

Full Length Research Paper

Anti-plasmodial and toxicological effects of methanolic bark extract of *Chrysophyllum albidum* in albino mice

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The anti-plasmodial, hematological, serum biochemical and pathological effects of *Chrysophyllum albidum* methanolic bark extract were evaluated using Swiss albino male mice as models. The plant is used in Southern Nigeria as a remedy for malaria and yellow fever. The LD₅₀ of the methanolic extract was 1850 mg/kg body weight. *C. albidum* methanolic bark extract (750 - 1500 mg/kg/day) exhibited significant ($P < 0.05$) schizontocidal activities both in a 4-day (early) infection and in an established (> 7 days) infection with a considerable mean survival time comparable to that of chloroquine. The plant extract treated mice did not develop appreciable anemia. This observation shows that the methanolic extract of *C. albidum* contains anti-plasmodial substance(s) which help to reduce parasitaemia and hence the rate of erythrocyte destruction during infection. Organ and tissue pathology during infection was milder at low doses, compared to the untreated mice and insignificant at higher doses of the extract, showing that the extract is non-toxic. It also validates the local consumption of the extracts of *C. albidum* as an anti-malarial agent. Further studies need to be done to identify and characterize the active principles/ substances in the extract. This study has implications in future development of antimalarial drugs with little or no cytotoxic effect.

Key words: *Chrysophyllum albidum*, anti-plasmodial, haematology, serum biochemistry, organ pathology, albino mice.

INTRODUCTION

Malaria is a human protozoan disease widespread in the Tropical African region (Shiff, 2002). Annually, between 300 - 5000 million people get infected with the disease worldwide and of these; 1 - 3 million die (Sachs et al., 2002). Despite significant progress in the treatment of malaria, this disease has staged a huge comeback in large areas of the world, due to the development of drug resistant parasites (Najera, 2001; Shiff, 2002).

Malarial infection is accompanied by a variety of biological responses on the part of the host, including the activation of its cellular immune system and production of pro-inflammatory cytokines such as interleukin (IL) 1 and

tumour necrosis factor (TNF) - alpha. Malaria can kill by infecting and destroying red blood cells, leading to severe anemia, and by clogging the capillaries that carry blood to the brain (cerebral malaria) or other vital organs (WHO, Rollback Malaria Info sheet, 2004). The mechanism by which anemia occurs as concluded by Weatheral (1982) is multifocal and complex. It includes hemolysis and inappropriate bone marrow response.

During the 1990s, malaria deaths and illnesses escalated in Africa because of increased resistance to conventional malaria drugs like amodiaquine and the less expensive monotherapies like chloroquine and sulfadoxine - pyrimethamine (WHO, Rollback Malaria, 2004). Drug resistance is a major problem in the treatment and prophylaxis of malaria. Research into the identification and production of more effective, cheaper and potentially less toxic remedies for the treatment of

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malaria would therefore continue to be relevant (Didia et al., 2002).

Chrysophyllum albidum (Linn), also known as African star apple, belongs to the family *Sapotaceae*. It is primarily a forest tree species with its natural occurrences in diverse ecozones in Uganda, Nigeria and Niger Republic (Bada, 1997). Across Nigeria, it is known by several local names and is generally regarded as a plant with diverse ethno-medicinal uses (Amusa et al., 2003). The plant is known as 'Agbalumo' in Yoruba. The fruit of African star apple has been found to have a very high content of ascorbic acid with 1000 to 3,300 mg of ascorbic acid per 100 g of edible fruit or about 100 times that of oranges and 10 times that of guava or cashew (Amusa et al., 2003). Several other components of the tree including the roots and leaves are used for medicinal purposes (Adewusi, 1997). The bark is used as a remedy for yellow fever and malaria while the leaves are used as emollients and for the treatment of skin eruption, diarrhea and stomach ache (Adisa, 2000). Eleagnine, an alkaloid isolated from *C. albidum* seed cotyledon has been reported to have anti-nociceptive, anti-inflammatory and antioxidant activities (Idowu et al., 2006).

In spite of the rich component and vast local use of *C. albidum*, there is dearth of information on its effect as an anti-malarial. This study therefore was designed to determine the possible anti-plasmodial and toxic effects of the methanolic bark extract of *C. albidum* against *Plasmodium berghei berghei* infection of Swiss albino mice.

MATERIALS AND METHODS

Reagents

All reagents, including methanol, formalin etc are analytical grade obtained from BDH chemicals LTD, Poole England.

Plant materials, collection and identification

The fresh bark of *C. albidum* was collected from its natural habitat at Igbo Owe cash crop farm at Moniya, Akinyele Local Government Area of Oyo State, South-western Nigeria. It was collected between the months of November and April 2007. The plant was identified and given a voucher number FHI 107514 at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The plant materials were dusted and dried at room temperature for 3 weeks and then grounded to powder using a dry electric mill (Moulineux, UK).

Preparation of extract

1.5 kg of the powdered bark of *C. albidum* was exhaustively dissolved in 2.5 l of 70% methanol for 72 h. The mixture was filtered with Whatman's filter paper (No. 1) and the filtrate evaporated to a paste on a thermostatic controlled water bath at 40°C. The yield, a solid residue obtained was referred to as the extract. The evaporation produced 26 g of the extract that is, 1.73% yield. The dried methanolic extracts of *C. albidum* (1.0, 1.5 and 2.25 g of *C.*

albidum) were dissolved separately in 15 ml of normal saline each to produce doses of 750, 1000 and 1500 mg/kg/day, respectively. All preparations were stored at 4°C until use.

Phytochemical screening

Standard screening tests for the extract were carried out for various constituents like alkaloids, saponins, tannins, glycosides and volatile oils using standard procedures (Harbone, 1983; Trease and Evans, 1989).

Animals

Two hundred (200) Swiss albino male mice weighing between 19 - 22 g were used in this experiment. They were obtained from the Animal House of the College of Medicine, University of Ibadan, Nigeria. They were maintained under standard conditions (12 h light and 12 h dark) and had access to mice chow and clean water *ad libitum*.

Acute toxicity test: Determination of LD₅₀

The acute toxicity of *C. albidum* bark extract was estimated using 60 albino male mice. The mice were divided into 10 groups consisting of 6 mice per group. Each group of mice was injected intra-peritoneally with different doses 50 - 2000 mg/kg of the extract made in 0.2 ml after having fasted them over night. The number of deaths in each group within 24 h was recorded and various clinical signs exhibited by the mice were noted. The LD₅₀ was calculated according to Lorke (1983).

Parasites

Chloroquine-sensitive *P. berghei berghei* (NK 65) was obtained from the Institute of Malaria Research Laboratory (IMRAT) of the University College Hospital, Ibadan, Oyo state.

Drug

Chloroquine was obtained from Sigma (UK) and was used as a positive control drug to evaluate the *in vivo* efficacy of methanolic extract of *C. albidum*.

Experimental design

Extract administration

All oral administration of drugs and extract were carried out using orogastric tube. Two separate experiments were conducted:

Evaluation of schizontocidal activity in early infection (4 - day test): The schizontocidal activity of the methanolic bark extract of *C. albidum* was evaluated using the method described by Knight and Peters (1980). Eighty Swiss albino mice were used in this experiment. The animals were divided into eight groups of 10 mice each. Shortly after inoculation of each mice with 1×10^6 *P. berghei berghei*, they were administered with 750, 1000 and 1500 mg/kg/b.w./day doses of the *C. albidum* extract, chloroquine 10 mg/kg/day (both dissolved in normal saline) and an equivalent volume of distilled water (negative control) for 4 consecutive days (days 0 to 3). Percentage parasitaemia was determined using standard laboratory procedures

described by Knight and Peters (1980). The groups are as indicated below:

- Group 1: Uninfected and untreated (normal animals).
- Group 2: Infected and untreated (negative control).
- Group 3: Infected and treated immediately with 1500 mg/kg/b.w/day *C. albidum*.
- Group 4: Infected and treated immediately with 1000 mg/kg/b.w./day *C. albidum*.
- Group 5: Infected and treated immediately with 10 mg/kg/b.w./day chloroquine (positive control).
- Group 6: Uninfected and treated with 1500 mg/kg /b.w./day *C. albidum* alone.
- Group 7: Uninfected and treated with 1000 mg/kg/b.w./day *C. albidum* alone.
- Group 8: Uninfected and treated with 10 mg/kg/day b.w chloroquine alone.

Evaluation of schizontocidal activity in established infection (curative or Rane test): The evaluation of the curative potential of the extract was done using the methods described by Ryley and Peters (1970). Fifty Swiss albino mice were used in the experiment. Seventy two hours after parasite inoculation of each mouse with 1×10^6 *P. berghei berghei*, the animals were divided into five groups of 10 mice each. These mice were treated with 1500, 1000 and 750 mg/kg/b.w./day doses of the *C. albidum* methanolic extract, chloroquine 10 mg/kg/day (both dissolved in normal saline) and an equivalent volume of distilled water (negative control) for 4 consecutive days. The drug or extract was given once daily to the appropriate group at 9:00 a.m. the levels of parasitaemia were determined using standard laboratory procedures (Knight and Peters, 1980). The group are as underlisted:

- Group 9: Infected and untreated.
- Group 10: Infected and treated on day 5 with 1500 mg/kg b.w. *C. albidum* for 3 consecutive days.
- Group 11: Infected and treated on day 5 with 1000 mg/kg b.w *C. albidum* for 3 consecutive days.
- Group 12: Infected and treated on day 5 with 750 mg/kg/day b.w *C. albidum* for 3 consecutive days.
- Group 13: Infected and treated on day 5 with 10 mg/kg b.w chloroquine for 3 consecutive days.

Hematology and serum biochemistry

Each mouse was sedated by ether suffocation and pooled blood, from mice in each group, was collected by cardiac puncture on days 3, 5, 7 into heparinized tubes for haematological studies - red blood cell (RBC) counts, hemoglobin (Hb) concentration, total white blood cell (WBC) counts and platelets counts according methods described by Dacie and Lewis (1991). Red blood cell indices such as mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated (Jain, 1996). Pooled blood samples from animals in each of the groups were also collected into plain vacutainer tubes to obtain sera for biochemical analysis - total protein, albumin, fibrinogen, total bilirubin, creatinine and serum activities of alanine transaminase (ALT) and aspartate transaminase (AST) were measured as described by (Taiwo et al., 2004).

Organ pathology

On days 3, 5 and 7, immediately after ether anaesthesia and blood samples collection, each mouse in all the groups was placed on

dorsal recumbency, dissected through the sternum and *Linea alba* and sections of the heart, liver, lungs, brain, kidney, spleen and stomach were harvested into labelled bottles containing 10% phosphate-buffered formalin for 24 h for proper fixation. Thereafter, the sections were embedded in paraffin, cut into 5 μ m sections, prepared routinely and stained with haematoxylin and eosin (H&E) for histopathological changes and photomicrography using a light microscope (Olympus, Germany) fitted with digital camera (HP Photosmart 735, Canada).

Statistical analysis

Data obtained for each group of experimental mice in the various parameters determined were expressed as the mean \pm SEM. Statistical comparisons between the groups were made using the 2-way analysis of variance (SAS, 1987) and Duncan's multiple range test (Duncan, 1959). The level of significant difference between the groups was evaluated at $P < 0.05$ at each level.

RESULTS

Phytochemical screening

Phytochemical screening of the methanolic bark extract of *Chrysophyllum albidum* revealed the presence of alkaloids, anthraquinones, cardenolides, saponin, and tannins in large quantities.

Acute toxicity test: Determination of LD₅₀

All the mice in the group treated with doses from 2500 - 3000 mg/kg b.w of *C. albidum* died. The intraperitoneal LD₅₀ of the plant extract in mice was calculated to be 1850 mg/kg b.w

Evaluation of schizontocidal activity in early infection (4 - day test)

The methanolic bark extract of *C. albidum* produced a dose independent schizontocidal (chemosuppressive) effect of 74.20 and 62.90% for 1000 and 1500 mg/kg/day b.w., respectively. The chemosuppression produced by the extract was significant ($P < 0.05$) when compared with the negative control (0% chemosuppression). Chloroquine at 10 mg/kg/day b.w produced a chemosuppressive effect of 74.20% similar to that of 1000 mg/kg/day of *C. albidum* methanolic extract.

Evaluation of schizontocidal activity in established infection (curative or Rane test)

From day 5 to day 7 in the established infection, a daily increase in the parasitaemia level of the infected untreated (negative) control group was recorded.

Table 1. Haematology of the experimental mice in each of the groups.

| Haematologic parameters | Mice groups | | | | | | | |
|-----------------------------------|-------------|-------|----------|----------|-------|--------------|--------------|----------|
| | UNITR* | PUT | PT1500Ca | PT1000Ca | PTCQ | 1500Ca alone | 1000Ca alone | CQ alone |
| PCV | | | | | | | | |
| Day 0 | 44 | | | | | | | |
| Day 3 | | 39 | 38 | 40 | 40 | 40 | 44 | 41 |
| Day 5 | | 35 | 35 | 40 | 43 | 40 | 47 | 45 |
| Day 7 | 43 | 31 | 38 | 41 | 38 | 39 | 43 | 43 |
| RBC ($\times 10^6/\mu\text{l}$) | | | | | | | | |
| Day 0 | 7.6 | | | | | | | |
| Day 3 | | 6.34 | 6.29 | 7.12 | 7.06 | 7.19 | 7.26 | 7.43 |
| Day 5 | | 5.81 | 5.37 | 6.67 | 7.34 | 6.73 | 7.93 | 7.46 |
| Day 7 | 7.4 | 5.34 | 6.32 | 6.92 | 6.48 | 6.40 | 6.54 | 7.24 |
| Hb Conc. (mg/dl) | | | | | | | | |
| Day 0 | 13.9 | | | | | | | |
| Day 3 | | 12.8 | 12.6 | 13.4 | 13.2 | 13.5 | 13.7 | 13.6 |
| Day 5 | | 11.5 | 11.7 | 13.2 | 14.6 | 13.3 | 15.4 | 14.8 |
| Day 7 | 13.7 | 9.3 | 12.6 | 13.6 | 12.5 | 12.6 | 14.3 | 14.2 |
| WBC ($\times 10^3/\mu\text{l}$) | | | | | | | | |
| Day 0 | 5700 | | | | | | | |
| Day 3 | | 6900 | 8350 | 6150 | 4800 | 4200 | 6200 | 6900 |
| Day 5 | | 10300 | 7100 | 6300 | 7400 | 4450 | 4600 | 5000 |
| Day 7 | 6100 | 10200 | 8600 | 11150 | 18900 | 6850 | 6300 | 6100 |
| MCV (fl) | | | | | | | | |
| Day 0 | 57.9 | | | | | | | |
| Day 3 | | 61.5 | 60.4 | 56.2 | 56.7 | 55.6 | 60.6 | 55.2 |
| Day 5 | | 60.2 | 65.2 | 60.0 | 58.6 | 59.4 | 59.3 | 60.3 |
| Day 7 | 58.1 | 58.1 | 60.1 | 59.2 | 58.6 | 59.4 | 65.7 | 59.4 |

Table 1. Contd.

| | | | | | | | | |
|----------------------|--------|--------|--------|--------|--------|--------|--------|--------|
| MCHC (%) | | | | | | | | |
| Day 0 | 31.6 | | | | | | | |
| Day 3 | | 32.8 | 33.2 | 33.5 | 33.0 | 33.8 | 31.1 | 33.2 |
| Day 5 | | 32.9 | 33.4 | 33.0 | 34.0 | 33.3 | 32.8 | 32.9 |
| Day 7 | 32.3 | 33.3 | 33.2 | 33.2 | 32.9 | 32.3 | 33.3 | 33.0 |
| Platelets (μ l) | | | | | | | | |
| Day 0 | 115000 | | | | | | | |
| Day 3 | | 89000 | 85000 | 156000 | 104000 | 88000 | 100000 | 90000 |
| Day 5 | | 104000 | 75000 | 72000 | 96000 | 102000 | 86000 | 94000 |
| Day 7 | 112000 | 89000 | 114000 | 103000 | 134000 | 110000 | 115000 | 104000 |

UNITR: Uninfected, untreated (control); PUT: Parasitized untreated mice; PT1500Ca: Parasitized mice treated with 1500 mg/kg *C. albidum*; PT1000Ca: Parasitized mice treated with 1000 mg/kg *C. albidum*; PTCQ: Parasitized mice treated with 30 mg/kg chloroquine.

However, a daily reduction in the parasitaemia levels was observed in the methanolic bark extract-treated groups and in the group treated with chloroquine. On day 7, the average percentage reduction in parasitaemia for the groups were 82.86, 97.14, 77.14 and 100% for 750, 1000 and 1500 mg/kg b.w. of the methanolic bark extract of *C. albidum* and chloroquine (10 mg/kg/day b.w), respectively. There was no reduction of parasitaemia in the infected untreated (negative control) group.

Haematology

No significant changes ($P > 0.05$) were observed in the haematologic parameters and red blood cell indices of mice in the uninfected and untreated group as well as those uninfected but administered with the drugs (extract and chloroquine) alone (Table 1). The infected and

untreated mice developed progressively severe ($P < 0.05$) normocytic normochromic anemia, while those infected and treated with 1,500 and 1,000 mg/kg/day chloroquine developed variable but mild normocytic normochromic anemia on days 3 and 5 with resolution on day 7. All infected mice developed severe and progressive leucocytosis ($P < 0.05$), and was most severe ($P < 0.01$) in mice infected and treated with chloroquine on day 7. No significant changes ($P > 0.05$) were observed in platelet counts of mice in all the groups (Table 1).

Serum biochemistry

The serum biochemical parameters of the experimental mice are as shown on Table 2. There were no significant changes in most of the parameters in the experimental mouse groups. However, there is slight hyperproteinaemia ($P < 0.05$), due to hyperglobulinaemia ($p < 0.05$) in

parasitized untreated mice. Also there were marginal, but significant increases ($P < 0.05$) in AST and ALT activities in infected mice treated with 1,500 mg/kg/day of *C. albidum* methanolic extract (Table 2).

Administration of chloroquine and *C. albidum* methanolic extract, in their various doses to the mice did not cause and significant changes in all the serum biochemical parameters studied (Table 2).

Organ pathology

Grossly, the livers of infected and untreated mice were pale and enlarged. At histology, they showed severe widespread hepatocellular necrosis, presence of protozoan schizonts in hepatocytes (Figure 1), periportal mononuclear cell aggregations and Kupffer cell hyperplasia. The spleens were reactive with lymphoid hyperplasia and

Table 2. Serum biochemical parameters of the experimental mice in each of the groups.

| Serum parameters | Mice groups | | | | | | | |
|-----------------------|-------------|-----|----------|----------|------|--------------|--------------|----------|
| | UNITR | PUT | PT1500Ca | PT1000Ca | PTCQ | 1500Ca alone | 1000Ca alone | CQ alone |
| Total protein (mg/dl) | | | | | | | | |
| Day 0 | 6.9 | | | | | | | |
| Day 3 | | 7.4 | 7.3 | 6.8 | 6.0 | 6.3 | 6.2 | 6.1 |
| Day 5 | | 7.5 | 6.8 | 7.8 | 5.6 | 6.5 | 6.2 | 5.5 |
| Day 7 | 6.8 | 7.2 | 6.6 | 6.7 | 5.8 | 6.5 | 6.2 | 6.0 |
| Albumin (mg/dl) | | | | | | | | |
| Day 0 | 3.6 | | | | | | | |
| Day 3 | | 3.4 | 3.4 | 2.8 | 2.7 | 2.9 | 2.7 | 3.1 |
| Day 5 | | 3.9 | 2.4 | 2.6 | 2.8 | 3.6 | 3.9 | 3.6 |
| Day 7 | 3.5 | 2.9 | 2.2 | 2.9 | 3.6 | 3.1 | 3.9 | 2.6 |
| Globulin (mg/dl) | | | | | | | | |
| Day 0 | 3.3 | | | | | | | |
| Day 3 | | 4.0 | 3.9 | 4.0 | 3.3 | 3.4 | 3.5 | 3.0 |
| Day 5 | | 3.6 | 4.4 | 5.2 | 2.8 | 2.9 | 2.3 | 1.9 |
| Day 7 | 3.3 | 4.3 | 4.4 | 3.8 | 2.2 | 3.4 | 2.3 | 3.4 |
| Fibrinogen (mg/dl) | | | | | | | | |
| Day 0 | 0.3 | | | | | | | |
| Day 3 | | 0.2 | 0.3 | 0.2 | 0.2 | 0.3 | 0.1 | 0.3 |
| Day 5 | | 0.2 | 0.2 | 0.3 | 0.2 | 0.3 | 0.3 | 0.2 |
| Day 7 | 0.2 | 0.1 | 0.4 | 0.4 | 0.3 | 0.3 | 0.2 | 0.3 |
| Bilirubin (mg/dl) | | | | | | | | |
| Day 0 | 0.4 | | | | | | | |
| Day 3 | | 0.3 | 0.3 | 0.4 | 0.6 | 0.3 | 0.3 | 0.3 |
| Day 5 | | 0.5 | 0.3 | 0.3 | 0.4 | 0.3 | 0.3 | 0.2 |
| Day 7 | 0.3 | 0.4 | 0.3 | 0.3 | 0.2 | 0.2 | 0.2 | 0.3 |
| Creatinine (mg/dl) | | | | | | | | |
| Day 0 | 27 | | | | | | | |
| Day 3 | | 28 | 27 | 27 | 16 | 29 | 29 | 26 |
| Day 5 | | 28 | 27 | 35 | 38 | 26 | 28 | 23 |
| Day 7 | 26 | 30 | 29 | 30 | 32 | 27 | 23 | 25 |
| AST (I.U.) | | | | | | | | |
| Day 0 | 37 | | | | | | | |
| Day 3 | | 28 | 47 | 37 | 26 | 32 | 29 | 36 |
| Day 5 | | 38 | 45 | 45 | 38 | 36 | 28 | 33 |
| Day 7 | 35 | 34 | 39 | 30 | 42 | 32 | 33 | 32 |
| ALT (I.U.) | | | | | | | | |
| Day 0 | 27 | | | | | | | |
| Day 3 | | 28 | 25 | 27 | 26 | 27 | 29 | 26 |
| Day 5 | | 38 | 27 | 35 | 38 | 26 | 28 | 28 |
| Day 7 | 29 | 34 | 24 | 30 | 42 | 28 | 30 | 26 |

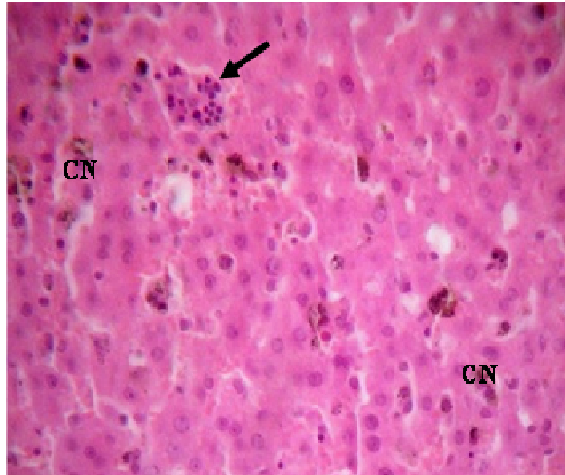


Figure 1. The liver of *Plasmodium*-infected and untreated mouse showing widespread coagulative necrosis of hepatocytes (CN) and presence of protozoan schizonts in dying hepatocytes (arrow) (H and E; $\times 450$).

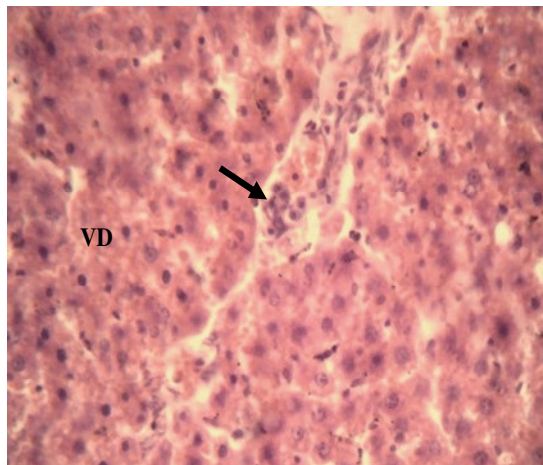


Figure 2. The liver of *Plasmodium*-infected mouse treated with 750 mg/kg body weight *C. albidum* extract showing a focal area of mild vacuolar degeneration of hepatocytes (VD) and mild periportal mononuclear cell aggregation (arrow) (H and E; $\times 450$).

presence of few haemopoietic islands within the red pulp. The hearts, brains, lungs, kidneys, livers (Figure 2), spleens and stomachs of all the infected and uninfected mice treated with the various doses of methanolic bark extract of *C. albidum* and chloroquine did not show any appreciable gross and histological changes mg/kg/day methanolic extract of *C. albidum* and However, a daily reduction in the parasitaemia levels was observed in the methanolic bark extract-treated groups and in the group treated with chloroquine. On day 7, the average percentage reduction in parasitaemia for the groups were

82.86, 97.14, 77.14 and 100% for 750, 1000 and 1500 mg/kg b.w. of the methanolic bark extract of *C. albidum* and chloroquine (10 mg/kg/day b.w), respectively. There was no reduction of parasitaemia in the infected untreated (negative control) group.

Haematology

No significant changes ($P > 0.05$) were observed in the haematologic parameters and red blood cell indices of mice in the uninfected and untreated group as well as those uninfected but administered with the drugs (extract and chloroquine) alone (Table 1). The infected and untreated mice developed progressively severe ($P < 0.05$) normocytic normochromic anemia, while those infected and treated with 1,500 and 1,000 mg/kg/day methanolic extract of *C. albidum* and 10 mg/kg/day chloroquine developed variable but mild normocytic normochromic anemia on days 3 and 5 with resolution on day 7. All infected mice developed severe and progressive leucocytosis ($P < 0.05$), and was most severe ($P < 0.01$) in mice infected and treated with chloroquine on day 7. No significant changes ($P > 0.05$) were observed in platelet counts of mice in all the groups (Table 1).

Serum biochemistry

The serum biochemical parameters of the experimental mice are as shown on Table 2. There were no significant changes in most of the parameters in the experimental mouse groups. However, there is slight hyperproteinaemia ($P < 0.05$), due to hyperglobulinaemia ($p < 0.05$) in parasitized untreated mice. Also there were marginal, but significant increases ($P < 0.05$) in AST and ALT activities in infected mice treated with 1,500 mg/kg/day of *C. albidum* methanolic extract (Table 2). Administration of chloroquine and *C. albidum* methanolic extract, in their various doses to the mice did not cause and significant changes in all the serum biochemical parameters studied (Table 2).

Organ pathology

Grossly, the livers of infected and untreated mice were pale and enlarged. At histology, they showed severe widespread hepatocellular necrosis, presence of protozoan schizonts in hepatocytes (Figure 1), periportal mononuclear cell aggregations and Kupffer cell hyperplasia. The spleens were reactive with lymphoid hyperplasia and presence of few haemopoietic islands within the red pulp. The hearts, brains, lungs, kidneys, livers (Figure 2), spleens and stomachs of all the infected and uninfected mice treated with the various doses of methanolic bark extract of *C.*

albidum and chloroquine did not show any appreciable gross and histological changes.

DISCUSSION

Results from this investigation suggest that the methanolic extract of the bark of *C. albidum* has anti-plasmodial activities and is non-toxic to mice when administered even at 1,500 mg/kg/day. It however appears to be more effective at a dose of 1,000 mg/kg/day. The life span of the mice infected with *P. berghei berghei* had earlier been carried out by Anigbogu and Fagbure (1997). This revealed that the lifespan of mice inoculated with *P. berghei berghei* is between the 7 to 10 days post-inoculation. This is in line with the drug treatment employed both in the suppressive and established or Rane test in this study. This time frame was used in order to prevent the death of animals before the end or drug treatment regime during the experiment.

Philipson and Wright (1991) as well as Christensen and Kharazmi (2001) reported that plants whose phytochemical compounds include alkaloids, anthraquinones and saponins may have antimalarial activities. These reports are similar to those obtained in this study as methanolic bark extract of *C. albidum* contains alkaloids, anthraquinones, saponins, cardenolides and tannins. These phytochemical compounds were also similar to those reportedly found in the leaves and stems of *C. albidum* by Smolenski et al. (1975) and Delande et al. (1979).

Saponins have been found to have antiprotozoan activities as well as possible defaunating agents in the rumen (Wallace et al., 1994; Newbold et al., 1997). This property has been exploited in the treatment of protozoal infections in other animals. Triterpenoid and steroid saponins have been found to be detrimental to several infectious protozoans, one of which is *P. falciparum* (Traore et al., 2000). This report supports what was observed in this experiment both in the suppressive and established infections. The mechanism of action by which saponins work might be through their toxicity to protozoans which may be widespread and non-specific. It might also be as a result of their detergent effect on the cell membranes (George) (Francis et al., 2002).

C. albidum has also been found to contain alkaloids and these have been associated with medicinal uses for centuries, though other possible roles have not been examined. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms like bacteria, viruses and protozoans to which malaria parasites belong. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Nobori et al., 1994). Alkaloids also possess anti-inflammatory, anti-asthmatic and anti-anaphylactic properties with

consequences of altered immunological status *in vivo* (Ganguly and Sainis, 2001; Staerk et al., 2002).

The significant reduction in parasitic load in infected mice treated with methanolic extract of *C. albidum* prevented rapid destruction of parasitized red blood cells and development of mild and insignificant anaemia on days 5 and 7. The results also show that chloroquine at 10 mg/kg/day is equally effective in prevention of anaemia due to its anti-protozoan effect in infected mice. It is noteworthy, however that all the infected mice treated or untreated developed leucocytosis, which was most severe in mice treated with chloroquine. The leucocytosis may be an indication of enhanced granulopoiesis and lymphocytosis as cellular and humoral responses, respectively to the protozoan infection (Jubb et al., 1996). This is corroborated by enhanced serum globulin levels (hyperglobulinemia) and reactive spleens in infected mice in this study. The lesions observed in the livers of infected and untreated mice are as a result of the destruction of hepatocytes during the hepatic phase of development of the malaria parasites. The relative milder and unnoticeable histological changes in the livers of infected and treated mice suggest that the drugs (both the extract and chloroquine) prevented or suppressed the build-up of parasites, in addition to enhanced immune response, in the mice and probably abrogated the hepatic phase of development of the protozoa.

Conclusion

The findings of present study support the use of this plant in the traditional treatment of malaria in Southwestern Nigeria. It is also observed that the extract, at the dosages used, are non-toxic to mice. However, further studies need to be carried out in humans to demonstrate its effectiveness and determine appropriate dosage regimens. The mechanism(s) of action of this extract is yet to be ascertained, but some main physiological possibilities have been postulated. These include the possibility that some active components in the methanolic extract of *C. albidum* could help in prevention of high parasitaemia, organ damage (notably the liver) and development of less oxidative stress and immune complex load in the body systems.

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