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Prophylactic Effect of Grapefruit Juice Against *Plasmodium berghei berghei* Infection in Mice

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Abstract: Grapefruit juice was assessed for chemoprophylactic activity against Chloroquine-sensitive *Plasmodium berghei berghei* infection in mice. A standard inoculum of 1×10^6 infected erythrocytes was used to assess the prophylactic effect of grapefruit juice (15 mL kg^{-1}) and this was compared with the prophylactic effect of high dose Ascorbic acid (150 mg mL^{-1} or 2.25 g kg^{-1}). The result of the experiment showed that grapefruit juice and high dose vitamin C significantly delayed the establishment of parasitaemia compared with the control group. Furthermore, grapefruit juice and ascorbic acid prolonged the mean survival time of the mice with corresponding decreases in mean peak percentage parasitaemia, respectively. Grapefruit juice however demonstrated a stronger chemoprophylactic activity than ascorbic acid ($p < 0.05$). These effects were however lower than the standard prophylactic drug (Pyrimethamine- 1.2 mg kg^{-1}). Regular intake of grape juice may protect against malaria infection. Further studies are necessary to elucidate possible mechanisms involved.

Key words: Grapefruit juice, *Plasmodium berghei berghei* malaria, ascorbic acid, chloroquine

INTRODUCTION

Grapefruit is the fruit of the citrus plant. Its botanical name is *Citrus paradisi*, family Rutaceae. It is believed to be native to Jamaica. This fruit is similar to the orange fruit (*Citrus sinensis*) but differs by being bigger than the orange fruit. Other differences are the thicker and smoother rind and the bitter taste of grapefruit juice.

Grapefruit, has very useful medicinal values. It is high in vitamin C and potassium (Mahan and Stump, 2000). It also contains bioflavonoids and other phytochemicals that protect against cancer and heart disease (Turner *et al.*, 2004).

Ascorbic Acid (AA), the reduced form of vitamin C, is an important cofactor in several enzyme reactions and plays a pivotal role in the defense against oxidative stress (Levine, 1986; Gambhir *et al.*, 1997); whereas the oxidized form, Dehydroascorbic Acid (DHAA) is considered a marker of oxidative stress (Ayori *et al.*, 2000).

Vitamin C, an antioxidant vitamin, present in grape juice prevents the free radical damage that triggers the inflammatory cascade and is therefore also associated with reduced severity of inflammatory conditions, such as asthma, osteoarthritis and rheumatoid arthritis (Kurl *et al.*, 2002).

There has been claims that consistent intake of grape juice which is high in vitamin C content will protect the individual against malaria infection (Hassan *et al.*, 2004).

We and others have demonstrated the prophylactic activity of other antioxidant vitamin preparations like vitamin A and Cod liver oil (Oreagba and Ashorobi, 2006; Awodele *et al.*, 2006; Levander *et al.*, 1989).

It has also been shown that *Plasmodium falciparum* infection could compromise the level of ascorbic acid, the reduced form of vitamin C (Isamah and Asagba, 2003).

These accounts suggest that grapefruit, an excellence source of vitamin C, may protect against malaria infection however scanty information is available to articulate this viewpoint

The aim of this study is therefore to investigate the chemoprophylactic effect of grapefruit juice against *Plasmodium berghei* infection in mice.

MATERIALS AND METHODS

The parasite: The NK65 strain of *Plasmodium berghei berghei* used in this study was obtained from Dr. G.O. Ademowo of the Chemotherapy Research Laboratory, Institute of Advance Medical Research and Training, (IMRAT) College of Medicine University of Ibadan Ibadan Nigeria and maintained in mice by weekly passaging into fresh mice. Each mouse was inoculated intraperitoneally with 0.1 mL of infected blood containing about 1×10^7 *Plasmodium berghei berghei* parasitized red blood cells obtained from a donor mouse having about 60% parasitaemia.

Thin blood films were made by collecting blood from the tail, this was stained with Geimsa stain and the percentage parasitaemia was determined by counting the number of parasitized red blood cells out of 1000 blood cells in 10 random microscopic fields.

The animal grouping and treatments: The repository activity of grapefruit juice was evaluated using the method described by Peters (1965).

Male Swiss albino mice, weighing 20-25 g kept under good ventilation and balanced diet, were used in this study. Animals were subdivided into five groups of 10 each. Group 1 (control) received distilled water only. Group 2 received high dose vitamin C (150 mg mL⁻¹) given 3 times daily for 7 days before parasite inoculation. Group 3 received grapefruit juice at a dose of 15 mL kg⁻¹ three times daily for 7 days before inoculating with malaria parasite. Group 4 received Pyrimethamine (1.2 mg kg⁻¹ day⁻¹) once daily for 7 days. Administration was by means of the oral cannular.

On the 8th day, the treated mice were passaged with *Plasmodium berghei* parasite. Microscopic examination of the blood film was made to determine percentage parasitaemia 72 h post inoculation. The average suppression of parasitaemia was calculated in comparison to control as shown below:

$$\text{Av. \% suppression} = \frac{\text{Av. \% parasitaemia in control} - \text{Av. \% parasitaemia in treated}}{\text{Av. \% parasitaemia in control}} \times 100$$

RESULTS

The result of the experiment showed that pretreatment of mice with grapefruit juice for 7 days delayed the establishment of parasitaemia compared with the control. On day 3, average parasitaemia of mice pretreated with distilled water was 5.21% while that of the mice pretreated with grapefruit juice was 2.22%, a difference of 2.99% (Table 1). Mice pre-treated with high dose vitamin C (150 mg mL⁻¹) recorded an average parasitaemia of 4.81% which showed mild prophylactic activity when compared with the control group (Table 1).

In addition, pretreatment of mice with grapefruit juice prolonged the average lifespan of mice, extending it by 6 days from 7 days in the control group to 13 days in the group pre-treated with grapefruit juice. The corresponding mean percentage parasitaemia were 37.5 and 23.08%, respectively. This was also the case with the group pre-treated with high dose vitamin C (150 mg mL⁻¹) which recorded a Mean Survival Time (MST) of 11 days (an extension of 4 days) and a Mean Percentage Parasitaemia (MPP) of 28.08% (Table 2).

Table 1: Prophylactic effect of grapefruit juice and vitamin C against *P. berghei* infection 3 days post inoculation

Drug	Dose (mg kg ⁻¹ day ⁻¹)	Average parasitaemia (%)	Average suppression (%)
Grapefruit juice	15 mL kg ⁻¹	2.22±0.35*	57.40
Vitamin C	150 mg mL ⁻¹	4.81±0.35*	7.70
Control *(distilled water)		5.21±0.19	-
Pyrimethamine (Std)	1.2	1.43±0.14	72.55
One-way ANOVA	F 5.07		p<0.05

Data expressed as mean±SEM for 10 animals per group df 4.45 *p<0.05 when compared to control; *: Equal volumes (0.3 mL) of distilled water and drugs were used

Table 2: Effect of grape juice and vitamin C on survival time during *P. berghei* infection

Agent	MST (days)	MPP (%)
Grapefruit juice	13±0.65	23.08
Vitamin C	11±0.28	28.08
Control *(distilled water)	7±0.82	37.50
Pyrimethamine (Std)	22±0.44	5.90

MST: Mean Survival Time, MPP: Corresponding Mean Percentage Parasitaemia, *: Equal volumes (0.3 mL) of distilled water and drugs were used

On day 5, it was noticed that while the stage of development of the parasite in the control group were mostly schizonts, those of the mice pretreated with grapefruit juice and vitamin C had a prolonged trophozoite stage indicating a delay in their growth rate.

DISCUSSION

Malaria parasites are highly susceptible to alterations in redox equilibrium of its system and environment and this offers great opportunities for chemotherapy and chemoprophylaxis. In the present study, we examined the chemoprophylactic effects of grapefruit juice, which is rich in ascorbic acid, against *Plasmodium berghei* infection in mice. Grapefruit juice indeed delayed the onset of parasitaemia as shown in our study indicating chemoprophylactic activity. This delay was similar to but stronger than that produced by pretreatment with high dose vitamin C alone indicating a form of synergistic interaction between vitamin C and other components of grapefruit juice like lycopene and bioflavonoids present in grapefruit juice. Incidentally a recent study suggests that higher plasma concentrations of lycopene at enrollment was significantly associated with clearance of malaria parasitemia by day 3 in young children (Amy *et al.*, 2001).

Although we did not study the antiplasmodial effect of grapefruit juice on established infection, this observation is nonetheless consistent with the study by Marva *et al.* (1992) which showed that Ascorbic acid caused stage-dependent destructive effects on the

in vitro development of *Plasmodium falciparum* especially the advanced forms of the parasite. Normally, as an antioxidant, ascorbic acid will help neutralize peroxides and other oxygen free radicals. However, in large doses ascorbic acid can change behavior and act like a pro-oxidant. The possible mechanism of action for the pro-oxidant effect of vitamin C has been well postulated. During the growth of *P. falciparum* the infected erythrocytes release increasing levels of iron-containing structures that are redox-active, vitamin C interacts with these structures by reducing iron and oxygen thereby inducing the conversion of hydrogen peroxide to yield highly reactive hydroxyl radicals (Udenfriend *et al.* 1954). It is speculated that hydroxyl radicals generated in this fashion is responsible for the pro-oxidant effect of vitamin C (Winter *et al.*, 1997).

Another possible mechanism which may compliment the above could be through the immunomodulatory effect of vitamin C and some other phytochemicals present in the juice e.g., bioflavonoids and coumarins, studies have shown that ascorbic acid appears to stimulate humoral immunity through increased antibody synthesis particularly IgG, IgA and IgM types (Ansari *et al.*, 1998). It also activate the macrophages which produce Reactive Oxygen Species (ROS) (Mohan *et al.*, 1994) as part of the host immune reaction against malarial parasites (Adelekan and Thurnham, 1998), a mechanism similar to that of vitamin A chemoprophylaxis against *P. falciparum* infection (Serghides and Kain, 2002) in non-immune host which in this case include present study animals.

Stocker *et al.* (1986) have shown that Ascorbic acid is taken up readily by *P. vinckei* parasitized red blood cells when administered and the uptake was increased in proportion to the rate of malarial infection. This may also be applicable to our findings which showed protection against *P. berghei* parasite by ascorbic acid and grapefruit juice.

The greater prophylactic effect of grapefruit juice compared with ascorbic acid may be associated with a higher rate of uptake into the parasitized erythrocytes. This mechanism may also account for the delay in the development of the parasite from the trophozoite stage to the schizont stage delay in the group that received grapefruit juice

One important limitation of this study was the absence of a separate group of mice placed on continuous administration of grapefruit juice even after parasite inoculation to determine the possibility of a permanent prophylactic effect. Since there is an increase in the concentration of plasma Thiobarbituric Acid Reactive Substances (TBARS) in malaria patients (Prasannachandra *et al.*, 2006) and Malondialdehyde

(MDA), in the blood of mice infected with *P. berghei* (Golenser and Chevion, 1989), a look at the chemoprophylactic effect of grapefruit juice on the level of these lipid peroxidation products would also be useful for future studies.

The results of this study has demonstrated the repository activity of grapefruit juice against *P. berghei* infection in mice. This confirms its use in ethnomedicine as a malaria prophylactic. Further studies are necessary to elucidate possible mechanisms involved.

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