African Journal of Biochemistry Research Vol. 5(11), pp. 328-332, 15 October, 2011
Available online at http://www.academicjournals.org/AJBR
ISSN 1996-0778 ©2011 Academic Journals

Full Length Research Paper

Anti-malarial activity of cocoa powder in mice

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Accepted 5 September, 2011

At present, malaria is one of the most important parasitic diseases in the world killing more than one million people annually. It is an important public health problem because many of the drugs that are being prescribed for the treatment of malaria have become ineffective to the disease. This study was therefore carried out to determine the anti-malaria activity of cocoa powder through the use of mouse model. Natural cocoa powder was used to compound mice feed and this was both pre-fed and post-fed to mouse that had been infected with *Plasmodium berghei*. The results indicated that cocoa powder had both therapeutic and prophylactic effects against *P. berghei.* The mean percentage plasmodial reduction expressed in mice post-fed with cocoa and those treated with chloroquine were 60.82 ± 8.47% and 60.09 ± 7.84% respectively. This is an indication that both agents exhibited plasmodial reduction almost at equal frequency. Though, percentage *plasmodium* reduction was more in mice pre-fed with cocoa than those post-fed with cocoa, but the difference was not significant (P > 0.05). The observation of higher percentage of plasmodial reduction in mice pre-fed with cocoa suggested it may possess an immune-booster effect which action is anti-malarial. There was a decline in weight of mice demonstrating that cocoa might contain some weight trimming ingredients.

Key words: *Plasmodium berghei,* chloroquine, therapeutic immune booster.

INTRODUCTION

Malaria is a potentially, fatal tropical parasitic disease that is spread through the bite of an infected female mosquito. Malaria parasites are members of the genus *Plasmodium (Phylum Apicomy plexa*). It is wide spread in tropical and subtropical regions including parts of the Americas, Asia and Africa. Malaria in humans is caused by one of four protozoan species of the genus *Plasmodium*: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, or *Plasmodium malariae* and *Plasmodium knowlesi*, a parasite of Old World monkeys also infects man. Investigations are ongoing to determine the extent of its transmission to humans (Mveller et al., 2007). Malaria is passed on by the female anopheles mosquito biting a person who has malaria parasites in their blood. Malaria can also be passed on by blood transfusions and the use of infected needles. Malaria causes an average loss of 1.3% of annual economic growth in countries with intense transmission. Severe malaria is almost exclusively caused by *P. falciparium* infection. The infected person may have feverish attacks, influenza-like symptoms, tiredness, diarrhea or a whole range of other symptoms (Trampuz et al., 2003). In most severe cases of the disease, fatality rates can exceed 20%, even with intensive care and treatment (Kain et al., 1998). In endemic areas, treatment is often less satisfactory and the overall fatality rate for all cases of malaria can be as high as one in ten (Mochenhaupt et al., 2004). Over the longer term, developmental impairments have been documented in children who have suffered episodes of severe malaria (Carter, 2005). It is responsible for the death of an estimated 10,000 pregnant women and up to 200,000 infants each year in Africa alone (Trampuz et al., 2003).

It is well-known that cocoa contains a wide range of polyphenols, especially procyanidins with a high degree of polymerization. Procyanidin is a class of polyphenolic

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polymer composed of flavan-3-ol (catechin) and epicathechin as monomers, moreover small amounts of flavonoids and phenolic acids have been found in various cocoa-derived products. Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavornols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages (tea, coffee, beer, wine and fruit drinks) (Ortega et al., 2008). Cocoa was reported to have high polyphenols content, which comprises 12 to 18% of the whole beans dry weight (Richelle et al., 2001). A study reported that in raw cocoa beans, 60% of total phenolics were flavonol monomers (epicatechin and catechin) and procyanidin oligomers (Dreosti et al., 2000). (–)-Epicatechin content in freshly prepared beans ranged from 21.89 to 43.27 mg/g dry defatted samples. Cocoa is rich in other polyphenols such as (+)-catechin, (–)-epicatechin, and oligomers of these monomeric base units, namely procyanidins, and anthocyanidins (Hammerstone et al., 1999). Kelm et al. (2006) later indicated that unfermented cocoa beans contain monomers up to tetradecamers. Cocoa contains flavanol which has potential beneficial effects on human health such as antiviral, anti-allergic, anti-platelet, anti-inflammatory, anti-tumor and anti-oxidant activities. Numerous studies have reported a relationship between the consumption of cocoa derivatives especially dark chocolate with beneficial health effects on cardiovascular diseases as a result of the antioxidant activity of procyanidins (Keen et al., 2005, Cooper et al., 2008). The flavonoids have aroused considerable interest recently because of their potential benefits on human health. Murakami (2003) have shown that diglycosides of flavanols (a specific type of flavonoids) retard life cycle of malaria parasites, whereas monoglycosides completely inhibited proliferation of trophozoite stage of parasites. The health benefits of consuming cocoa based products have recently been recognized, indeed reports have suggested that cocoa may protect against cancer (Masharinec, 2009), cardiovascular disease (Galleano et al., 2009; Corti et al., 2009) and various other medical conditions (Visioli et al., 2009). Such qualities of cocoa have largely been attributed to the catechins, which are polyphenols of the flavanol group, which function as antioxidants and which cocoa contains in significant amounts (Vinson et al., 2006). This study was therefore geared towards confirming the previously reported good attributes of cocoa in human health through the use of mouse models to determine its anti-malaria activity.

MATERIALS

Parasite

P. berghei was obtained from Malaria Research and Reference Repository Centre (MR4) 10801 University Boulevard, Manassas, Virginia United States of America (Batch Number S0594596) was used. It was collected from the Institute of Medical Research and Training (IMRAT), College of Medicine, University of Ibadan, Nigeria and maintained by mouse-to-mouse passage.

Mice

Male and female Naïve BALB/C mice of 8 to 12 weeks old (14 to 22 g) were used for this study. They were purchased from commercial animal breeding house in Ibadan Oyo State, Nigeria.

Cocoa

Natural cocoa powder (non-alkalized) as packaged by Cocoa Research Institute of Nigeria (CRIN), Idi-Ayunre, Ibadan, Oyo State, Nigeria, was used for this experiment.

Chloroquine

Chloroquine injection produced by Pfizer Nig. Ltd was purchased at Ogun State University Teaching Hospital Pharmaceutical Centre at Shagamu, Ogun State, Nigeria.

Modified cocoa feed

The experimental feed was specially formulated on request to be made of the normal rat diet with inclusion of 2% natural cocoa powder. The modified feed consisted of maize starch, sucrose, soybean oil, fibre (cellulose powder), mineral premix, choline bitartrate, tert-butyl-hydroquinone (Katsuhiko et al., 2009) and 2% cocoa powder. This was made into rat feed pellet by Pfizer feed mill, Iwo Road, Ibadan.

Experimental animals

Mice were housed in cages and maintained under standard condition (12 h light/dark cycle 25± 3°C, 45 to 65% humidity). The animals had access to modified standard mouse feed and water ad libidum. All the animals were acclimatized to laboratory condition for 3 days before commencement of the experiment as described by Karunakar et al. (2009). Experimental mice were randomly put grouped into six groups randomly containing 5 animals each, according to their weight and sex. Each group comprises of both male and female mice.

Group A - normal (rat) feed
Group B - compounded cocoa feed
Group C - normal (rat) feed + infection with *P. berghei*
Group D - normal (rat) feed + infection with *P. berghei* + treatment with chloroquine
Group E - normal (rat) feed + infection with *P. berghei* + post feeding with cocoa feed
Group F - pre-fed with cocoa feed (one week before) + infection with *P. berghei* + post feeding with cocoa feed
Group G - normal (rat feed) + infection with *P. berghei* + cocoa feed + chloroquine.

Inoculation of mice

Infected blood containing *P. berghei* was obtained from donor mouse with 20 to 40% parasitemia. Blood for innocular was obtained by cardiac bleeding using 3% sodium citrate as an

Table 1. Parasitaemia (cells/µl) in cocoa and chloroquine treated mice.

Groups	$\overline{X}$ ± SEM	F	P-value
Untreated (control)	23,937.50 ± 1499.77	27.20	< 0.05
Treated with chloroquine	3,412.50 ±1499.77		
Post-fed with cocoa	2,989.59 ± 361.80		
Pre-fed with cocoa	3,166.67 ± 2333.34		
Treatment with cocoa and chloroquine	2,375.00 ±125.00		

Table 2. Variation in parasitaemia (cells/µl) in cocoa and chloroquine treated mice.

Groups	$\overline{X}$ ± SEM (%)	t	P-value
Untreated (control)	+258.33 ± 56.49	0.96	< 0.05
Treated with chloroquine	-60.09 ± 7.84		
Post-fed with cocoa	-60.82 ± 8.47		
Pre-fed with cocoa	-73.81 ± 6.86		
Treatment with cocoa and chloroquine	-52.38 ± 11.60		

anticoagulant. Experimental mice received one innoculum of 0.2 ml of *P. berghei* which was applied intraperitoneallly (IP).

Determination of parasitemia

For the detection of malaria infection, thin and thick blood smears were prepared from all experimental mice before the start of experiment. Less than 1 mm section of the distal end of the tail was cut and approximately 3 µl of blood was sported unto a microscope slide. Slides were air dried, and smears were stained with a 10% Giemsa solution and were observed in a light microscope with X 1000 objective (oil immersion). The percentage parasitemia and parasite density were taken at each follow-up day that is day 0, 3, 5, 7, 11 and 14. The mean weights were taken, parasite counts were expressed as parasite density per microlitre (µl) of blood, (Cheesebrough, 2005). Also, percentage change in parasite density was determined as stated below:

$$\text{Percentage (\%) change in parasite density} = \frac{\text{Initial MP density before treatment - Density of MP after treatment}}{\text{Initial MP density before treat}} \times 100$$

Note: MP = Malaria parasite

Weight gain/loss

Weights of the experimental mice were taken before the commencement of the experiment (that is, day 0) and on days 3, 5, 7, 11, and 14 days. Mean weights were taken and percentage weight loss or gain was determined through comparison of the mean weight before and after infection with *P. berghei*.

Statistical analysis

Data analysis was carried out with (SPSS version 15). Comparison of weights and parasite density in mice was done by using Analysis of Variance (ANOVA) and Student's t-test. Level of significance was determined at 95%.

RESULTS AND DISCUSSION

The results of the experiment on plasmodium infection on mice and the effect of cocoa powder ingestion as compared with chloroquine treatment as demonstrated in this study. In Table 1, the result of parasitaemia (cells/µl) in cocoa and chloroquine treated mice were indicated, the highest parasitaemia (plasmodial density) of 23,937.50 ± 2,782.25 cells/µl of blood was recorded in the untreated mice ($P< 0.05$) while others showed decrease in their plasmodial densities. On comparison of percentage change in parasite density as indicated in Table 2, the untreated mice recorded a mean percentage increase of +258.33 ± 56.49% while mean percentage decrease was recorded by various groups of treated mice such as those injected with chloroquine (-60.82 ± 8.47%), post-fed with cocoa (-60.82 ± 8.47%), pre-fed with cocoa (-73.81 ± 6.86%) and those treated with chloroquine and cocoa (-52.38 ± 11.60%). Observation of significantly lower parasite density in blood of mice group treated with chloroquine and cocoa ($P< 0.05$) showed that both agents have anti-plasmodial activities. This experiment was in agreement with the research findings of de Monbrinson et al. (2006) who observed that flavonoid derivatives such as dehydrosilybin and 8-(1,1)-DMA-kaempferide, which were extracted from cocoa, exerted significant anti-malarial activity against five strains of *P. falciparum*. The author also recommended that flavonoid derivatives could be used as adjunct to already available anti-malarial drugs to delay the spread of resistance in *P. falciparum*. The mean percentage plasmodial reduction

Table 3. Weight variations in cocoa treated mice.

Period of treatment (weeks)	Values $\overline{X}$ ± SEM	F	P-value
Before treatment (0)	17.60 ± 1.27	1.41	< 0.05
1st week of treatment (1)	16.65± 1.63		
2nd week of treatment (2)	15.60 ± 0.10		

Table 4. Total parasite density in male and female mice.

Total parasite density (cells/µl of blood)			
Sex	**$\overline{X}$ ± SEM (%)**	**t**	**P-value**
Male	45666.867 ± 10443.83	0.061	< 0.05
Female	44900.200 ± 7154.43		

expressed in mice post-fed with cocoa (60.82 ± 8.47%) and those treated with chloroquine (60.09 ± 7.84%) indicated that both agents exhibit plasmodial reduction almost at equal level. In this study, therapeutic and prophylactic effects of cocoa against *P. berghei* were compared in mice. Though, higher percentage of plasmodium reduction was observed in mice pre-fed with cocoa (73.81 ± 6.86%) than those post-fed (60.82 ± 8.47%), but the difference was not significant (t = 0.96, P> 0.05).

Insignificant difference in the percentage plasmodial reduction between mice pre-fed with cocoa and those post-fed with cocoa (P> 0.05) is an indication that both method of anti-malaria treatment are equally effective. However, the observation of higher percentage of plasmodial reduction in mice pre-fed with cocoa suggests that cocoa may contain immune-booster whose action is anti-malarial. The effect of cocoa consumption on mice weight was determined in Table 3, a systematic decline was observed in the weight of mice with mean weight of 17.60 ± 1.27 g before administration of cocoa, 16.65 ± 1.63 g after first week of treatment and 15.60 ± 0.00 g after second week of treatment. The difference was however, found to be insignificant (F = 1.41, P> 0.05).

The systematic decline in weight of mice after being fed with cocoa for a period of two weeks demonstrated that cocoa may contain some weight trimming ingredients. The insignificant observation may be attributable to the short duration of this study. The result of total parasite density in male and female mice as observed in Table 4 showed comparison between the total parasite density of male and female mice. The result of this analysis indicated that there was no significant difference in the level of parasitaemia observed in mice from both gender (t = 0.61, P> 0.05).

Conclusion

From this study it can be concluded that cocoa powder exhibited *in vivo* anti-malaria activity. It further confirmed that cocoa powder possesses both therapeutic and prophylactic effects against *P. berghei*. This means that regular consumption of cocoa will reduce the occurrence of malaria attack. However, extensive research work is required for the study of its anti-malaria agents and their possible, precise mechanism of action.

RECOMMENDATION

From the outcome of this study, we hereby recommend that cocoa consumption should be promoted in areas where malaria is endemic. This will surely reduce incessant cases of drug-resistance and as well prevent the devastating effect of malaria on human. Since cocoa powder is a food drink, it is not likely for malaria causing parasite (*Plasmodium spp*.) to become resistance to cocoa powder. The immune booster effect of cocoa powder is an area to look into in the nearest future.

ACKNOWLEDGEMENT

We are grateful to the Executive Director of Cocoa Research Institute of Nigeria Prof. G. Iremiren for the permission given to publish this paper. We also appreciate Mrs Oyelami for her secretarial assistant during the course of this project.

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