Arch. Bas. App. Med. 2 (2014) 106 - 111

ARCHIVES OF BASIC & APPLIED MEDICINE

Research Article

In Vivo Antimalarial Activities of the Crude Methanolic Extract and Chromatographic Fractions of the Bulb of *Crinum jagus*

Akintola A[1], Kosoko A[2], Kehinde A[3] and Ademowo O.G[2]*

Department of Science Laboratory Technology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria[1]
Institute for Advanced Medical Research & Training, College of Medicine, University of Ibadan, Ibadan, Nigeria[2]
Department of Medical Microbiology & Parasitology, College of Medicine, University of Ibadan, Nigeria[3]

Accepted in final form: 10th April 2014

Abstract

This study was carried out to investigate in-vivo antiplasmodial activity of crude methanolic extract and chromatographic fractions of the bulb of *Crinum jagus* in *Plasmodium berghei* infected mice. Swiss albino mice were inoculated with *P. berghei* intraperitoneal . The crude extract and fractions were administered orally daily for four consecutive days starting from the day infection was established (Day 3). The control group received tween 80, while chloroquine (10mg per kg body weight) and arteether (3mg per kg body weight) given for 3 days were were used as standard reference drugs. Treatment of infected mice with crude extract and fractions caused suppression of parasitaemia in the treated groups. On Day 6 post infection, the crude extract at different doses administered (10, 25, 50 and 75 mg per kg body weight) produced chemosuppression of 70.1%, 76.2%, 80.0% and 87.2% respectively. Fractions 1, 2 and 3 at 10mg per kg body weight; chloroquine and arteether had chemosuppression of 89.3%, 76.1%, 77.7%, 100% and 86.6% respectively. The difference between serum levels of liver enzymes AST, ALT and ALP following 30 days administration of 10 and 25mg/ kg bw of crude extract and control was not significant ($p > 0.05$). These findings showed that the crude extract and chromatographic fractions of the bulb of *Crinum jagus* possess antiplasmodial activity comparable to that of the reference drugs. This supports the use of the plant extract as a source of natural antimalarial agent. Further studies on mechanism of its antimalarial activity, toxicity and identification of active principle are ongoing.

Keywords; Antimalarial, *Crinum jagus, Plasmodium berghei*

INTRODUCTION

Despite attempts to eradicate malaria, it remains one of the worst diseases in terms of morbidity and mortality. Approximately one million people die of the disease yearly with majority of them being children (Greenwood et al., 2005; WHO, 2011). The reported re-emergence of malaria in many parts of the world is due to rapid increase of resistance to most of the commonly available antimalarial drugs, as well as resistance of vectors to insecticides (Ridley, 2002; Zirihi et al., 2005; WHO, 2011). Drug resistant strains of P*lasmodium falciparum* have been found in many endemic areas of the world and many of the conventional antimalarial drugs have been associated with treatment failures. Furthermore, the difficulty of creating efficient vaccines and also adverse effects of some existing antimalarial drugs highlight the urgent need for development of well tolerated antimalarial medicines.

Plants have always been considered as an important source of medicines against malaria and other infectious diseases (UNESCO, 1998; Krettli et al., 2001; Bhat and Surolia, 2001; Okeola et al., 2011). For example, quinine and artemisinin were derived from plant sources. Although due to resistance to conventional antimalarial drugs, artemisinin derivatives in combination with other drugs have been recommended by the World Health Organization (WHO) as the first- line treatment of malaria (WHO, 2011), resistance to artemisinin derivatives has also been recently described in Southeast-Asia (Dondorp et al., 2010; Pyae Phyo et al., 2012).This has encouraged the continuous search for new antimalarial agents from natural products.

Crinum jagus is a bulbous plant with umbels of lily-like flowers. It belongs to the family of Amaryllidaceae, Phyllum - Angiospermae and Subphylum - Lilifloral. It's local name in Yoruba is *Ogede Odo*. The plant has been used traditionally to cure various ailments and diseases due to the activity of alkaloids, flavonoids and other bioactive components in it (Adesanya et al., 1992).

Crinium jagus has been reported to have various medicinal uses such as antibacterial and anti-fungal activities (Adesanya et al., 1992), anticholinergic action **(**Houghton, et al., 2004), anti-convulsant activity (Edema and Okeimen, 2002; Azikiwe et al., 2012), anti-asthmatic activity (Ogunkunle and Olopade, 2011) and antioxidant actions (Ode et al., 2010). Osakwe et al. (2011) reported ethnomedicinal use of *Crinum jagus* for the treatment of malaria.

This study was therefore aimed at investigating in-vivo antimalarial activity of the crude extract and purified fractions of the bulb of *Crinum jagus* to determine the extent of its antimalarial activity.

**Author for correspondence* -
E-mail: ademowo_g@yahoo.com, ogademowo@comui.edu.ng
Phone (mobile): +234 (0) 8023342856

MATERIALS AND METHODS

Plants material: The bulb of *Crinum jagus* were collected from Omi-Adio area, a suburb in Ibadan, Oyo State and were authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen of the plant (FHI-10911) was deposited in the herbarium of the institute.

Animals, Parasite and drugs: Male Swiss albino mice used for the study were obtained from the animal house of the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. The animals were housed in well-aerated plastic cages, fed with standard mouse cubes (Ladokun Feeds, Nigeria, Ltd) and supplied with clean drinking water *ad libitum*. Chloroquine sensitive strain of *Plasmodium berghei* (NK65) used in this study was donated to the laboratory of one of us (AOG) by Malaria Research and Reference Reagent Resource (MR4) Centre. Handling of animals and other protocols conformed to the guidelines of the National Institutes of Health (NIH) for care of laboratory animals. The study was approved by the Animal Use Ethical Committee of the University of Ibadan, Nigeria. Standard antimalarial drugs (chloroquine and arteether) used as references for the anti-plasmodial study were obtained from Laborate Pharmaceutical, India.

Preparation of the Crude Extract : Fresh samples of the plant materials were chopped, air dried and ground into powdery forms. 1.127grammes of the powdered plant was loaded into soxhlet extractor and then extracted with boiling petroleum ether, followed by 2.5litres of methanol for 24 hours. The free petroleum ether and methanol were allowed to evaporate using water bath. A brown viscous solid was obtained and transferred into a clean dry bottle, weighed and labeled.

Preparation of the Fractions: The crude methanolic extract of the plant was fractionated by column and thin layer chromatography. Glass column was packed with silica gel. The crude extract was adsorbed with the gel and packed onto the column layer. Three solvents (hexane, ethylacetate and methanol were used based on their polarity) to elute the column using different solvent systems. Twenty one fractions were obtained. The fractions were pooled together by thin layer chrormatography (TLC). This reduced the number of the fractions to five. Fractions 1 ,2 and 3 were used to test for biological activities of the plant as there was no yield for fractions 4 and 5 hence could not be used for assay. The extract was screened for presence of alkaloids, flavonoids, tannins, phenols, steroids, protein, carbohydrate, cardiac glycosides, reducing sugars using a method described by Ode et al., 2010.

Evaluation of antimalarial activities of the crude extract and chromatographic fractions of Crinum jagus bulb.
In-vivo antimalarial assay was carried out using the method of Ryley and Peters (1970). Eighty mice were infected with the parasite and randomly divided into 10 groups of eight animals each. Treatments commenced 72 hours after inoculation to allow for the establishment of infection during which parasitemia reached 5-10% while chloroquine and arteether were dissolved in tween 80.

The crude methanolic extract and chromatographic fractions were also dissolved in tween 80. The mice in Group 1 were administered with 0.3ml tween 80 (negative control), Group 2 were given 10mg/kg body weight chloroquine (positive control), Group 3, 3mg/kg body weight arteether (positive control) while Groups 4, 5, 6 and 7 received 10, 25, 50, and 75mg/kg body weight of the crude extract. Groups 8, 9 and 10 were administered with 10mg/kg body weight each of fractions 1, 2, and 3 respectively for four conservative days. To monitor parasite response to treatment in *P. berghei* infected mice, thin blood films were made from the tail of each infected mice daily from the 3rd day till day 7 and weekly till day 28 post - infection. Blood films were fixed with methanol, stained with Giemsa stain and examined microscopically to assess the level of parasitemia. The average percentage suppression of parasitemia was calculated in comparison with the control as follows:

$$\frac{\text{parasitaemia in -ve control - parasitaemia in the test group}}{\text{Average \% parasitaemia in negative control}} \times 100$$

Packed cell volume (PCV) was determined from capillary blood from day 3 to day 6 after drug administration using Hawksley centrifuge and reader.

Evaluation of biochemical effect of the crude extract of Crinum jagus: Mice in Groups 4, 5, 6 and 7 were fed with 10, 25, 50, and 75mg/kg body weight of the crude extract for 30 days while group 1 which served as control received 0.2ml normal saline. Thereafter, they were sacrificed 24 hours after the last treatment. Blood samples were collected by cardiac puncture into plain bottles and then allowed to stand for 1 hour, then centrifuged at 3000g for 10 minutes to obtain serum. Liver and kidney samples were removed and washed in ice cold 1.15% KCl, dried, weighed and homogenized in phosphate buffer. The homogenate was centrifuged at 10,000g for 20 minutes to obtain the supernatant used for the assay. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were assayed using the procedure described by Rietman and Frankel (1957). Alkanine phosphate (ALP) was assayed by using the procedure of Wright et al, 1972 while lactate dehydrogenase (LDH) was assayed by the method of Pesce and Kaplan (1984).

RESULTS

Phytochemical screening revealed the presence of alkaloids, including flavonoids and saponins, phenols, steroids, protein and reducing sugars.

Animals in the control group which were infected but not treated showed a gradual daily increase in parasitemia level. By day 6 post - infection, the percentage parasitemia in the groups treated with 10, 25, 50 and 75mg/kg body weight of the crude extract were 4.99%, 4.46%, 3.95% and 2.29% respectively, while the percentage parasitemia of 2.13% and 18.72% were recorded for arteether treated and negative control groups respectively (Table 1). Whereas at the same day 6, there had been complete clearance of parasitemia by chloroquine. (Table1 and Figure 1). The groups treated with fractions 1, 2 and 3 had percentage parasitemia of 2.00%, 4.47% and 3.50% respectively (Tables 1&2).

Concerning average percentage suppression following administration of 10, 25, 50 and 75mg/kg bw doses of crude extract, chemosuppression of 70.10%, 76.17%, 78.90% and 87.17% respectively were produced.

Table 1:
Antimalarial activity of the crude methanol extract and chromatographic fractions of the bulb of *Crinum jagus* on established *Plasmodium berghei* infection in Mice (Mean Percentage Parasitaemia ± SD)

Groups	Treatment	D3	D4	D5	D6	D14	D21	D28
1 (control)	0.3ml (Tween 80)	5.21 ± 0.54	7.12 ± 0.39	11.45 ± 1.06	18.72 ± 1.50	34.47 ± 2.85	43.58 ± 1.91	___
2	10mg/kgbw Chloroquine	5.22 ± 0.38	*3.69 ± 0.38	*1.14 ± 0.05	0.00	0.00	0.00	0.00
3	3mg/kgbw Artheether	5.12 ± 0.39	*4.24 ± 0.76	*3.04 ± 0.32	*2.13 ± 0.30	*4.22 ± 0.46	*11.80 ± 0.54	16.86 ±0.17
4	10mg/kgbw crude extract	5.38 ± 0.34	*5.53 ± 0.36	*5.21 ± 0.20	*4.99 ± 0.20	*12.09 ± 0.60	*18.92 ± 0.64	25.64 ± 0.39
5	25mg/kgbw crude extract	5.07 ± 0.48	*5.24 ± 0.40	*4.96 ± 0.26	*4.46 ± 0.48	*12.43 ± 0.51	*20.21 ± 0.38	24.53 ± 0.89
6	50mg/kgbw crude extract	5.23 ± 0.30	*5.58 ± 0.37	*3.62 ± 0.77	*3.95 ± 0.55	*10.96 ± 0.78	*18.31 ± 0.73	26.79 ± 1.28
7	75mg/kgbw crude extract	5.40 ± 0.38	*3.78 ± 0.35	*3.17 ± 0.48	*2.29 ± 0.20	*9.48 ± 0.48	*16.92 ± 0.70	21.01 ±1.03
8	10mg/kgbw F1	5.31 ± 0.36	*3.75 ± 0.27	*2.62 ± 0.41	*2.00 ± 0.35	*8.92 ± 0.30	*16.69 ± 0.37	19.82 ± 0.64
9	10mg/kgbw F2	5.38 ± 0.31	*5.50 ± 0.46	*5.00 ± 0.43	*4.47 ± 0.15	*11.46 ±0.40	*19.36 ± 0.50	24.84 ±1.10
10	10mg/kgbw F3	5.31 ± 0.42	*5.49 ± 0.35	*4.61 ± 0.34	*3.50 ± 0.53	*11.35 ± 6.44	*18.92 ± 0.21	21.92 ± 0.74

Values are expressed as mean ± SD (n = 8) * = significantly different from control D = Days of observation .

Table 2
Suppressive activities of the crude extract and fractions of the bulb of *Crinum jagus* on established *Plasmodium berghei* infection in mice

Treatment	**Dose (mg/kg/bw)**	**Average % Parasitaemia (Day 3)**	**Average % Parasitaemia (Day 6)**	**% Suppression**
Tween 80 (Control)	0.3ml	5.21±0.54	I8.72±1.50	
Chloroquine	10	5.22±0.38	0.00	100
Artemether	3	5.12±0.39	2.13+0.30	88.6
Crude extract	10	5.38±0.34	4.99±0.20	70.10
	25	5.07±0.48	4.46±0.48	76.17
	50	5.23±0.30	3.95±0.55	78.90
	75	5.40±0.38	2.29±0.20	87.17
Fraction 1	10	5.31±0.36	2.00±0.35	89.33
Fraction 2	10	5.38±0.31	4.47±0.51	76.12
Fraction 3	10	5.31±0.42	3.50±0.53	77.70

Values are expressed as mean ±SD

Fractions 1, 2 and 3 had chemosuppression of 89.33%, 76.12% and 77.70% respectively while chloroquine and arteether had 100% and 88.60% suppression respectively (Table 2).

Mean survival times (MST) of 25 and 23 days were observed for chloroquine and artemether treated groups compared with 19, 20, 21 and 22 days observed for groups treated with crude extract at doses of 10, 25, 50 and 75 mg/kg bw respectively. Values of MST were expressed as mean ± standard deviation (SD), the SD of the number of animals used and MST values were shown in tables 1 & 3. Animals treated with fractions 1, 2 and 3 had a MST of 25, 21, and 21 days respectively. Whereas untreated control groups survived for only 12.5 days (Table 3). Crude extract at 75mg/kg bw caused 12.87 rise in PCV from 42.72 ± 8.45 to 55.59 ± 4.72. Table 4 shows effects of crude extract and the chromatographic fractions of *Crinium jagus* on PCV of mice infected with *P. bergei*.

Concerning serum enzymes following 30 days administration of crude extract, there was no significant difference between serum levels of AST, ALT and ALP in the groups (4 and 5) administered with 10 and 25mg/kg bw and the control group ($p > 0.5$) while that of the groups 6 and 7 administered with 50 and 75mg/kg bw respectively was significant when compared with the control ($p<0.05$). Both lower and higher doses of the extract did not produce any significant difference in the serum level of LDH when compared with the control group (Figure 3).

DISCUSSION

Chemotherapy remains the mainstay of global malaria control programme. Previously efficacious drugs such as chloroquine, sulfadoxine, pyrimethamine and amodiaquine have failed as prophylactic and therapeutic antimalarial agents in many endemic countries of Africa including Nigeria (WHO, 2011). This has prompted the change of policy to artemisinin- based combination therapy thereby narrowing the drug of choice for treatment of malaria. This has prompted the search and discovery of new drugs for the treatment of malaria. The results observed from this study showed that the crude extract and chromatographic fractions of the bulb of *Crinum jagus* possess considerable antiplasmodial activities as shown by chemosuppression of parasitemia and prolongation of the life of infected mice treated with the extract or fractions (Table 1&2 and Figures 1&2).

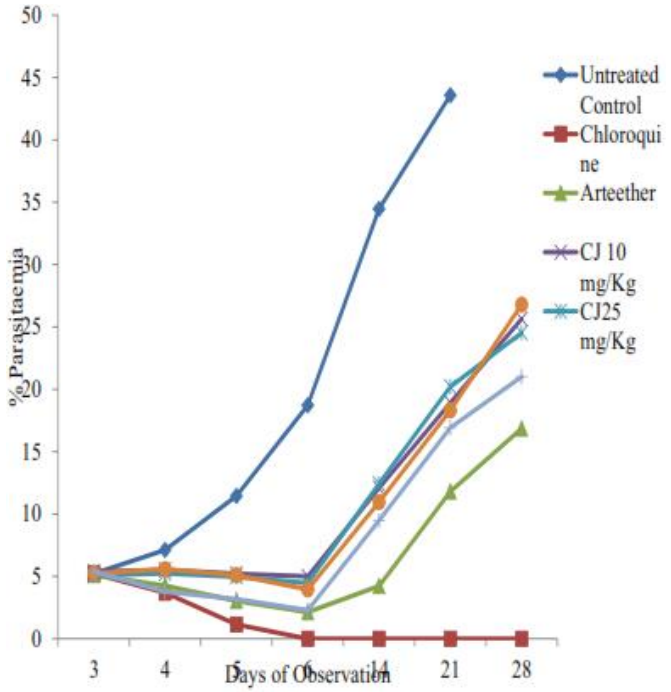

Figure 1.
Anti-malarial activity of the crude methanolic extract of the bulb of *Crinum jagus* (CJ) on established *Plasmodium berghei* infection in mice

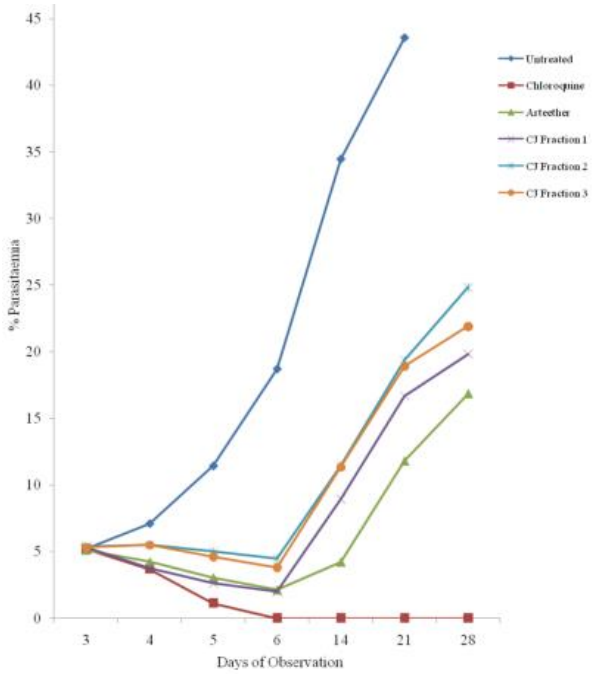

Figure 2.
Anti-malarial Activity of the Purified Fractions of the Bulb of *Crinum jagus* (CJ) on established *Plasmodium berghei* infection in mice

Table 4
Effect of crude methanolic extract and chromatographic fractions of the bulb of *Crinum jagus* on packed cell volume (PCV) of mice infected with *Plasmodium berghei*

Treatment	Dose	PCV(%) (Day 3)	PCV (%) (Day 6)	PCV Change	P-value
Tween 80	0.3ml	50.17±9.00	28.03±3.12	-22.14	0.000
Chloroquine (mg/kg)	10	45.45+5.13	62.89±3.12	17.44	0.000
Arteether (mg/kg)	3	48.89±6.17	58.25±2.04	9.36	0.001
Crude extract (mg/kg)	10	43.24±11.19	43.86±6.50	0.62	0.894
	25	48.65±9.11	48.95±6.02	0.30	0.939
	50	41.87±8.23	48.04±3.88	6.18	0.076
	75	42.72±8.45	55.59±4.72	12.87	0.002
Fraction 1 (mg/kg)	10	47.16±9.46	58.49±6.54	11.33	0.015
Fraction 2 (mg/kg)	10	48.36±9.46	48.98±5.44	0.62	0.875
Fraction 3 mg/kg	10	50.79±8.78	50.88±7.52	0.08	0.983

Values are expressed as mean ± SD, n = 8
PCV change is the difference in PCV at pre- treatment (day 3) and post-treatment (day 6). The value is negative for tween 80 because of decrease in PCV post-treatment

The antimalarial activity of the crude extract was considerable but low due to the crude nature of the extract. Purification of the crude extract enhanced its antimalarial activity as evident in the higher suppressive activities of the fractions. This is supported by the fact that 89.33% chemosuppression of parasitemia obtained at 10mg/kg bw of fraction 1 was higher than the values obtained for crude extract at 10, 25 and 50 mg/kg bw but similar to the 87.17% for 75mg/kg of crude extract (Table 2). This may be due to the higher concentration of fraction 1 in 75 mg/kg bw crude extract. *Crinium jagus* has been reported to contain some phytochemicals such as alkaloids, terpenes saponins, flavonoids, steroids and phenols (Ode et al., 2010). Some of these secondary metabolites are known to have antiplasmodial activities. Among these metabolites are flavonoids and triterpenoids (Kirby et al., 1989; Phillipson and Wright, 1991; Saxena et al., 2003).

Table 3
Mean survival time of mice treated with doses of crude extract and chromatographic fractions of the bulb of *Crinum jagus* on established *Plasmodium berghei* infection

Treatment	Dose	Mean survival time (Days)
Tween 80	0.3ml	12.5
Chloroquine (mg/kg)	10	25
Arteether (mg/kg)	3	23
Crude extract (mg/kg)	10	19
	25	20
	50	21
	75	22
Fraction 1 (mg/kg)	10	25
Fraction 2 (mg/kg)	10	21
Fraction 3 (mg/kg)	10	21

Values are expressed as mean ±SD, n = 8

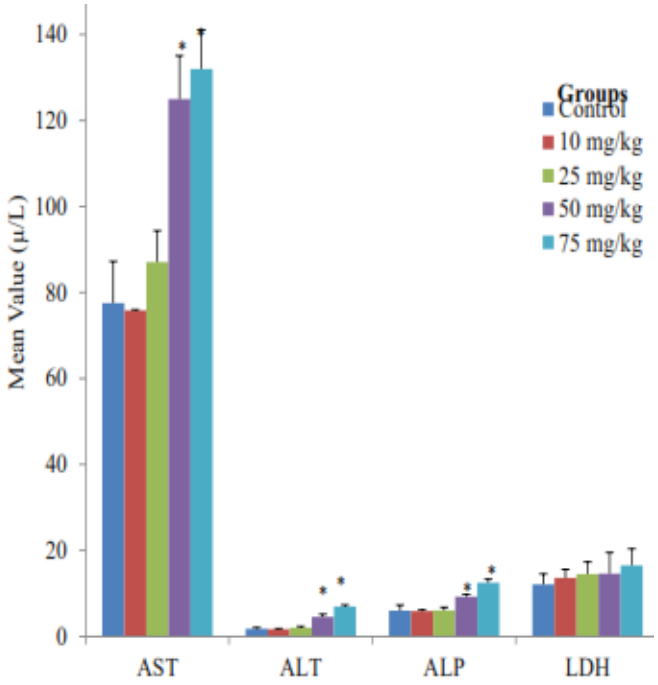

Figure 3.
Effect of 30 days oral administration of crude extract of the bulb of *Crinum jagus* on serum enzymes.
* = Significantly different from control

Flavonoids are reported to chelate nucleic acid base pairing of the parasite (Lui et al., 1992) while triterpenes are potent protein inhibitors (Liao et al., 1976). These compounds may in part contribute to antiplasmodial activity of the plant.
The average percentage suppression of 89.33% obtained for fraction 1 was similar to the 88.6% and 100.0% obtained for the two standard drugs (arteether and CQ respectively) used in this study (Table 2). This implies that fraction 1 has antiplasmodial activity which may be explored for development of antimalarial drug. The fact that CQ had 100.0% average percentage suppression in this study is due to the fact that the *Plasmodium berghei* strain (NK65) used is known to be chloroquine sensitive.

The mean survival times (MST) obtained for the crude extract and chromatographic fractions treatment groups were similar to that of CQ and arteether treatment groups while the MST was as expected considerably shorter in the untreated (control) group (Table 3). Fraction 1 had the same MST with that of CQ which was markedly higher than what was obtained in other fractions and the crude extracts treatment groups. This indicates that both the crude extract and chromatographic fractions have antiplasmodial activities which are similar to that of the two reference drugs with fraction 1 being most potent.

Anaemia is a constant feature of *Plasmodium* infections (Jones et al., 2002) caused by several factors, among which are oxidative damage to the membrane components of erythrocytes and suppression of erythropoiesis (Omodeo-Sale et al., 2003). It was observed that in addition to the suppression of parasitemia, both the crude extracts and the chromatographic fractions prevented a drastic reduction in haematocrit as a result of *P. berghei* infection in mice. This is an indication of its efficacy in ameliorating anaemic conditions in malaria (Table 4). The amelioration of the anaemic condition may be attributable to its scavenging effects towards the generated reactive oxygen species and thereby reducing the oxidative attack to which the erythrocytes membranes are exposed in the infected mice (Adaramoye et al., 2014).

The fact that there were no significant differences between serum levels of liver enzymes AST, ALT and ALP following 30 days administration of 10 and 25 mg/kg bw of crude extract and the control indicates that the extract was not toxic at a lower doses, but high doses of the plant (50 and 75 mg/kg bw) pose toxicological risks . Exposure of the animals for thirty days to higher doses (50 and 75 mg/kg bw) resulted in the elevation of serum AST, ALT and ALP. This suggests that the extract is safe when used at lower doses and can be explored as a prospective new antimalarial medicine.

It can be concluded from the study that crude methanolic extract and chromatographic fractions of the bulb of *Crinium jagus* have parasite suppressive effect in *P. bergei* infected mice comparable to chloroquine and arteether. Fraction 1 has the most notable antimalarial potential in terms of degree of chemosuppression produced and prolongation of the lives of infected mice. Further studies are on-going to identify it's active principles and mechanism of its antiplasmodial properties.

Acknowledgments
We acknowledge the technical assistance rendered in the animal house by Mrs V. Thomas.

REFERENCES

Adaramoye, O., Akinpelu, T., Kosoko, A., Okorie, P., Kehinde, A., Falade, C., Ademowo, O. (2014). Antimalarial potential of Kolaviron, a biflavonoid from *Garcinia kola* seeds, against *Plasmodium berghei* infection in Swiss albino mice. Asian Pacific Journal of Tropical Medicine. 7(2), 97-104.

Adesanya, S.A., Olugbode, T. A., Odebiyi, O.O., and Aladesanmi, J. A. (1992). Antibacterial alkaloids in *Crinum jagus*. International Journal of Pharmacognosy. 4, 303-307

Azikwe, C.C. A., Siminialayi, M., Brambaifa, N., Amazu, L. U., Enye, J.C., and Ezeani, M.C., (2012). Anticonvulsant activity of fractionated extract of *Crinum jagus* bulbs in experimental animals. Asian Pacific Journal of Tropical Biomedicine. 9, 21-9

Bhat, G.P., and Surolia, N. (2001). *In vitro* antimalarial activity of extracts of three plants used in the traditional medicine of India. American Journal of Tropical Medicine & Hygiene. 65(4), 304-8.

Dondorp, A.M., Yeung, S., Ngguon, C., Day, N.P., Socheat, D., and Von Seidlein, L.(2010).Artemisinin resistance: current status and scenarios for containment. Nature Review Microbiology. 8, 4, 272-80.

Edema, M.D. and Okiemen, F.E. (2002). Chemical and anticonvulsant screening of *Crinum jagus*. Nigerian Journal of Chemistry. 7, 25-28

Greewood, B.M., Bojang, K., Whittly, C.J., and Targett, G.A. (2005). Malaria. Lancet. 365, 1487-1498.

Houghton, P. J., Agbedahunsi, J. M., and Adegbulugbe, A. (2004). Choline esterase inhibitory properties of alkaloids in *Crinum glaucum* and *Crinum jagus* . Phytochemistry. 65(21), 2893-2896.

Jones, T.R., Stroncek, D.F., Gozalo, A.S., Obaldia, N., Andersen, E., Lucas, M.C., Narum, D.L., Magill, A.J., Sim, B.K., and Hoffman, S.L. (2002). Anemia in parasite and recombinant protein-immunized Aotus monkeys infected

with *Plasmodium falciparum*. American Journal of Tropical Medicine & Hygiene. 66(6), 672-9.

Kirby, G.C., O.Neill, M.J., Phillipson, J.D., and Warhurst, D.C. (1989). In vitro studies on the mode of action of quassinoids with chloroquine resistant *Plasmodium falciparum*. Biochemical Pharmacology. 36, 4367-4374

Krettli, A.U., Andrade-Neto, V., Brandao, M.L., and Ferrari, W.M.S. (2001). The search for new antimalarial drugs from plants used to treat fever and malaria or plants randomly selected: a review. Mem Institute Oswaldo Cruz. 96(1), 1033-42.

Liao, Y.F., Kupchan, S. M., and Horwitz, S.B. (1976). Mode of action of anti-tumour compound bruceantin, an inhibitor of protein synthesis. Molecular Pharmacology. 12, 167-176

Lui, K.C., Yang, S.C., and Roberts, M.F. (1992). Antimalaria of artemisia annua flavonoids from whole plants and cell cultures. Plant Cells. II, 637-640

Ode, O. J., Nwaehuyor, C. O., and Onapka, M.M. (2010). Evaluation of antihaeamorrhagic and antioxidant potentials of *Crinium jagus* bulb. International Journal of Applied Biology and Pharmaceutical Technology. 1(3), 1330-6

Ogunkunle, A.T., and Olopade, R. O. (2011). Studies on asthma coughs plants. *Crinum jagus* (Amaryllidaceae) in Nigeria. African Journal of Plant Science. 5, 108-114

Okeola, V.O., Adaramoye, O.A., Nneji, C.M., Falade, C.O., Farombi, E.O., and Ademowo, O.G. (2011). Antimalarial and antioxidant activities of methanolic extract of *Nigella sativa* seeds (black cumin) in mice infected with *Plasmodium yoelli nigeriensis*. Parasitology Research. 108(6),1507-12.

Omodeo-Sale, M.F., Motti, A., Basilico, N., Parapini, S., and Olliaro, P. (2003). Accelerated senescence of human erythrocytes cultured with *Plasmodium falciparum*. BLOOD. 102(2), 705-11.

Osakwe, S.A., Diete, S., and Imbolo, F.A. (2011). Review of medicinal plants and pharmaceutical properties of some selected Nigerian plants. Biosciences and Biotechnology Research. 08 (1), 110-5

Pesce, A. J., and Kaplan, L. A. (1984). Lactate dehydrogenase isoenzymes. *In* Clinical chemistry: theory, analysis and correlation. A. J. Pesce and L. A. Kaplan, editors. C V Mossby Company, St. Louis. 1127 - 30

Phillipson, J.D., and Wright, C. (1991). Anti-protozoal compounds from plant resources. Planta medical. 57, 553-559

Pyae - Phyo, A., Nkhoma, S., Stepniewska, K., Ashley, E.A., Nair, S., McGready, R., Moo, C.l., Al-Saai, S., Dondorp, A.M., Lwin, K.M., Singhasivanon, P., Day, N.P.J., White, N.J., Anderson, T.J.C., and Nosten, F.(2012). Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. Lancet. 379. 1960–66.

Ridley, R.G. (2002). Medical need, scientific opportunity and the drive for antimalarial drugs. Nature. 415, 686-693

Ryley, J.F., and Peters, W. (1970). The antimalarial activity of some quinine ester: Annals of Tropical Medicine and Parasitology. 84, 209-22

Rietman, S., and Frankel, S.(1957). A colorimetric method for the determination of serum GOT and GPT. American Journal of Clinical Pathology. 28: 56-63

Saxena, S., Pant, N., and Bhakuni, R.S. (2003). Antimalarial agents from plant sources. Curriculum Science. 85(1), 1314-26.

United Nations Educational Scientific and Cultural Organization (UNESCO) (1998). Terminal report. Promotion of ethnobotany and sustainable use of plant resources in Africa. Page 60 Paris

World Health Organization. (2011). World malaria report. WHO Geneva Switzerland. www.who.int/malaria/world_malaria_report_2011. accessed 23rd July, 2013

Wright, P. J., and Plummer, D.V. (1972). The use of urinary enzyme measurement to detect renal changes caused by nephrotoxic compounds. Biochemistry and Pharmacology. 12: 65

Zirihi, G.N., Mambu, L., Guede-Guina, F., Bodo. B., and Grellier, P. (2005). In vitro antiplasmodial activity and cytotoxicity of 33 West African Plants used for the treatment of malaria. Journal of Ethnopharmacology. 98, 281-285.