti-malarial activity may be attributed to many classes of phytochemicals, such as flavonoids and steroids, which have been reported to have anti-malarial, anti-inflammatory and immunomodulation activities (Panday et al., 2014). The use of M. charantia as a potential anti-malarial has been reported in Nigeria (Dike et al., 2012). In another study, Akanji et al., (2016) reported that a leaf extract of M. charantia has been shown anti-malarial activity and the steroids, tannins and flavonoids identified in M. charantia extract may contribute to the development this activity of M. charantia.

In addition, anti-oxidant activity of M. charantia may help reduce the oxidative stress besides having anti-malarial activity (Tcheghebe et al., 2016). Previous study demonstrated that the traditional medicinal plant extract in combination therapy against P. berghei in infected mice (Onaku et al., 2011) may be a useful strategy in order to develop new anti-malarials. The researchers resorted to utilise medicinal plants in combination treatment with standard anti-malarial drug (Somsak et al., 2016) to increase the efficacy against parasite development. The combination therapy of M. charantia with CQ has yet to be evaluated. The development of ACT is an alternative strategy to high-cost anti-malarial drugs and may address the parasite resistance in poor areas where malaria is endemic. CQ administered at low doses suppressed the development of parasitaemia in dose-dependent manner. The activity significantly increased as the dose increased and prolonged the median survival time of the mice compared with that of the control group on day 4. In this context, CQ is a common, cheap and effective anti-malarial drug and this treatment is successful when it is combined with medicinal plants or drugs in vitro and in vivo (Adebajo et al., 2014; Sahu et al., 2014). This study assessed the M. charantia extract to provide the potential anti-malarial efficacy of M. charantia in combination with CQ in inhibiting Plasmodium sp growth. The results indicated that the anti-malarial activity of M. charantia reduced the development of parasitaemia in a dose-dependent manner. Hence, the use of M. charantia in combination with standard anti-malarial drug was beneficial to the treatment of malaria and the reduction of the risk of resistance. The results showed that the combination treatment elicited better anti-malarial activity and improved the survival rates of the mice compared with the effect of the monotherapy of M. charantia extract or CQ. A high dose of M. charantia aqueous extract at 100 mg/kg with 3 mg/kg of CQ presented the best anti-malarial activity as compared to other doses of the combination treatment on day 4. Low CQ doses are also effective in reducing parasitaemia level, because CQ possesses a long half-life in murine malarial infection (Moore et al., 2011). It is due to inhibit hemozoin crystallisation (Aminake and Pradel, 2013).

This indicated presence of synergistic action and absence of antagonistic between M. charantia and CQ. These results displayed the ability of M. charantia aqueous extract in combination with CQ to provide an effective solution for malarial control by using traditional medicinal plants in poor and developing countries where treatment for malaria is expensive. Additionally, the effect of M. charantia aqueous extract is important because it offers possibility for a new therapy as an anti-malarials. This study was the first to report the anti-malarial effect of M. charantia in combination treatment. Thus, M. charantia combined with CQ provided evidence supporting the feasibility of developing potential anti-malarial combination therapy and enhancing the anti-malarial activity of M. charantia as a medicinal plant.

V. CONCLUSION

It is hoped that the outcome from this study will provide scientific evidence on the potential use of M. charantia in combination treatment against malaria which is a plausible strategy to address parasite resistance by using effective, cheap and easily available medicinal plant.
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