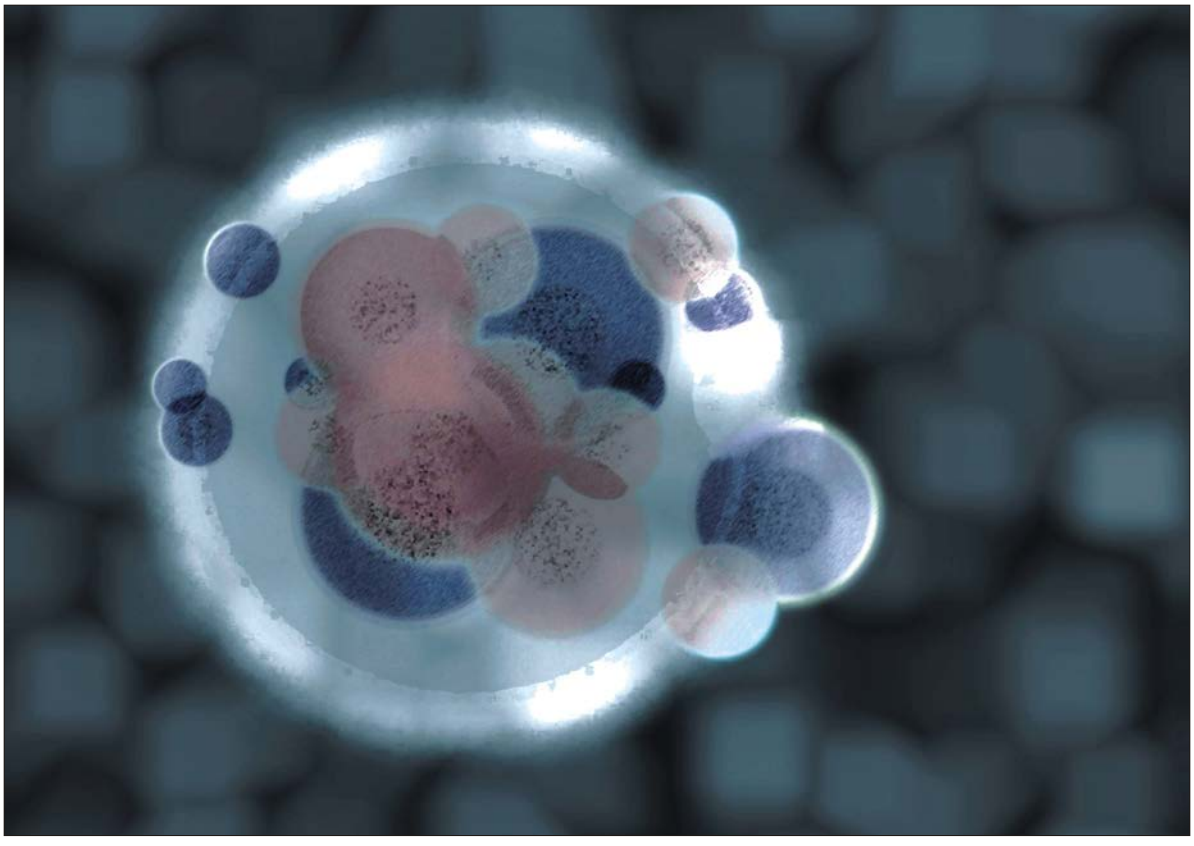

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Anti-pyretic and Antiplasmodial Activities of a Polyherbal Formula (Joloo)

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Abstract: To investigated the polyherbal formula (Joloo) for its antimalarial and anti-pyretic properties on mice and rats. In the antimalarial study mice were infected with *Plasmodium berghei* using three doses (1600, 800 and 400 mg kg^{-1} b.wt.). Pyrexia was induced in the rats by the administration of 10 mg kg^{-1} b.wt., of 2,4-Dinitrophenol intraperitoneally while measurement was by inserting a clinical thermometer into their anal cavities for about 2 min. Chloroquine and acetylsalicylic acid were used as reference drugs in mice and rats respectively, while water served as control for both. The antiplasmodial study involved two phases; the suppressive where mice were administered plant extract per os for four days immediately after inoculation and blood smear prepared on the fifth day and the curative phases where mice were inoculated with parasites three days before administration of extract so as to allow for full development of parasites. They were administered the extract orally for five consecutive days and blood smears prepared during the period of administration and five days post administration. During the 5-days suppressive study, the herbal formulation (Joloo) showed significant daily dose-dependent decrease in parasitaemia and compared favourably with the reference drug (Chloroquine). It was observed however that the 1600 and 800 mg achieved a total 100% chemosuppression of parasitaemia, while 400 mg kg^{-1} b.wt. dose was 44.5%. During the curative study, there was significant dose-dependent decrease in parasitaemia during the 5-days period of administration and subsequent increase in parasitaemia in the remaining 5-days post administration. In both suppressive and curative assays, chloroquine achieved 100% chemosuppression while Joloo achieved 100% chemosuppression in the suppressive assay. Besides, Joloo inhibited parasitaemia only during administration in the curative study after which a progressive increase in parasitaemia was observed during post-administration. In the anti-pyretic study, Joloo significantly reduced hyperthermia in rats dose-dependently. This clearly suggests that Joloo contain biologically active substances with the potential of managing and treating malaria and fever. It provides scientific evidence to support the isolation and development of biologically active components as anti-malarial and antipyretic agents.

Key words: Antiplasmodial activity, anti-pyretic activity, Joloo, malaria, *Plasmodium berghei*

INTRODUCTION

Malaria is the major cause of mortality and morbidity in tropical Africa and still remains one of the major threats concerning world public health. As devastating as it could be, WHO (1999) estimated death occurring from malarial infection annually in Africa as 2 million, with Nigeria accounting for a quarter of all the malaria cases in Africa, ranked the most common disease in Nigeria (Adebayo and Krettli, 2011; Federal Ministry of Health, 2004). Also over 250 million are infected by this disease with 90 million of clinical cases annually (Hilou *et al*., 2006).

Having a vast population of about 300 million people, sub-Saharan West Africa represents the region with the highest population exposed to high levels of malaria transmission (Soh and Benoit-Vical, 2007) and over 2 billion people have limited access to effective antimalarial treatment across the world (Andrade-Neto *et al*., 2008). In recent years mortality has been on the increase mainly due to increased resistance to antimalarial medicine (Gathirwa *et al*., 2011), lack of new therapeutic targets and unaffordable antimalarial drugs (Soh and Benoit-Vical, 2007). Medicinal plants have traditionally been the most common source of drugs, as it has long been recognized that they characteristically possess; high chemical diversity, biochemical specificity and other molecular properties that predispose them as new drug-leads and drug entities (Clardy and Walsh, 2004). Herbal medicines have been used to treat and manage various infections including malaria for thousands of years in various part of the world; its use in Africa still contributes significantly in malaria treatment.

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Joloo, the preparation used for these studies, is a polyherbal formula traditionally used in southwestern Nigeria against an array of diseases. It comprised of seven medicinal plants jointly extracted together, which include; *Allium ascalonicum* Linn. {(Liliaceae: Alliaceae) (alubosa elewe)}, *Butyrospermum paradoxum* Gaertn {(Sepotaceae) (eso ori)}, *Hoslundia opposite* Vahl {(Lamiaceae) (efinrin odan)}, *Olax subscorpioidea* Olive {(Olacaceae) (egbo ifon)}, *Xylopia aethiopica* Dunal A. Richard {(Annonaceae) (eru awonka)}, *Securidaca longepedunculata* Fresen {(*Polygalaceae*) (egbo ipeta)} and *Tetrapleura tetraptera* Schum/Thonn {(Leguminosae: Mimosidae) (aidan onigun)}. Joloo was reported to possess anti-inflammatory and analgesic properties (Oloyede *et al*., 2008), it also exhibited positive antimicrobial properties (Oloyede *et al*., 2012a). Also Joloo was screened for a plethora of toxicosis, such as Acute (Oloyede *et al*., 2009), subchronic (Oloyede *et al*., 2011) and chronic toxicity (Oloyede *et al*., 2012b). Others include; genotoxicity (Oloyede *et al*., 2009), cytotoxicity (Oloyede *et al*., 2012c) and gonadotoxicity (Oloyede *et al*., 2012a). The present investigation therefore was to establish if Joloo posses any antimalarial and antipyretic properties to ascertain its wide ethnobotanical uses.

MATERIAL AND METHODS

Extract preparation: The total plant materials (weighing 570 g) comprised of *B. paradoxum* seed, *S. longepunculata* bark, *T. tetraptera* stem, *H. opposita* leaves, *X. aethiopica* seed, *O. subscorpioidea* stem and *A. ascalonicum* in ratio 3:5:2:1:4:1:3, respectively, were made into a cocktail (traditional herbalist) to produce the desired pharmacological action (Oloyede *et al*., 2008). Samples were air dried, powdered, weighed and then allowed to stand in 1600 mL of absolute (95%) cold ethanol for 72 h. They were thereafter decanted and filtered using a muslin cloth. The extract was further evaporated to dryness using a freeze-dryer at Biochemistry Department University of Lagos. Finally, the dried extract weighed 29.27 g. The cocktail was then reconstituted to concentrations of 400, 800 and 1600 mg mL^{-1}.

Animals: Swiss albino mice (20-25 g) used for antiplasmodial studies and rats (100-180 g) used for anti-pyretic activity of both sexes were obtained from the Redeemer's University Animal House, Ogun state, Nigeria. The animals were housed in standard cages and acclimatized for a period of 10 days. The mice were maintained on standard feed and water *ad libitum*. Approval for the study was obtained from the Animal Ethics Committee, Redeemer's University.

Parasite inoculation: The chloroquine sensitive *Plasmodium berghei* was obtained from Biochemistry Department of Nigerian Institute of Medical Research, Lagos, Nigeria and maintained in mice by passaging. The inoculum consisted of 5×10^7 *P. berghei* parasitized red blood cells per mL. This was prepared by determining both the percentage parasitaemia and the red blood cell count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations. Each mouse was inoculated on day 0, intraperitoneally, with 0.2 mL of infected blood containing about 1×10^7 *P. berghei* parasitized red blood cell.

Drug administration: The drugs and Joloo used in the antiplasmodial and antipyretic studies were orally administered with the aid of a stainless metallic feeding cannula.

Evaluation of schizontocidal activity on early infection (5-day test): Schizontocidal activity of the Joloo was evaluated using the method described by Okokon *et al*. (2007). Each mouse was intraperitoneally inoculated on the first day (day 0), with 0.2 mL of infected blood containing about 1×10^7 *P. berghei* parasitized erythrocytes. The animals were divided into five groups of five mice each and orally administered, shortly after inoculation with 400, 800 and 1600 mg kg^{-1} day doses of Joloo, chloroquine 5 mg kg^{-1} day and an equivalent volume of distilled water (negative control) for four consecutive days (days 0-3). On the fifth day (day 4), thin and thick films were made from the tail blood of each mouse and the parasitaemia level was determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Average percentage chemosuppression was calculated as 100((A-B)/A), where A is the average percentage parasitaemia in the negative control group and B, average percentage parasitaemia in the test group.

Evaluation of schizontocidal activity on established infection (curative or rane test): Evaluation of curative activity of Joloo was done according to Okokon *et al*. (2007). The mice were injected intraperitoneally with standard inoculum of 1×10^7 *P. berghei* infected erythrocytes on the first day (day 0). Seventy-two hours later, the mice were divided into five groups of five mice each. The groups were orally administered with Joloo (400, 800 and 1600 mg kg^{-1} day), chloroquine (5 mg kg^{-1}) was given to the positive control group and an equal volume of distilled water to the negative control group. The treatment, control and chloroquine were administered once daily for 5 days. Thin and thick films stained with

Giemsa stain were prepared from tail blood of each mouse daily for 5 days to monitor the parasitaemia level.

Anti-pyretic activity: Anti-pyretic activity of Joloo was carried out using the methods of Berken *et al*., (1991). Rats of both sexes weighing (100-180 g) were fasted for 24 h but allowed water *ad libitum*. They were randomized into five groups of ten rats per group. The baseline body temperatures of the rats were taken by inserting a clinical thermometer into their anal cavities for 2 min. The steady temperature readings obtained were recorded as the pre-treatment temperatures. Pyrexia was induced in the rats by the administration of 10 mg kg^{-1} b.wt., of 2,4-Dinitrophenol (DNP) intraperitoneally. Hyperthermia developed 30 min later after DNP administration. Different doses of Joloo (ranging between 400-1600 mg kg^{-1} b.wt.) were given orally, aspirin (100 mg kg^{-1} b.wt. i.p) and distilled water (10 mL kg^{-1} b.wt.) was administered orally to the treatment and control groups of animals. Rectal temperatures were obtained at 30 min interval for 5 h.

Statistical analysis: Data obtained from the study were analyzed statistically using Student's test and values of $p<0.05$ were considered significant.

RESULTS

Joloo achieved 100% chemosuppression of parasitaemia at 1600 and 800 mg kg^{-1} b.wt., thereby comparing favourably with the chloroquine which also recorded a 100% chemosuppression. However, the 400 mg kg^{-1} b.wt. dose did not produce significant chemosuppression when compared to control (Table 1).

After established parasitaemia in 72 h post-inoculation, the animals administered with Joloo, showed dose-dependent activities against parasites. The 1600 mg kg^{-1} b.wt. dose significantly reduced parasitaemia. As the days of infection increased animals appeared weak and critically sick in the 1600 and 400 mg kg^{-1} b.wt. doses respectively. At the 8th day three mice died in the 400 mg kg^{-1} b.wt. dose group, while all the mice in the controlled group died. However 100% clearance was observed in the group administered with 5 mg kg^{-1} b.wt. Chloroquine (Table 2).

The administration of 2,4-dinitrophenol elevated the rectal temperature of the rats. Treatment with Joloo at 400, 800 and 1600 mg kg^{-1} significantly decreased the rectal temperature dose-dependently. The antipyretic activity started from the first 30 to 300 min after Joloo administration The formulation showed a biphasic pattern of activity. All doses (400-1600) reduced hyperthermia dose-dependently. The activity of Joloo was significantly different from the control. The formulation also compared favourably with the reference drug (acetylsalicylic acid 100 mg). The standard drug also reduced hyperthermia significantly at all-time intervals (Table 3).

Table 1: Suppressive activity of Joloo on *P. berghei* infection in mice (5-day test)

Dose of drug/extract (mg kg^{-1} b.wt./day)	Parasitaemia (Mean±SEM)	Chemosuppression (%)
1600	0	100.0
800	0	100.0
400	2.7±0.8	44.5
Chloroquine (5 mg)	0	100.0
Control (0.2 mL)	4.8±1.1	0.0

Values are Mean±SEM (N = 5)

Table 2: Curative activity of Joloo on *P. berghei* infection in mice (10-day test)

Treatment	Days									
(mg kg^{-1} b.wt.)	0	1	2	3	4	5	6	7	8	9
1600	1.6±1.5	5.2±0.2	1.9±0.3***	1.9±0.3***	4.3±0.7***	5.2±0.9**	5.4±1.2***	10.1±0.1***	11.5±0.2	6.1±0.8
800	2.2±3.4	5.5±0.4	4.4±0.4	2.8±0.4**	4.6±0.3***	5.7±0.3**	10.7±0.8	10.7±0.8***	15.7±0.5	21.8±12
400	2.8±1.6	2.8±0.4	3.4±0.3	5.5±0.1	5.7±0.3	11.1±0.1**	13.3±0.8	12.9±0.5	16.2±0.9 (3 died)	26.3±0.8 (3 died)
Chloroquine (5 mg)	5.1±2.4	4.1±0.4	0	0	0	0	0	0	0	0
Control	5.8±3.6	6.1±3.8	7.9±5.6	11.3±10.9	26.0±16.5	27.5±35.4	28.8±12.8	28.9±23.3	All died	All died

Values are Mean±SEM (N = 5), *p<0.05, **p<0.01, ***p<0.001 significantly different from control (student's t-test)

Table 3: Effect of Joloo on DNP-induced pyrexia

Treatment	Time (min)									
(mg kg^{-1} b.wt.)	30	60	90	120	150	180	210	240	270	300
Control	39.1±0.1	39.1±0.1	39.5±0.2	39.4±0.1	38.9±0.2	38.2±0.2	38.9±0.1	39.0±0.1	39.1±0.1	38.9±0.1
400	38.2±0.3*	37.7±0.2*	38.2±0.2*	37.9±0.2**	37.8±0.2**	37.7±0.2**	37.6±0.1**	37.6±0.1[c]	37.6±0.1***	37.5±0.1**
800	39.4±0.2*	38.6±0.2*	39.0±0.2	38.8±0.1	38.3±0.3	38.8±0.2**	38.3±0.3**	38.1±0.2**	37.8±0.2***	37.6±0.1***
1600	36.5±0.3***	35.9±0.4***	36.5±0.4***	36.3±0.3***	36.6±0.2***	36.4±0.2***	37.2±0.1***	37.5±0.1***	37.5±0.1***	37.5±0.1***
Acetylsalicylic acid	38.7±0.2**	38.3±0.2**	37.7±0.2***	37.6±0.2***	38.4±0.1	37.4±0.2**	38.3±0.1	38.2±0.1*	38.1±0.9**	37.9±0.1***

Values are Mean±SEM (N = 10), *p<0.05, **p<0.01, ***p<0.001 significantly different from control (student's t-test)

DISCUSSION

In this study, the antimalarial and antipyretic activity of a polyherbal formula 'Joloo' was evaluated on mice and rats. It was found out that the polyherbal 'Joloo' formula significantly reduced the parasitaemia dose-dependently in the suppressive model with 800 and 1600 mg kg^{-1} doses, recording 100% chemosuppression. In the curative studies there was dose-dependent reduction in parasitaemia during the period of administration and subsequently parasitaemia gradually increased in the post administration period of observation. Studies have implicated some phytochemicals compounds like alkaloids, terpenes and their derivatives as antiplasmodial (Akpan *et al*., 2012). The presence of these phytochemicals as reported by Oloyede *et al*. (2012a) may have contributed to the antiplasmodial activity of the polyherbal formula.

Pyrexia could be induced by tissue damage, inflammation, infections, malignancy and other disease-states. The infected or damaged tissue serves as a pyrogenic stimulus and the pyrogens are phagocytized by the Kupffer cells, monocytes, macrophages etc. leading to the release of cytokines (cytokines e.g. interleukins and TNF-α), which increase the synthesis of prostaglandin E2 near the pre-optic hypothalamic area, thereby stimulating the hypothalamus to elevate the set point of normal body temperature (Spacer and Breder, 1994). Dinitrophenol (DNP) acts as a proton ionophore, across biological membranes. It defeats the proton gradient across mitochondrial membranes, collapsing the proton motive force that the cell uses in the production of most of its ATP chemical energy. Instead of producing ATP, the energy of the proton gradient is lost as heat (Leftwich *et al*. 1982), which usually result in increased level of intracellular calcium, muscle contraction and hyperthermia. A dose-dependent significant reduction in pyrexia was observed in rats treated with Joloo in all the concentrations used and through-out the study. This suggests that Joloo possess a significant antipyretic effect in DNP-provoked hyperthermia in rats and its effect was comparable to the acetylsalicylic acid. In general, Non-steroidal Anti-inflammatory Drugs (NSAIDs) produce antipyretic action by inhibiting the production of prostaglandins in the hypothalamus (Binny *et al*. 2010). Since the hypothalamus regulates the body temperature, Joloo may have inhibited COX-2 expression and consequently inhibiting PGE_2 biosynthesis so as to reduce the elevated plasma level. Beside prostaglandin synthesis, there are several multiprocesses underlying the pathogenesis of fever. Inhibition of such mediators may cause antipyresis (Sahu *et al*., 2012). Joloo may also enhance the production of body own antipyretics like vasopressin and arginine (Okokon, 2011) and may have caused genetic down regulation of COX-2 expression in certain cell types.

The therapeutic activities of traditional remedies are often attributed to a combination of active constituents like titerpenes, flavonoids, alkaloids, steroids, tannins and glycosides, for example flavonoids are known to target PGs involved in late phase of acute inflammation and pain perception (Narayana *et al*., 2001) and have also been linked with analgesic, anti-inflammatory and antipyretic activities (Mutalik *et al*., 2003; Venkatesh *et al*., 2003). It is not surprising therefore to have observed antipyretic activity, since Joloo had been reported to possess analgesic and anti-inflammatory properties (Oloyede *et al*., 2008) and also contains flavonoids (Oloyede *et al*., 2012a).

CONCLUSION

From this study, Joloo has demonstrated considerable antiplasmodial and antipyretic activities. Joloo can specifically be a prophylaxy in the treatment of malaria. This validates its use as antimalarial in folkloric medicine.

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