IN-VIVO ANTIPLASMODIAL ACTIVITY OF ETHANOLIC EXTRACT OF *CASSIA ALATA* AND *PHYLLANTUS AMARUS*

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ABSTRACT
The antiplasmodial property of ethanol extracts of *Cassia alata* leaf and *Phyllantus amarus* (whole plant) was evaluated. Besides, effects of the extracts on certain biochemical parameters were also investigated in this study using mice. The extracts demonstrated significant antimalarial effects with the leaf of *C. alata* causing a reduction in parasitaemia (from 8.02±0.0 to 2.79±0.01), while the extract of *P. amarus* equally caused a decrease in the parasitemia from 8.01±0.03 to 3.96±0.01. The reduction in parasitemia observed for both plants extracts were significantly ($P < 0.05$) higher than the reference drug. On the seventh day of this study, biochemical analyses were done to assess the levels of alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), urea, creatinine and triacylglyceride as indices of liver and renal functions, respectively. A significant decrease ($P < 0.05$) and an increase ($P < 0.05$) in the serum ALT and triacylglyceride respectively were observed in the treated mice. However, there was no significant change noticed in the serum AST for the groups treated with extracts. There was a significant reduction ($P < 0.05$) noted in the serum urea level for the group treated with *C. alata*. Whereas, a reduction in the serum ALP level was seen for both plant groups, it was significant ($P< 0.05$) only in the *P. amarus* group. Thus, the study has shown that extracts of both plants posses' significant ($P< 0.05$) suppressive effects against *P. berghei* infection in Swiss albino mice, and has also confirmed their traditional usages for the treatment of malaria.

Key words: Antiplasmodial, *Plasmodium berghei, Cassia alata*, *Phyllantus amarus*, medicinal plants, biochemical parameters.

INTRODUCTION
Malaria is said to be responsible for 1 to 3 million deaths universally, with most cases occurring in sub - Saharan Africa (Breman, 2001). The malaria endemic is a foremost reason behind childhood mortality, and also adult morbidity in parts of the world and has continued to be the world most destructive human infection, affecting over 500 million people and resulting in the death of 1.7 to 2.5million people yearly (WHO, 2010). Millions of people have been projected in Nigeria (50% of adult population) to be at the risk of malaria experiencing one episode at least annually. Malaria is the main cause of death to children and mothers in Nigeria (30 and 11% mortality respectively). The disease is said to have adversely affected the nation's economy as billions of naira are lost to its treatment yearly (FMOH, 2005 a and b).

The main drawback in the fight against the disease had been the incidence of resistance to currently available antimalarial drugs, especially *Plasmodium falciparum* resistance (Wongsrichanalai et al., 2002). This has therefore necessitated the continuous search for new efficacious antimalarial drugs from plants, as plants had been the main source of medicines (Kayembe et al., 2010). Plants have always been the fundamental source of high-tech traditional medicine for centuries, and were contributory to early drug discovery (Elujoba et al., 2005). Basic sources of information that serves as a lead to eventual research on Africa medicinal plants have been herbal sellers, traditional medicine practitioners and local indigenous people (Baba et al., 1992).

Cassia alata is a tree that grows up to 12 m high in grasslands, and it is extensively found in the tropics. The herb is used in folklore medicine, and it is also use as timber and firewood (Rai, 1987). The uses of the plant in the

treatment of diabetes, constipation, hemorrhoids, inguinal hernia, blennorrhagia and syphilis have been reported (Makinde et al., 2007). The antifungal properties of the plant have also been stated (Reezal et al., 2002). Other uses of *C. alata* reported include: as a remedy for urinary and gastrointestinal tract infections, boils, diarrhea, wound and scarlet fever (Benjamin and Lamikaura, 1981; Lindley, 1981). The leaves of *C. alata are* usually employed by herbalists in Delta state for the treatment of malaria among other diseases.

Phyllanthus amarus is a broad spectrum herbal plant that is broadly found in the tropical and subtropical parts of the world (Burkill, 1935; Srividiya et al., 1995). The plant is used for the treatment of jaundice, malaria and diabetes (Etta, 2008). The active ingredients in *Phyllanthus* include: lignans phyllanthine, phylochrysine, phyllanthenol, hypophyllanthine, phylltetralin, quercertin, bioflavanoids, quercertol, rutin, quercitrin the alkaloids, saponins, glycosides and catechins (Thyagarajan et al., 1998; Khanna and Srivastava, 2002).

With the increase in incidences of antimalaria drug resistance and the magnitude of the malaria problem worldwide, the need for discovery of more potent antimalarial drugs have been necessitated now more than ever before (WHO, 1997; Peters, 1998; Wongsrichanalai et al., 2002; Milijaona et al., 2003). Consequently, this study aims to examine the antimalarial properties of two herbs, *C. alata* and *P. amarus* used in the traditional treatment of malaria in Delta State, Nigeria, and to also assess the effects of the plants treatment on some biochemical parameters. The findings would provide a scientific backing in the use of these herbs against malarial disease.

MATERIALS AND METHODS

Sample collection

Two medicinal plants namely, *C. alata* (leaves) and *P. amarus* (whole plant) were obtained from bushes in Abraka, Delta State, Nigeria. The plants were chosen following leads from local traditional healers and they were identified by a taxonomist, Dr J.K. Ebigwai in the Department of Botany, Delta State University, Abraka, Nigeria.

Extraction procedure

The plant samples were washed with distilled water and air-dried at 30±0.5°C. Thereafter, they were pulverized using pestle and mortar. The powdered samples were extracted using 232 g: 1 L of the sample: 98% ethanol respectively for 72 h with daily shaking for appropriate extraction (Abosi and Raseroke, 2003). The herb-ethanol mixtures were filtered using filter paper (Whatman No 1), and the filtrate was concentrated with a vacuum rotary evaporator (at reduced pressure and low temperature) and water bathe (at 45°C). Before being use for the experiment, the concentrated residue was reconstituted in distilled water to yield a dose of 125 mg kg-1.

Mouse strain

Healthy albino mice, weighing 18 to 24 g, about 5 weeks old were procured from the Department of Anatomy's animal house of the Delta State University, Abraka, Nigeria. They were acclimatized for a week, and the animals were fed on livestock feed and water *ad libitum* throughout the study which was done in the Biochemistry Laboratory of Delta State University, Abraka, Nigeria.

Parasites and drug

The chloroquine sensitive *Plasmodium berghei* (NK - 65) used in this study was obtained from the National Institute of Medical Research (NIMR), Lagos, Nigeria. While the standard chloroquine (Diphosphate salt –50-63-5 EC No. 200-055-2) was obtained from Sigma-Aldrich Company POB 1450B, Louis MD 63178, United States of America (USA), and was employed as standard reference for the study.

Inoculation and treatment

The standard Peter's 4 day test (Peter and Anatoli, 1998; David et al., 2004) was employed to assess the blood schizontocidal action against *P. berghei* using twenty four mice that were randomly divided into four groups (of six per cage). The experimental groups (two) were each orally given 125 mg kg-1 of the extracts per day. While the other two groups were given either 5 mg kg-1 chloroquine per day (positive control) or

0.2 ml of phosphate buffered saline (concentration of 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, pH 7.4) for the negative control. All experiments were done in triplicate.

Estimation of parasitemia

On the fourth day of the test, parasitemia estimation was done and the monitoring of parasitemia continued for seven days (Devi et al., 2000; Dikaso et al., 2006). The suppression of parasitaemia (%) was estimated by comparing the parasitaemia of infected (controls) with those in treated mice for each dose level. Average suppression (%) of parasitaemia was computed using the formula:

$$A = \frac{B - C}{B} \times 100$$

Where:

A = Average (%) suppression
B = Average (%) parasitaemia of control group
C = Average (%) parasitaemia of test group (Abosi and Raseroke 2003)

Biochemical studies

On day seven of the study, an airtight glass chamber that was saturated with chloroform was employed to euthanize the mice. The animals were each opened up surgically, and blood samples used for the analyses were collected. The Biochemical parameters (Creatinine, Urea, Transaminases, Alkaline phosphatase and Triacylglyceride) were estimated in the plasma using standard commercial kits of acceptable protocols.

RESULTS

A significant difference ($P < 0.05$) was noticed between the % parasitemia of the infected/treated (*Cassia alata*) mice and infected/untreated mice (Table 1). Comparisons were done between days 3 and 7. This same trend was also observed for the *P. amarus* treated group when compared with the infected/untreated mice (Table 1). Although, the parasitemia was not entirely cleared as seen in the test groups, it was however reduced considerably in the groups treated with either extracts, and the reduction was more in the *C. alata* treated group than the *P. amarus* treated group, while the reduction observed in parasitemia was nominal in the positive control group when it was compared with other groups as shown in Table 1.

Table 1. *In vivo* anti-malarial effect of ethanol extracts of *C. alata* and *P. amarus.*

Drug/plant extract	n	Drug/plant extract dose (mg/kg)	Average parasitemia(%)				
			Day 3	Day 4	Day 5	Day 6	Day 7
C. alata	6	125	8.02±0.00	6.34±0.89	4.36±0.95	3.86±1.05	*2.79±0.01
P. amarus	6	125	8.01±0.03	5.42±1.07	4.21±0.83	4.07±0.81	*3.96±0.01
Untreated control (negative control)	6		8.01±0.00	8.73±1.32	8.85±1.32	8.96±0.14	8.96±0.01
Chloroquine (positive control)	6	5	8.02±0.00	7.82±0.24	7.02±0.05	6.89±1.05	*6.93±0.01

Values indicate mean percentage parasitemia and are expressed as Mean ± SEM (n=6). * differ statistically at $p<0.05$; n = number of animals per group.

Results for the biochemical parameters in both treated and untreated groups are shown in Table 2. The ALT of infected/treated mice of both plant extracts showed significant ($p <0.05$) reduction relative to the infected/untreated mice (negative control). Similarly, the AST of positive control was also, significantly ($p<0.05$) reduced compared to the infected/untreated mice (negative control), but no statistical difference ($p<0.05$) was observed when the urea levels were compared between groups. However, the serum triacylglyceride levels were

Table 2. Effect of ethanol extracts of *C. alata* and *P. amarus* on Biochemical parameters.

Group	Urea	TRIG	CR	ALP	AST	ALT
C. alata	13.00±0.58*	98.00±0.58*	0.68±O.01	21.47±0.78*	7.13±0.45	3.63±0.24*
P. amarus	19.67±1.20	125.33±2.91*	1.12±0.10*	21.67±5.24	7.87±0.09	3.97±0.26*
Untreated control (negative control)	18.00±1.15	141.00±2.08	1.03±0.15	27.50±0.29	8.87±0.20	6.03±0.15
Chloroquine (positive control)	20.33±2.40	92.67±1.86*	3.23±0.19*	25.67±1.20	7.33±0.28*	3.37±0.12*

Data are expressed as Mean ± SEM for three determinations. * differ statistically (P<0.05); Where TRIG= triacylglyceride; CR= Creatinine; ALP= alkaline phosphatase; AST= aspartate transaminase; ALT= alanine transaminase.

statistically ($p<0.05$) reduced in both plant extracts, and the positive control when compared with the infected/untreated group. Also, comparing with the infected/untreated group, a statistical increase was seen in the *P. amarus* treated mice and the positive control group for serum creatinine. While a reduction ($p<0.05$) was seen in the ALP levels for the *P. amarus* treated mice as compared with the control (Table 2).

DISCUSSION

The results of this study suggest very good activities of *C. alata* (leaves) and *P. amarus* (whole plant) ethanol extracts against *P. berghei* malaria parasite (at 125 mg kg-1dosage). A significant level of inhibition was displayed by *C. alata* (2.79±0.01%) than both the extract of *P. amarus* (3.96±0.01%) and the positive control (6.93±0.01%) when compared with the negative control (8.96±0.01%) on the 7th day of this study.

Significant decrease in parasitemia was noticed in both plant extracts than the standard drug. Because the parasites used were chloroquine sensitive, it may be that the strength of the respective extracts (at 125 mg kg-1) was greater than the chloroquine (at 5 mg kg-1). A clearance rate of 100% parasitaemia has been reported by Ajaiyeoba *et al*. (2006) and Devi et al. (2000) in their respective studies with chloroquine sensitive *P. berghei* parasites at a total dose of 25 and 20 mg kg-1, respectively. It should however be noted that whereas both researchers used $1.0x10^7$ parasites, but in the present study a total dose of 20 mg kg-1 and $1.1x10^7$ parasites was used in this study. Thus, an increase in the parasite count was noticed in the infected/untreated group of this study, which agrees with previous reports (Ajaiyeoba et al., 2006; Devi et al., 2000).

Broad phytochemical investigations on numerous species of *Phyllanthus* have shown a number of bioactive components like lignans, alkaloids, terpenes, tannins and flavones (Matsunga et al., 1993; Foo, 1995; Bila et al., 1996; Lee et al., 1996; Houghton et al., 1996; Zhang et al., 2001, 2002; Chang et al., 2003). Similarly, some flavonoid compounds such as sennosides, anthroquinones and kaempferol have been isolated from *C. alata* which are useful in the treatment of fistula, constipation, diabetes, inguinal hernia, blennorrhagia, intestinal parasitosis and syphilis (Kochar, 1981; Adjanahoun et al., 1991). Therefore, the anti-malarial effects of the extracts seen in this study may be ascribed to the occurrence of some of these bioactive components/ secondary metabolites in the plants used.

Some biochemical changes were also seen in the animals when the ethanol extract of both plants (*C. alata* and *P. amarus*) was administered at 125 mg/kg to the mice. A significant decrease was seen in serum triacylglyceride and ALT in the infected/treated animals that received either plant extracts or the positive control group. A dropping off in serum level of ALT possibly will imply absence of side effects of the extracts on hepatic tissues. Dioka et al. (2002) reported a significant elevation in the hepatic function parameters they tested in albino rats, employing rinbancin (trademark preparation), which is a homeopathic drug (at dose levels of 26.25 and 52.50 g/L).

A high activities level of liver enzymes like AST, ALT and ALP are said to be a usual sign of liver disorders (Adolph and Lorenz, 1982). Nonetheless, a decline in hepatic enzymes level was seen with the extracts of this study,

which may imply that the liver's integrity was not affected adversely by the extracts. This observation however does not agree with that of Akah et al. (1991) that the intake of certain herbal extracts could result in elevation of serum liver enzymes. This suggests that the plants used in the present study may be safe to an extent, and are nontoxic for human use.

An elevated level of serum creatinine was observed in both the *P. amarus* treated mice, and the group given the reference drug when compared with the infected/untreated animals. Although, a decline in levels of serum creatinine was seen in the mice treated with extract of *C. alata*, the decrease was nevertheless non-significant.

Also, the results demonstrated a significant reduction in the levels of serum urea for the group treated with *C. alata*. Urea is a major end product of catabolism of protein. The deamination of amino acids usually occurs in the liver, which doubles also as the site of urea cycle. Here, ammonia is transformed into urea and then excreted via the urine. Urea levels changes with the intake of protein, and also varies with excretion rate. A decrease in urea is common in serious liver diseases where there is damage of hepatic cells resulting in loss of the urea cycle (Ranjna, 1999). High levels of serum urea can be ascribed to damage the kidney, which may be due to alkaloidal fraction of drug/ plant extracts (Adeoye et al., 2004).

A decrease in levels of serum creatinine seen in this study could be as a result of boosted uptake of creatinine from circulation that occurred in the infected/treated groups, which denotes that integrity of skeletal muscles in the animals treated with either extracts are sufficiently protected (Adeoye and Oyedapo, 2004).

CONCLUSION

This study has revealed that the extracts of both plants posses' significant suppressive effects against *P. berghei* infection in Swiss albino mice, and has confirmed their traditional use for the treatment of malaria.

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Conflict of interests

The authors have not declared any conflict of interests.

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