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The Inhibitory Effects of Neem Oil against the Development of *Plasmodium berghei* in Mice

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Although neem oil extract is widely used in Africa and Asia for the treatment and prevention of malaria, its inhibitory effect on the growth of malaria parasites *in vivo* has not been fully tested. In the present study, the inhibitory effects of neem oil extract against the growth of rodent malaria parasite in the mice were investigated. The results revealed that administration of neem oil to the mice infected by rodent malaria parasite reduces the rate of development of *Plasmodium berghei*. These results show that neem oil extract could be an effective antimalarial agent in humans that merits further investigations using human parasites.

Key words: Neem oil, inhibitory effect, *Plasmodium berghei,* mice

INTRODUCTION

A variety of antimalarial drugs including chloroquine have been used extensively to control and treat malaria [1-2]. However, prevention of patients with malaria parasites from continuing to infect mosquitoes after treatment with current antimalarials has not been achieved [1]. The available evidence shows that the incidences of resistance to chloroquine are on the increase [1-4]. This for instance prompted the Kenyan government to discontinue chloroquine use in the country.

The problem of chloroquine-resistant strains of malaria parasite has led many researchers to focus their attention to the use of traditional herbs for the control and treatment of malaria [5-7]. Extracts from traditional herbs such as *Uvaria spp* [8], *Artemisia spp* [9] and from parts of the neem tree have been tested *in vitro* and found to be active against chloroquine-resistant strains of *Plasmodium falciparum* at various stages of development [10-12].

The importance of the traditional uses of neem concoctions for the control and treatment of malaria and other diseases in Africa cannot be overlooked. The active component in neem oil extract, gedunin, has been found to be very effective against the chloroquine-resistant strains of *Plasmodium falciparum in vitro* [8-9]. Most of the available evidence concerning antimalarial activities of neem oil has been obtained from *in vitro* experimental models.

In view of the importance of reports on the *in vitro* antimalarial activities of neem oil extracts, there is a need for additional evidence to validate these reports *in vivo*. The purpose of the present study was therefore to further determine the activity of neem oil against *Plasmodium berghei* in infected mice.

MATERIALS AND METHODS

Neem oil

Neem oil extract from the seeds of neem tree used for this study was obtained from the Department of Chemistry, Moi University. It was diluted to 10% (v/v) with phosphate buffered saline solution, pH 7.4. The diluted oil suspension in volumes of 25 µl, 50 µl, 100 µl and 150 µl were orally administered to each mouse in their respective set of test groups using a feeding needle.

Plasmodium berghei culture and passaging

Plasmodium berghei culture was maintained in the outbred original mice by passaging as previously described [13]. The parasites were introduced into the host mice to develop and then extracted and transferred into another host after every five days. The development of the plasmodium parasites in the mice was

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determined by microscopic examination of blood smears and cell counts. Subsequently, 50 µl of blood were collected into heparinized tubes from the mice which had parasitemia of about 35% and diluted to 5 ml (1:100 dilution) with phosphate buffered saline, pH 7.4. The diluted parasitized blood (100 µl) were administered to each mouse by intraperitoneal injection.

Treatment of animals

Adult mice weighing on average 24 g were used for this study. They were housed in plastic cages under controlled temperature between 22-25°C and had free access to food and water. They were initially divided into two study groups for the purpose of determining the potency of neem oil as a therapeutic agent and its protective action against malaria parasite. The groups were treated as follows.

***Group 1*:** The purpose of this group was to investigate the potency of the inhibitory action of neem oil on the growth of *Plasmodium berghei* in mice. All the animals in this group were infected with parasites 24 h before treatment with neem oil was started. Each animal received a single intraperitoneal injection of 100 µl of parasitized blood and allowed to stay in the same environment for 24 h. They were then subdivided into five batches of 10 animals each and treated with appropriate amounts of neem oil per batch per animal. The control batch was treated with distilled water. The development of parasitemia was recorded every 24 h.

***Group 2*:** This group was set up to investigate the protective role of neem oil against malaria parasites. All the animals were subdivided into five batches of 10 animals each and treated with an appropriate dose of neem oil by gastric intubation. After 24 h, each animal received a single intraperitoneal injection of 100 µl of parasitized blood. The control batch was treated with the parasitized blood and distilled water.

Blood smears and staining

Blood samples for the preparation of smears were drawn from the tails of the mice by squeezing out a drop on a glass slide. The smears were allowed to dry in air and then fixed in absolute methanol. They were air dried and stained in Giemsa stains for 15 min. After staining, the slides were carefully rinsed in water and then dried in air before they were examined under microscope for parasitemia. Up to 1000 cells were counted for estimation of the percentage growth of parasitemia and recorded every 24 h.

RESULTS AND DISCUSSION

Figure 1 is graphical illustration of the percentage growth rate of *Plasmodium berghei* in infected mice after treatment with neem oil extract. The results show that neem oil when administered at doses of 50 µl, 100 µl and 150 µl caused a significant decrease of the growth of rodent malaria parasite in the mice. While the growth rate of parasitemia in the control group was 29%, it was reduced to 9%, 5% and 4% after treatment with 50 µl, 100 µl and 150 µl of neem oil respectively.

Figure 2 illustrates the protective effect of neem oil against *P. berghei* parasitization. The results indicate that administration of neem oil to the mice before infecting them with the parasite causes a significant inhibition of the growth of *P. berghei*. Whereas the growth rate was 17% in the control group, it was 3%, 2% and 0.6% in mice treated with 50 µl, 100 µl and 150 µl respectively.

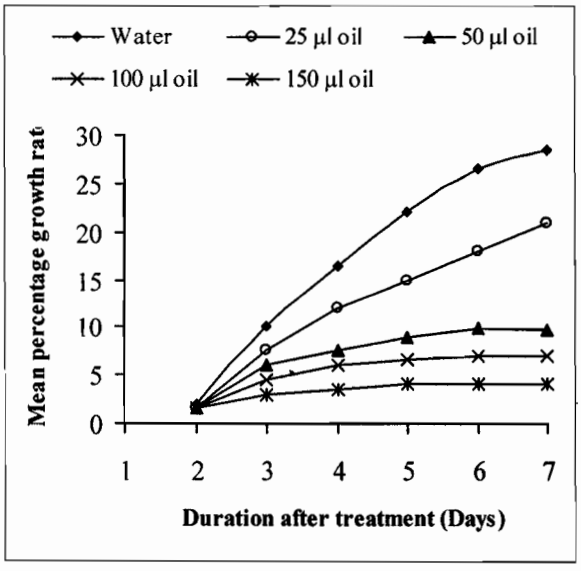

Figure 1: Effect of neem oil on the growth rate of *Plasmodium berghei* in infected mice.

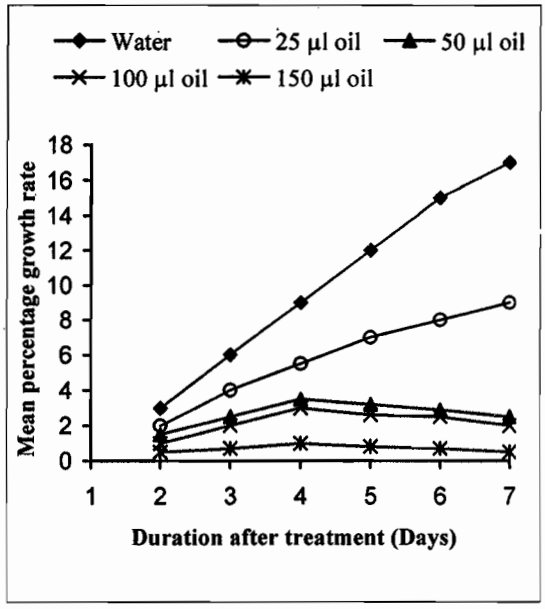

Figure 2: The growth rate of *Plasmodium berghei* in mice treated with purified neem oil.

Despite its recognized importance in elimination of *P. falciparum*, neem oil extract and its traditional uses for the treatment and prevention of malaria have yet to be validated. This study confirmed that neem oil extract is an effective inhibitor of *P. berghei*. Despite the possibility of interference from the interactions with plasma proteins and nutritional deficiencies on the growth of parasites, it can be concluded that neem oil extract is a powerful inhibitor of rodent malaria parasite that merits further investigation as a potential antimalarial agent in humans.

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