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Concurrent administration of aqueous extract of *Cryptolepis sanguinolenta* reduces the effectiveness of Artesunate against *Plasmodium berghei* in Rats

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ABSTRACT

Concurrent administration of aqueous extract of *C. sanguinolenta* was earlier reported to alter the pharmacokinetics of artesunate consequently resulting in an increase in the clearance rate, which could cause sub-therapeutic levels of dihydroartemisinin of artesunate leading to reduced efficacy. In the present study, the effect of aqueous extract of *C. sanguinolenta* (Cryp) on the effectiveness of artesunate (Art) against *Plasmodium berghei* in male Sprague-Dawley rats was investigated. Twenty (20) female Sprague-Dawley rats were divided into four (4) treatment groups (1 to 4). Groups 2 (Cryp) and 3 (Cryp/Art) served as test groups, which were pre-treated with aqueous extract of *C. sanguinolenta* for two weeks whilst groups 1 (no drug) and 4 (Art) served as negative and positive controls, respectively and were given water. The rats were inoculated with *P. berghei* after the two weeks pretreatment period and were either not treated (no drug) or treated with artesunate (Art) or *C. sanguinolenta* (Cryp and Cryp/Art) 2 days post-inoculation. Parasitemia and chemosuppression were then determined in 4 and 6-day suppressive tests. The parasitemia in group 3 rats (Cryp/Art) was significantly ($p < 0.05$) higher (270%) than in group 4 (Art) on day 8. Consequently, the chemosuppression of artesunate only group was significantly ($p < 0.05$) higher (133%) than that of the concurrently administered Cryp/Art group. The findings show that concurrent usage of *C. sanguinolenta* and artesunate reduces the effectiveness of artesunate as an antimalarial. Therefore, patients should be advised on the implication of the practice of taking *C. sanguinolenta* and artesunate concurrently.

INTRODUCTION

Malaria is a widespread infectious disease caused by the *Plasmodium* parasite (Deressa *et al*., 2003) and is a major public health problem, particularly in sub-Saharan Africa (Cibulskis *et al.*, 2011). Presently, the primary form of control of malaria is chemotherapy (Kar and Kar, 2010). Previously, malaria was treated with chloroquine which was an affordable and a very efficient chemotherapy for malaria until the emergence of chloroquine-resistant strains of the *Plasmodium* parasite (Kar and Kar, 2010; Wellems, 2002). This led to the discontinued use of chloroquine (Mwai *et al*., 2009) and its replacement with artemisinin and its derivatives as the recommended first-line drugs for malaria (Adjuit *et al.*, 2002; Li and Weina, 2010; Rosenthal, 2008).

However, the emergence of artemisinin-resistant *Plasmodium* parasites in certain parts of the world has provoked global alarm (White, 2008). Such resistance may arise as a result of exposure of the parasite to sub-therapeutic levels of the drug (Bowerman, 1978). Recently in Ghana, laboratory strains of artesunate-resistant *Plasmodium falciparium* have been produced by continuous exposure to sub-therapeutic doses (unpublished observation). The exposure to sub-therapeutic levels may result from non-compliance to dosage regimen by patients or be caused by drug interactions. As the patronage of herbal medicines increases across the world, herbs have been increasingly used with other drugs rather than in place of drugs (Delgodaa *et al.*, 2011) raising the concern about herb-drug interactions, which may be adverse or beneficial (De Smet, 2006). The practice of concurrent administration of antimalarial herbal preparations, most of which contain *C. sanguinolenta*, and some orthodox antimalarials has been observed in Ghana (unpublished observation).

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It has been reported recently that concurrent administration of *C. sanguinolenta* and artesunate enhances the cytochrome P450 1A isozyme activity (Ocloo *et al.*, 2012) leading to increase in the elimination rate constant and clearance and decrease in bioavailability, volume of distribution and half-life of dihyhroartemisinin, the most potent metabolite of artesunate (Sakyiamah *et al.*, 2011).

These observations suggest that concurrent usage of *C. sanguinolenta* and dihydroartemisinin could cause sub-therapeutic levels of dihydroartemisinin of artesunate leading to reduced effectiveness, which could contribute to the development of artemisinin resistance. In the present study the effect of concurrent use of *C. sanguinolenta* and artesunate on the effectiveness of artesunate against *Plasmodium berghei* was investigated in rats.

MATERIALS AND METHODS

Experimental Animals

A total of twenty (20), aged matched female Sprague-Dawley rats were obtained from the Animal Unit of the Centre for Plant Medicine Research (CPMR), Mampong Akuapem, Ghana and standard laboratory chow diet was obtained from Agricare, Ghana.

Plant Material

Powdered dried roots of *Cryptolepis sanguinolenta* was obtained from and authenticated by the Plant Development Department (PDD) of the Centre for Plant Medicine Research (CPMR), Mampong Akuapem, Ghana.

Preparation of plant extract

The plant extract was prepared as described previously (Sakyiamah *et al.*, 2011). About 360 g of *Cryptolepis sanguinolenta* was boiled in 6 L of water for 45 min. and allowed to cool. The resultant extract was filtered through a cotton wool and lyophilized into powder using a freeze dryer (EYELA, Tokyo Rikakikai Co Ltd. Japan). The dried powdered extract was then weighed and stored in a refrigerator until ready to use.

Inoculum preparation

Plasmodium berghei NK65 strains were brought from University of Copenhagen, Denmark and maintained in the Department of Immunology, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, Accra, Ghana in liquid nitrogen with occasional passage in Balb/c mice. The *P. berghei* were activated by injecting intraperitoneally into Sprague-Dawley rats and subsequently maintained in the laboratory by serial blood passage from rat to rat (Pedroni *et al.*, 2006). A stock of parasitized erythrocytes, with a minimum peripheral parasitemia of 20%, was then obtained from the infected rats by cardiac puncture. Blood was collected into heparin-coated tube. The cell concentration of the stock was determined and diluted with physiological saline such that 2 ml of final inoculum contained 30×10^6 parasitized RBCs.

Treatment of animals

The rats were randomly divided into four (4) groups of five (5) each. They were fed on the standard laboratory chow and water *ad libitum*, acclimatized for a week and treated as follows: Rats in group 1 (no drug) received drug vehicle throughout the study period and served as negative control; groups 2 (Cryp) and 3 (Cryp/Art) were pre-treated with freeze dried *C. sanguinolenta* reconstituted in distilled water at the therapeutic dose of 36 mg/kg (CPMR, unpublished data) for two weeks whilst group 4 (Art) received vehicle treatment for the same period and served as positive control.

The animals in all the groups were inoculated intraperitoneally with 2 ml of final inoculum which contained 30×10^6 parasitized RBCs after the two weeks. They were left for two days without administration of extract/drug, after which, group 1 was treated with drug vehicle for six (6) days, group 2 was treated with the reconstituted freeze dried *C. sanguinolenta* for six (6) days and groups 3 and 4 were treated with artesunate obtained from Ernests Chemists Limited (Tema, Ghana) for six (6) days at a therapeutic dose of 2.5 mg/kg with a double dose on the first day. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care (NIH, No.85-23, revised 1985). Ethical clearance was obtained from the CPMR's ethics committee.

Determination of parasitemia

Parasitemia was determined 2, 6 and 8 days after inoculation or 0, 4 and 6 days after artesunate/extract administration as described by Berhan *et al*. (2012). Thin blood smears were prepared from the tail vein of each rat. Each smear was air-dried, fixed in methanol, air-dried again and stained with Giemsa for 15 minutes.

The thin blood smear was then examined under oil immersion with a microscope. Each slide was observed at three different fields and the parasitized RBCs counted were recorded. The percentage parasitemia (% P) and the percentage chemo-suppression (% C), also known as the Activity, were estimated using the following formulae:

$$\%\ parasitemia = \frac{Number\ of parasitised\ \ RBCs}{Total\ number\ of RBCs}\ x100$$

$$\%\ Suppression = \frac{Parasitemia\ of\ negative\ control - parasitemia\ of\ study\ group}{Parasitemia\ of\ negative\ control}\ x\ 100$$

The % survival for each treatment group was also monitored for fourteen days after treatment. The % survival was calculated as.

$$\frac{Number\ of\ dead\ rats\ in\ a\ group}{Total\ number\ of\ rats\ in\ the\ group}\ x\ 100$$

Statistical analysis of results

Values were expressed as means ± standard error mean (S. E. M.) of 5 determinations. Comparisons between means were performed and significance was evaluated by analysis of variance (ANOVA) followed by Duncan's protected least significant difference test. Probability value of $p < 0.05$ was used as the criteria for significant differences.

RESULTS AND DISCUSSION

Malaria is a widespread infectious disease which is a major public health problem, particularly in sub-Saharan Africa (Cibulskis *et al.,* 2011) and is a major cause of mortality among children under five years and pregnant women (WHO, 2011). The devastating effect of the malaria disease combined with the incidence of resistance to antimalarials such as chloroquine (Wellems, 2002) and artesunate (White, 2008) is of great concern. Such resistance may arise as a result of exposure of the parasite to sub-therapeutic levels of the drug (Bowerman, 1978).

The practice of patients taking herbal antimalarials, most of which contain *C. sanguinolenta*, and orthodox antimalarials concurrently is on the rise in Ghana (unpublished observation). This may result in herb-drug interactions which may be adverse or beneficial. The consequent of drug interaction, a phenomenon caused by concomitant or concurrent use of drug with other substances is that it leads to physiological effects which are entirely different from those associated with the individual drugs (De Smet, 2006) and herb-drug interactions have been widely studied. Ginger is reported to cause increase in the bioavailability and half-life, and decrease in the clearance and elimination rate constant of metronidazole (Okonta *et al.,* 2008). *St. John's Wort* causes up to 81% reduction in concentration of indinavir leading to treatment failure (Piscitelli, *et al.,* 2000).

Concurrent use of *C. sanguinolenta* was shown to result in significant reduction of plasma concentration of chloroquine (Sakyiamah *et al.*, 2012).

According to Sakyiamah *et al.,* (2011), the concurrent administration of *C. sanguinolenta* and artesunate caused an increase in the elimination rate constant and clearance of dihydroartemisinin, the most potent metabolite of artesunate, leading to a decrease in the bioavailability, volume of distribution and half-life of dihyhroartemisinin of artesunate. It was therefore hypothesized that the concurrent administration of *C. sanguinolenta* and artesunate could cause sub-therapeutic levels of dihydroartemisinin leading to reduced effectiveness of artesunate which may eventually lead to resistance. The present study therefore sought to investigate the effect of the concurrent use of aqueous extract of *C. sanguinolenta* and artesunate on the effectiveness of artesunate against *P. berghei* infected rats.

The 4 and 6-day *P. berghei* chemo-suppressive test was used to determine the parasitemia level and chemo-suppression in four groups of rats. Expectedly, parasitemia was significantly higher in the negative control group during the 6 days period of monitoring (Figs. 1 and 2). Furthermore, parasitemia was significantly lower in rats that were given the aqueous extract of *C. sanguinolenta* for two weeks before inoculation (Cryp and Cryp/Art) (Figs. 1 and 2) demonstrating a prophylactic property of *C.* sanguinolenta, which is consistent with previous observations (Gbedema *et al*., 2011).

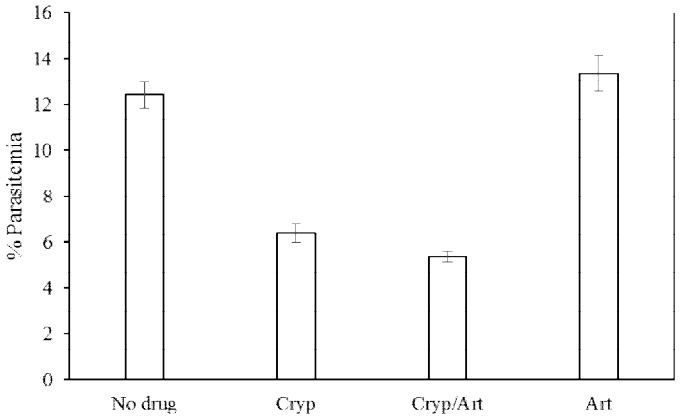

Fig. 1: Percentage parasitemia in *P. berghei* infected rats before treatment (two weeks after pretreatment and two days after inoculation). *Values are Mean ± SEM of n= 5; * - Values significantly different from no drug control (p<0.05); # - Values significantly different from artesunate control (p<0.05)*

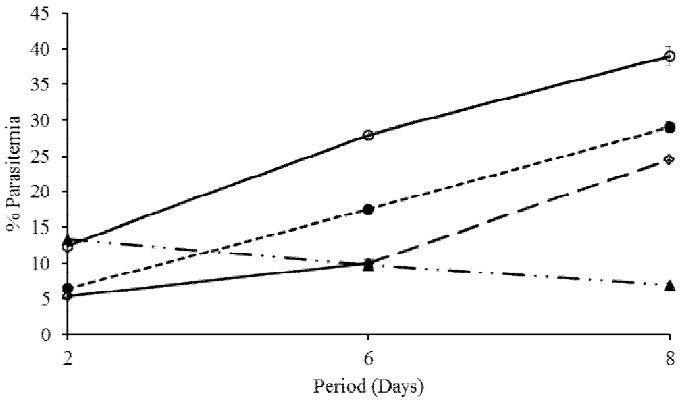

Fig. 2: Percentage parasitemia in *P. berghei* infected rats monitored over a period of 8 days after inoculation or 6 days of drug/extract treatment. No drug control (———); artesunate control (Art) (— · — · —); *C. sanguinolenta* (Cryp) (·····) and *C. sanguninolenta/artesunate* (Cryp/Art) (--------). *Values are Mean ± SEM of n = 5.*

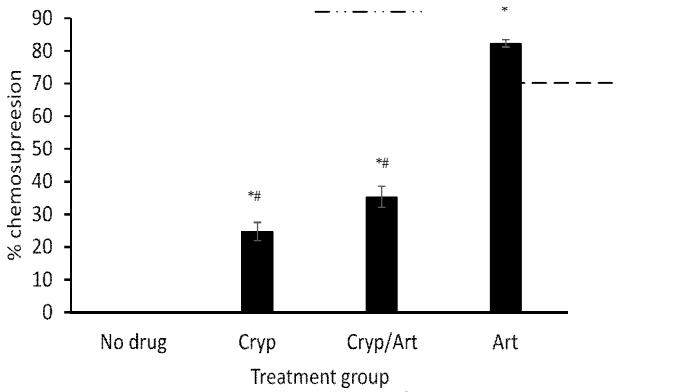

Fig. 3: Percentage chemosuppression on the 6th day of extract/drug treatment. *Values are Mean ± SEM of n= 5; # - Values significantly different from artesunate control (p<0.05). * - Values significantly different from no drug control (p<0.05).*

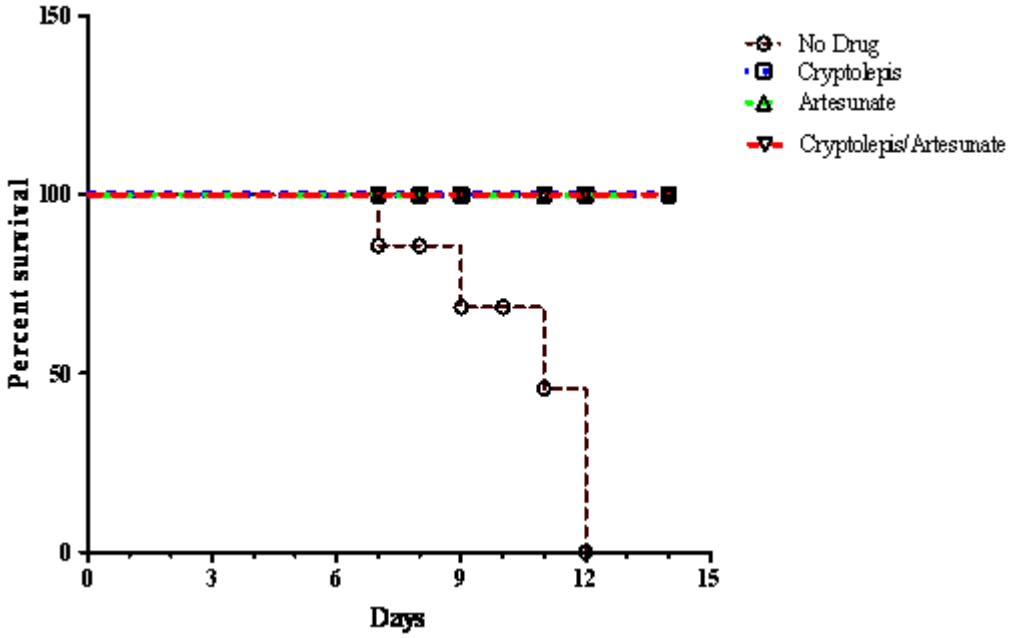

Fig. 4: Percentage survival of the *P. berghei* infected rats: The rats in each group were monitored for fourteen days after the end of treatment and number of deaths recorded.

Intriguingly, parasitemia decreased progressively in rats that were not pre-treated with the extract before inoculation but treated with artesunate (Art) (positive control) from (13.3 ± 0.8 %) on the day of start of treatment to (6.9 ± 0.4%) six days after start of treatment whilst parasitemia increased significantly in all the other groups. Parasitemia in the no drug group increased from (12.4 ± 0.5%) to (39.0 ± 1.3 %), that in the *C. sanguinolenta* only (Cryp) from (6.4 ± 0.4%) to (29.0 ± 0.7%) and that in *C. sanguinolenta*/artesunate (Cryp/Art) from (5.4 ± 0.2%) to (24.5 ± 0.5%) (Fig. 2). This observation is completely consistent with our hypothesis that concurrent use of *C. sanguinolenta* would reduce the effectiveness of artesunate. Percent chemo-suppression which is an alternative way of expressing parasitemia followed a similar trend (Fig. 3).

Furthermore, although 100% mortality was recorded in the negative (no drug) control, none of the rats in the other group died 0% mortality during the 14 day monitoring after drug administration (Fig. 4) suggesting that even though concurrent use of the extract reduces the effectiveness of artesunate, the individual animals were able to recover from the reduced level of the parasite as compared to the no drug controls. It however, has an implication for development of resistance since the parasite may have enough time to get adjusted to the sub-therapeutic levels and with time develop resistance to the drug.

CONCLUSION

The present study has demonstrated the potential of *C. sanguinolenta* to reduce the effectiveness of artesunate. It is, therefore, recommended that patients must be cautioned against such practices as it has an implication for the development of artesunate resistance.

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