DRUG DISCOVERY

Clinico-biochemical and histopathological alterations in sub-chronically exposed mice (*Mus Musculus)* to polyherbal antimalarials

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ABSTRACT

Introduction: Concerns have been raised in the safety of antimalarial remedies for them to be acceptable for use. The present study is an assessment of safety profiles of two plant-based antimalarial cocktail treatments, namely: Cocktail treatment A (CtA) and Cocktail treatment B (CtB). *Materials and methods:* The treatments have been prepared from a defined mixture of hot water extracts of 6 plant parts, based on how they are used locally as antimalarials. CtA comprised *Enantia chlorantha* Oliv., *Cymbopogon citratus* Stapf, *Curcuma longa* L., *while* CtB comprised *Enantia chlorantha* Oliv., *Carica papaya* L., *Alstonia boonei* De Wild and *Mangifera indica* L. Safety assessments were done as post antimalarial exposures of CtA, CtB administered in mice at 200, 400 and 800 mg/kg dose levels against *Plasmoduim berghei berghei*. Haematological parameters, biochemical parameters, and histopathological changes of vital organs were equally evaluated for mice sacrificed immediately after exposures on day 8 (D8) (suppressive), day 9 (D9) (curative), day 12 (D12) (prophylactic), day 6 (D6) (treated/unparasitized). These results of acute effects of the treatments were compared with corresponding curative, suppressive, prophylactic, treated/unparasitized groups of treated mice which were sacrificed on day 25 (D25) for sub-chronic effects of the cocktails aganist *P. berghei* infected mice. Data was analyzed by using SPSS version 23.0. ($p<0.05$). *Results:* The treatments increased concentrations of packed cell volume, haemoglobin, red blood cells and white blood cells, liver enzymes and oxidative stress biomarkers. However, these concentrations returned to normal levels by day 25 (D25). Heart and spleen morphology was normal in all the treatments. But pathological damages were observed in the kidney, liver and brain, indicating multi-organ toxicities. The damages were mostly observed in groups administered 400 and 800 mg/kg doses. *Conclusions:* Overall, findings demonstrated some toxicities associated with CtA and CtB antimalarials that could be transient, as demonstrated in some groups of the experimental mice where haematological and some biochemical parameters were returning to normal levels within 25 days post-treatment. Cautious administrations of these

polyherbal cocktails within acceptable doses are safe for antimalarial therapies and their standardization is therefore encouraged.

Keywords: Polyherbal remedies; Antimalarials; Safety assessments; Haematological biochemical and histopathological toxicities; *Mus Musculus*

1. INTRODUCTION

Malaria remains one of the most prevalent infectious diseases in the world today. The World Health Organization reported that nearly half of the world's population lives in areas at risk of malaria transmission in 87 countries and territories (World Health Organization, 2020). Malaria control has relied upon the success recorded by antimalarial quinine and artemisinin derived from plant sources (Ginsburg and Deharo, 2011). However, there have been challenges in curbing the disease. The challenge is mainly caused by the resistance of parasites to available drugs World Health Organization, (2019), and the limited availability and affordability of pharmaceutical antimalarials especially in poor malarious countries. In many ways, these challenges continue to pose serious threat to malaria control programs.

Most importantly, the challenges have left the poor masses in some endemic countries, including Nigeria, heavily reliant on medicinal plants for malaria treatment. These challenges have also necessitated the global search for new antimalarials. Malaria is known to be a mosquito-borne disease with various clinical presentations (Otubanjo, 2008). The disease induces acute injuries to vital organs, with the most pronounced effects being observed in the spleen, liver and kidney of the infected host (Vineet et al., 2014). Alterations in the haematological indices are some of the commonest complications of malaria infection that play major role in its pathogenesis. Malaria-infected patients tend to have significantly lower PCV, RBCs and Hb levels (Bakhubaira, 2013). As a disease, malaria overworks the immune system of the body. As a consequence, there is an increase in oxidative stress through the release of reactive oxygen species such as superoxide ions, hydrogen peroxide, and other related species in patients (Eze et al., 1990).

Many antimalarial plants have shown promising therapeutic potential in pre-clinical and clinical investigations (Julianti et al., 2014; Mueller et al., 2014). However, herbal remedies are also known to be capable of producing a wide range of undesirable or adverse reactions. Some of the undesirable reactions can cause serious injuries, life-threatening conditions, and even death. Numerous irrefutable poisoning cases have been reported (Cosyns et al., 1999). Drug combinations can act in synergy, potentiation or antagonism to induce alterations in enzyme biochemistry, biological molecules (reactive oxygen species) and cell membrane (Essa et al., 2012). There are increasing reports that some plants are highly toxic despite their high chemo suppression of parasitaemia (Idowu et al., 2010). Toxicity evaluation of polyherbal remedies indicates that they have toxic effects on the liver and kidney of experimental animals. The studies also showed that polyherbal remedies reduce survival time.

In some other cases, they have some effects on biochemical variables (most commonly liver function tests) that have been reported (Idowu et al., 2015a). So far, only a very few pharmacological studies have reported safety profiles of polyherbal antimalarial cocktail remedies (Idowu et al., 2015a; Nwabui, 2002; Martey et al., 2013; Arrey-Tarkang et al., 2014; Okpok et al., 2014; Okpe et al., 2016; Ibukunoluwa, 2017; Orabueze et al., 2018). Reports have stated that not many consumers of herbal remedies really know their adverse effects and toxicities (Agbaje and Babatunde, 2005). These knowledge gaps encourage studies such as this to ultimately guide their development as effective antimalarial options towards achieving Sustainable Development Goal 3.b. SDG 3.b seeks the eradication of malaria epidemic by 2030 by providing more support for research and development of vaccines and medicines, and ensuring more access to affordable essential antimalarials. Nigeria has rich flora diversity, and many of the plant species are used for the management of malaria (Odugbemi et al., 2007; Aiyeloja and Bello, 2006).

The medicinal plants studied are *Cymbopogon citratus* Stapf, *Curcuma longa* L., *Enantia chlorantha* Oliv., *Mangifera indica* L., *Carica papaya* L., *Alstonia boonei* De Wild (names checked with "World Flora Online"). Antimalarial activities of these plants have been reported as monotherapies Adebajo et al., (2014), Arome et al., (2016), Agbedahunsi et al., (2016), Zeleke et al., (2017), Iyiola et al., (2011), Omoya, (2016) and as herbal formulations namely: Cocktail treatment A (CtA) and Cocktail treatment B (CtB) (Omagha et al., 2021a; Omagha et al., 2021b). Results showed plasmodium inhibition was dose dependent. At 800mg/kg inhibition with CtA and CtB was respectively: 96.95 % and 99.13 % on established infection; 96.46 % and 78.62 % on early infection; 65.05 % and 88.80 % on residual infection. The investigation into herbal toxicity is therefore necessary to ensure safety profile, and encourage wider use for alleviating malaria burden. Clinico-biochemical activities and histomorphology assessments were therefore carried out so as to evaluate acute and sub-chronic effects of two herbal formulations Omagha et al., (2021a), Omagha et al., (2021b) aganist *P. berghei* infected mice.

2. MATERIALS AND METHODS

Drugs, animals and parasite species for study

As discussed in Omagha et al., (2021b), the plant parts used for this study were collected from medicinal plants growers at Oje, Ibadan. Identification and authentication was done by a plant taxonomist in the Department of Botany, University of Lagos, Nigeria. Voucher specimens with LUT numbers 7817, 7818, 7819, 7820, 7821, 7822 for the specimens *Alstonia boonei* (stem bark), *Carica papaya* (fruits), *Cymbopogon citratus* (leaves), *Curcuma longa* (roots), *Magnifera indica* (stem bark) and *Enantia chlorantha* (stem bark) respectively was deposited at the herbarium unit of the Department of Botany, University of Lagos, Nigeria. Each of the plants parts were sorted, properly washed under running tap water, cut in pieces and then dried separately at 38 0C. Powdered *E. chlorantha* (stem bark), *C. citratus* (leaves), *C. papaya* (unripe fruits), *M. indica* (stem bark), *C. longa* (roots) and *A. boonei* (stem bark) were then separately extracted with hot water, dried, labelled and stored. The extracts were combined in ratios in order to obtain Cocktail treatment A (CtA) and Cocktail treatment B (CtB). CtA was prepared by dissolving 5.70 g + 2.87 g + 1.43 g of *E. chlorantha, C. citratus and C. longa* in 200 mL distilled water equivalent to 50 mg/mL concentration. In the same vein, CtB was prepared by dissolving 5.00 g + 2.33 g + 1.27 g + 1.27 g of *E. chlorantha, A. boonei*, *C. papaya* and *M. indica* in 200 mL distilled water equivalent to 50 mg/mL concentration.

The results of the combinations were separately heated over a water bath for 30 minutes and left to cool. They were then labelled and refrigerated at 4 °C in air-tight bottles. Chloroquine phosphate (CQ) and Pyrimethamine (PY) produced by Vitabiotics Limited, and SKG-Pharma Limited respectively in Lagos, Nigeria makes up the standard drugs used. The doses required for each of the standard drugs, 25 mg/kg and 5 mg/kg respectively Iwalokun, (2008), Alli et al., (2011), were administered on the animals, according to the weight of their body. They were then prepared by diluting 250 mg tablet of CQ in 25 mLs of distilled water (10 mg/mL), and 25 mg tablet of PY in 5 mLs of distilled water (5 mg/mL). Distilled water (DW) was also administered as control.

A total of 312 adult male mice of about 7 – 12 weeks old and weighing between 16 – 26 g were obtained from the Animal House, University of Lagos, Lagos Nigeria. Before being subjected to experimentation, the animals were left for two weeks to acclimatize to laboratory conditions. They had constant access to feed on a standard rodent's diet and tap water. The animals were also kept in plastic cages with metal covers for free passage of air, and at room temperature of about 27 oC. Chloroquine-sensitive *Plasmodium berghei berghei* parasites were obtained from the Institute for Advanced Medical Research and Training, (IMRAT), University of Ibadan, Ibadan, Nigeria, by intraperitoneal inoculation of uninfected mice with 0.2 ml of the diluted blood from previously infected mice maintained by successive intra-peritoneal inoculation of parasite-free mice every four days.

The donor mice were then transported to the University of Lagos animal house where they were kept under standard laboratory conditions with constant access to food and water until the desired level of parasitemia was achieved. Infected blood from donor mouse was obtained by cardiac puncture. Infected red cells/µl was calculated using the relative value method, count of infected red cells X 5000000/total red cells counted (infected + non-infected). This was done to determine the required standard inoculum of 1 × 106 using thin blood films of donor mice. 5 millilitres normal saline, a quantity determined by the level of parasitaemia of the infected donor mice was used to dilute 2 mL of the donor blood. This was previously described in (Omagha et al., 2021b).

Antimalarial activities of CtA and CtB

In all, 120 mice were used for this study. These were divided into 3 groups. The groups were utilized to evaluate antimalarial curative test according to Ryley and Peters, (1970), and Peters, (1965) 4-day suppressive and prophylactic tests as similarly followed in (Alli et al., 2011). Thirty-six mice were also exposed as the unparasitized/treated. Another 156 mice divided into 3 groups consisting of 40 mice for suppressive, 40 mice for curative, 40 mice for prophylactic and 36 mice for unparasitized/treated groups were similarly treated and maintained daily. The weights of this group of mice were recorded weekly until they were sacrificed and used for sub-acute safety assessment studies. Experimental layout showing concentrations for treatments administered and number of animals per group is shown in (Table 1).

Table 1 Experimental animal grouping, polyherbal concentrations and administration

Tests	Acute exposures (Curative Day 9, Suppressive Day 8, Prophylactic Day 12, Treated/Unparasitized Day 6)			25 days sub-chronic exposure studies for Curative, Suppressive, Prophylactic, Treated/Unparasitized		
	Groups	No of mice	Treatments and Doses (mg/kg)	Groups	No of mice	Treatments and Doses (mg/kg)
Curative	1	5	CtA200 mg/kg	1	5	CtA200 mg/kg
	2	5	CtA400 mg/kg	2	5	CtA400 mg/kg
	3	5	CtA800 mg/kg	3	5	CtA800 mg/kg
	4	5	CQ25 mg/kg	4	5	CQ25 mg/kg
	5	5	DW	5	5	DW
	6	5	CtB200 mg/kg	6	5	CtB200 mg/kg
	7	5	CtB400 mg/kg	7	5	CtB400 mg/kg
	8	5	CtB800 mg/kg	8	5	CtB800 mg/kg
Suppressive	1	5	CtA200 mg/kg	1	5	CtA200 mg/kg
	2	5	CtA400 mg/kg	2	5	CtA400 mg/kg
	3	5	CtA800 mg/kg	3	5	CtA800 mg/kg
	4	5	CQ25 mg/kg	4	5	CQ25 mg/kg
	5	5	DW	5	5	DW
	6	5	CtB200 mg/kg	6	5	CtB200 mg/kg
	7	5	CtB400 mg/kg	7	5	CtB400 mg/kg
	8	5	CtB800 mg/kg	8	5	CtB800 mg/kg
Prophylactic	1	5	CtA200 mg/kg	1	5	CtA200 mg/kg
	2	5	CtA400 mg/kg	2	5	CtA400 mg/kg
	3	5	CtA800 mg/kg	3	5	CtA800 mg/kg
	4	5	PY5 mg/kg	4	5	PY5 mg/kg
	5	5	DW	5	5	DW
	6	5	CtB200 mg/kg	6	5	CtB200 mg/kg
	7	5	CtB400 mg/kg	7	5	CtB400 mg/kg
	8	5	CtB800 mg/kg	8	5	CtB800 mg/kg
Unparasitized/ Treated	1	4	CtA200 mg/kg	1	4	CtA200 mg/kg
	2	4	CtA400 mg/kg	2	4	CtA400 mg/kg
	3	4	CtA800 mg/kg	3	4	CtA800 mg/kg
	4	4	CQ25 mg/kg	4	4	CQ25 mg/kg
	5	4	PY5 mg/kg	5	4	PY5 mg/kg
	6	4	DW	6	4	DW
	7	4	CtB200 mg/kg	7	4	CtB200 mg/kg
	8	4	CtB400 mg/kg	8	4	CtB400 mg/kg
	9	4	CtB800 mg/kg	9	4	CtB800 mg/kg

Key: DW (Distilled water), CQ (Chloroquine), PY (Pyrimethamine), CtA (Cocktail treatment A), CtB (Cocktail treatment B).

Sample collection

At the end of antimalarial evaluations, mice were fasted overnight. Each of their body weight was measured before they were sacrificed. Their samples were then analysed. On day 25, samples were also collected and analysed for the groups observed for chronic exposure. Blood collection was performed after each animal was anesthesized using diethyl ether (Parasuraman et al., 2010). Blood was collected from the left eye (ocular) of the mice, with the aid of micro haematocrit tubes through a 5 mL pipe tubes into Ethylenediaminetetraacetic acid (EDTA) bottles for hematological analysis, and into lithium-coated serum separator tubes for biochemical analysis.

Mice were sacrificed by cervical dislocation. Collection of animals' organs was done following the method described by (Onifade et al., 2016). The liver, right kidney, spleen, heart and brain were harvested, rinsed with ice-cold physiological saline,

blotted dry with clean tissue and weighed using analytical weighing balance. The relative weights of each organ were immediately determined using the formula: Organ weight/ body weight of animal on day of sacrifice × 100 g (Sellers et al., 2007). Preservation of the organs was done in properly labelled specimen bottles containing 10 % solution of neutral buffered formalin for histological analysis.

Haematological analysis

Within 24 hours after sample collection, blood from each exposed animals were used for the haematological analysis following the method of Dacie and Lewis (Dacie and Lewis, 1995).

Determination of packed cell volume

Blood sample was collected into capillary tubes containing anticoagulant. The capillary tubes were correctly placed in the numbered slots in the haematocrit (Hawskley Japan). The tubes were then centrifuged at 13000 rpm for 5 minutes. The percentage PCV was determined using a haematocrit reader Hawksley 850179, England.

Determination of haemoglobin

For each sample, 20 µl of heparinized blood was added to 5 mL of transformation solution in a test tube and stirred immediately. The absorbance of the test was measured using a spectrophotometer (TC-3300plus, USA) against the transformation solution at a wavelength of 540-546 nm. The haemoglobin content was determined from a calibration curve drawn using the cyanohaemoglobin standard and the transformation solution.

2Determination of total white blood cell

A small volume of 0.38 mL of WBC diluting fluid was dispensed into test tubes using automatic micropipettes. Twenty microliter of anticoagulated whole blood was added into the tube and mixed properly to haemolyse the RBCs, leaving the WBCs to be counted. The leukocytes (white cells) were then counted in a counting chamber under X1000 magnification microscope Olympus CX21FSI, Japan. The number of WBCs counted was reported as cells per microlitre of blood.

Determination of red blood cell counts

Colorimetric method was used to determine the red blood cell (erythrocyte count). 20 µl of blood was transferred into 10 mL pawinski solution and thoroughly stirred. The absorbance measurement was read in a spectrophotometer (TC-3300plus, USA) at wavelength of 600 nm. The concentration of the erythrocyte was determined using a calibration curve.

Biochemical parameters

Blood samples in the lithium tubes were kept at 4 °C for 4 hours to clot. The clotted blood samples were centrifuged at 5000 rpm for 10 minutes to obtain the serum which were refrigerated at −20 °C until used for biochemical analysis. The biochemical parameters evaluated at all doses were Aspartate Aminotransferase (AST), Alanin Aminotransferase (ALT), and Alkaline Phosphatase (ALP). The analyses were performed using randox commercial kits (Idowu et al., 2015b). Concentration estimations of AST and ALT were determined from the absorbance readings of the samples using a spectrophotometer (TC-3300plus, USA) against the standard curve (Reitman and Frankel, 1957). ALP was determined quantitatively using the colorimetric method according to manufacturer's instruction. 20 µl of serum sample was pipette into a test tube containing 2 mL cuvette (1 cm light path) and 1 mL of the ALP reagent. Absorbance values were read after 1.2 and 3 minutes at 405 nm wavelength using spectrophotometer (TC-3300plus, USA). Calculation was by the formular: absorbance at 405 nm/min x 2760 (U/I).

Oxidative stress biomarkers

Liver samples from exposed animals were kept at −20 °C refrigerator until used to evaluate biochemical markers of oxidative stress. According to standard protocols, Catalase (CAT) activity Quinlan et al., (1994), Superoxide Dismutase (SOD) Gao et al., (1998) and Malondialdehyde (MDA) Aristidou et al., (2015), Rath et al., (2010) concentrations were measured using diagnostic kits. All reactions' absorbances were measured spectrophotometrically (TC-3300plus, USA).

Histopathological analysis

Preserved organs were histologically examined for pathological changes at different levels of treatment. Tissue sections of each sample were cut and prepared on clean slides for Hematoxylin-Eosin staining before mounting in neutral DPX medium (Adeoye et al., 2015; Mebratu et al., 2013). Prepared slides were examined under X 400 magnification microscope with a camera attached for photomicrographs.

Determination of percentage survival of mice

Percentage survivors of the treated mice was calculated as: (Number of animals that survived at the end of exposure/total number of animals in the group at the beginning of exposure) X 100

Data analysis

Data from haematological and biochemical assays were analyzed using the 2016 version of Microsoft Excel and SPSS version 23.0. Descriptive statistics was performed on different variables. Where necessary, results obtained were expressed as mean ± standard error of mean. The differences between means of treated and control groups were compared for significance using one way Analysis of Variance (ANOVA), followed by Dunnett's multiple post hoc tests. Differences were considered significant to negative control at Probability value ($P<0.05$).

3. RESULTS

Haematological and biochemical parameters of treated mice

Post antimalarial curative, suppressive and prophylactic evaluations took place on Days 9, 8 and 12 respectively, packed cell volume, haemoglobin, white blood cell and red blood cell counts were significantly ($p<0.05$) increased. Haematological parameters on day 25 were significantly ($p<0.05$) elevated. In the unparasitized/treated test groups measured on Days 6 and 25, increased levels of PCV, Hb, WBC and RBC were observed (Figures 1 (a-d) and Table 2). Table 3 shows liver enzymes markers aspartate aminotransferase, alanine transaminase and alkaline phosphatase in most of the groups immediately after antimalarial evaluations.

The unparasitized/treated test groups were measured to be significantly ($p<0.05$) decreased, but increased in the groups measured on Day 25. Concentrations of oxidative stress biomarkers which were determined from Superoxide Dismutase, Catalase and Malondialdehyde are depicted in Table 4. Concentrations of SOD, CAT and MDA were significantly lower ($p<0.05$) in the curative and suppressive groups. However, levels were increased in the prophylactic and treated/unparasitized groups. High enzyme activities immediately after treatments (acute exposures) were observed to be significantly decreased ($p<0.05$) in groups observed on Day 25.

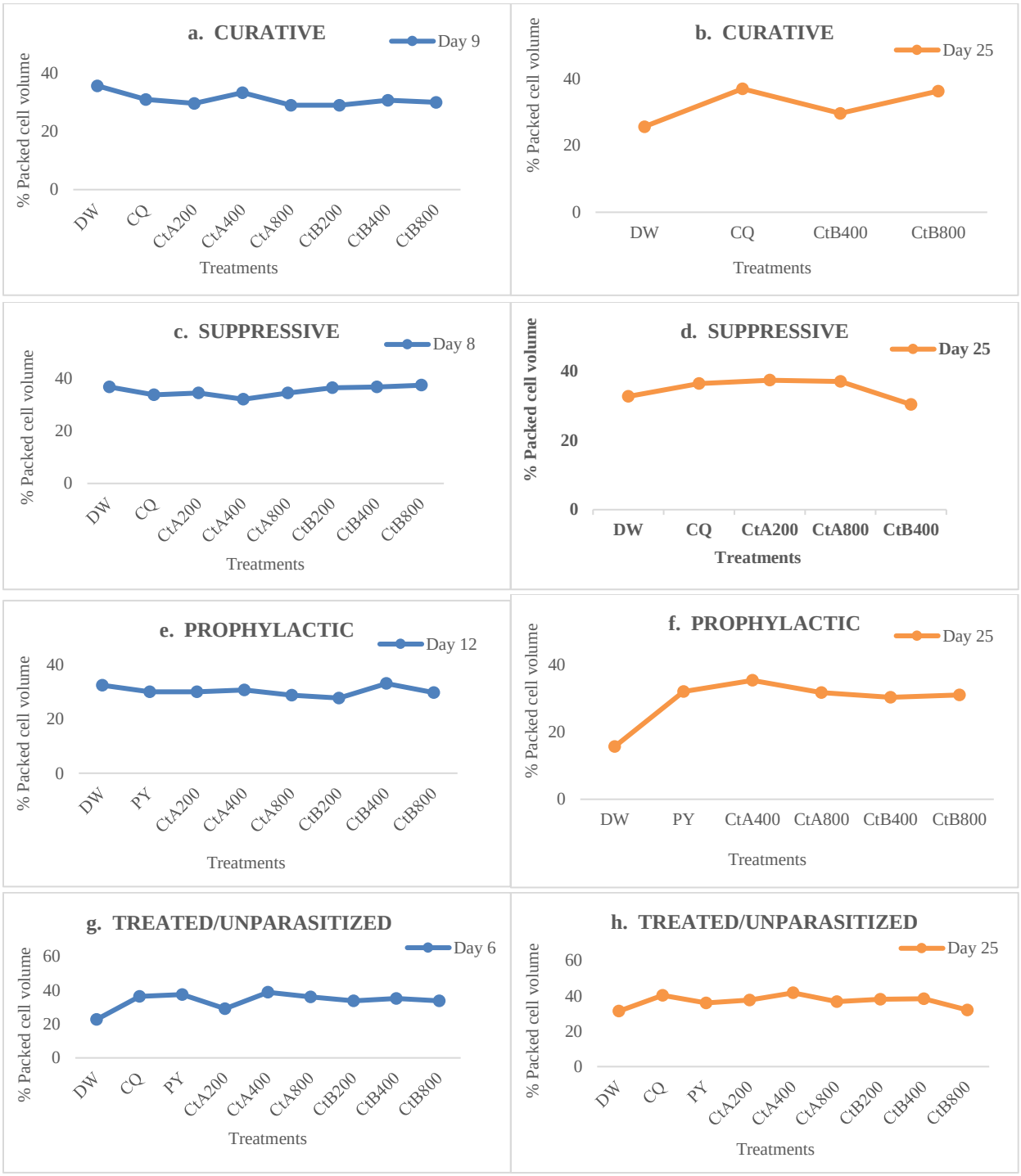


Figure 1 (a-d) Packed cell volume of the treatments

NB: The 0 points are groups with 100 % mortality.

Key: DW (Distilled water), CQ (Chloroquine), PY (Pyrimethamine), CtA (Cocktail treatment A), CtB (Cocktail treatment B).

Table 2 Effects of CtA and CtB antimalarials on haematological parameters of mice

Tests	Treatments mg/kg	Haematology post antimalarial evaluation (Curative Day 9, Suppressive Day 8, Prophylactic			Haematology on Day 25		
		HB g/dl	WBC mm3	RBC	HB g/dl	WBC mm3	RBCX1012/L
Curative	DW	12.82±0.35	3816.67±202.07	4.11±0.18	9.25±0.22	2066.67±104.08	3.09±0.27
	CQ	10.63±0.85	3283.33±464.58*	3.29±0.11	13.16±1.12	6533.33±1858.31^	4.98±0.83
	CtA200	10.51±1.04	3116.67±246.64	3.23±0.17	ND	ND	ND
	CtA400	11.98±0.18*	5633.33±513.16^	3.85±0.14*	ND	ND	ND
	CtA800	9.64±0.37	2783.33±175.59	3.17±0.07	ND	ND	ND
	CtB200	9.92±0.81	3133.33±416.33	3.15±0.05	ND	ND	ND
	CtB400	11.07±0.08*	2433.33±251.66	3.14±0.13	10.06±0.65^	3066.67±115.47	3.37±0.32^
	CtB800	10.21±0.61	4483.33±583.81	3.17±0.07	12.94±0.16	2633.33±251.66	4.99±0.08
Suppressive	DW	13.16±0.45	5666.67±292.97	4.69±0.55	11.64±0.32	1450.00±86.60	3.74±0.23
	CQ	12.19±0.41*	3033.33±251.66	3.46±0.10	13.03±1.79^	6400.00±871.78	4.97±1.19
	CtA200	12.36±0.66*	3583.33±160.73	4.05±0.18*	12.85±0.89^	3000.00±132.29	4.16±0.19^
	CtA400	11.62±0.70*	4233.33±448.14	4.12±0.73^	ND	ND	ND
	CtA800	12.38±0.40*	2633.33±568.62*	4.15±0.38^	13.44±0.68^	3616.67±212.79	4.56±0.35
	CtB200	13.02±0.56^	3850.00±350.00^	4.64±0.39^	ND	ND	ND
	CtB400	13.22±1.02^	3216.67±340.34*	4.35±0.89^	10.58±0.34	8866.67±57.74	3.44±0.22
	CtB800	13.38±0.42^	4233.33±251.66	4.77±1.01	ND	ND	ND
Prophylactic	DW	11.36±0.46	3200.00±264.58	3.86±0.18	5.88±0.21	2233.33±585.94	2.21±0.21
	PY	10.37±0.75*	5066.67±602.77^	3.19±0.29	10.83±1.44	4100.00±132.29	3.76±0.54
	CtA200	10.63±0.85*	3150.00±217.94*	3.18±0.20	ND	ND	ND
	CtA400	11.10±0.38*	3316.67±256.58^	3.28±0.09	12.63±0.15	6066.67±1331.67	4.44±0.33
	CtA800	10.12±0.94*	3866.67±321.46^	3.04±0.15	12.03±0.89	6666.67±472.58	3.55±0.49
	CtB200	9.21±0.84	3016.67±175.59*	2.90±0.32	ND	ND	ND
	CtB400	11.67±0.34^	3550.00±278.39^	3.33±0.16*	11.22±0.13	7683.33±125.83	3.55±0.28
	CtB800	6.77±5.86*	3250.00±312.25	3.20±0.19*	11.31±0.30	6616.67±325.32	3.69±0.24
Treated/ Unparasitized	DW	8.41±084	1850.00±180.28	3.07±0.16	10.65±0.99	1783.33±256.58	3.65±0.51
	CQ	12.84±0.63	4783.33±332.92	4.53±0.24	14.13±0.75	3516.67±104.08	5.53±0.29
	PY	13.19±0.62^	4800.00±360.56^	4.53±0.36	11.92±0.28^	2766.67±321.46	4.36±0.55
	CtA200	10.13±0.74	4850.00±638.36	3.25±0.11^	12.75±0.28	4133.33±321.46^	4.78±0.19
	CtA400	13.70±1.01^	3766.67±251.66^	5.07±0.74	14.08±0.66	4383.33±202.07	5.63±0.51
	CtA800	12.75±0.31^	2900.00±217.95^	4.48±0.25^	12.64±0.34	3366.67±256.58	4.58±0.26
	CtB200	11.94±0.46^	2150.00±30414^	4.13±0.22	13.49±0.82	4883.33±480.45	4.85±0.62
	CtB400	11.93±0.88^	3050.00±217.94^	3.92±0.13^	13.66±0.52	4016.67±76.38	5.06±0.23
	CtB800	11.34±0.32	4100.00±264.58	4.14±0.80	11.63±0.34^	3033.33±378.59	3.93±0.24^

N.B: Values are represented as mean of three replicates ± standard deviation. (P<0.05)^* was considered significant to negative control using Dunnett's multiple post hoc test.

Key: HB (Haemoglobin), RBC (Red blood cell), WBC (White blood cell), DW (Distilled water), CQ (Chloroquine), PY (Pyrimethamine), CtA (Cocktail treatment A), CtB (Cocktail treatment), ND (Not determined).

Table 3 Effects of CtA and CtB antimalarial exposure on liver function markers of mice

Tests	Treatments mg/kg	Liver enzymes levels post antimalarial evaluation (Curative Day 9, Suppressive Day 8, Prophylactic			Liver enzymes levels on Day 25		
		AST U/L	ALT U/L	ALP U/L	AST U/L	ALT U/L	ALP U/L
Curative	DW	87.81±10.34	38.33±10.27	99.36±2.76	39.20±0.45	41.59±0.58	93.52±0.50
	CQ	62.45±33.06*	20.16±3.40*	74.52±7.30	35.52±7.74*	46.33±4.87^	74.52±5.52
	CtA200	79.89±8.88*	30.26±3.56	77.28±5.52	ND	ND	ND
	CtA400	98.83±1.94^	39.73±0.49^	92.64±1.41*	ND	ND	ND
	CtA800	94.53±23.28^	32.91±4.29*	102.12±5.52^	ND	ND	ND
	CtB200	97.12±29.18	37.05±4.68	76.36±6.95	ND	ND	ND
	CtB400	72.01±20.93*	32.77±0.78*	76.89±2.84	26.37±0.55	38.71±0.70*	85.79±4.59*
	CtB800	70.30±4.94	40.38±0.30^	92.92±1.59*	67.14±0.51	50.96±0.35	100.14±3.07^
Suppressive	DW	46.14±5.10	45.24±2.37	100.28±5.75	76.46±0.57	39.67±0.53	100.75±1.71
	CQ	55.63±14.86	24.67±1.36*	70.84±4.22*	34.78±0.52	42.72±0.26^	62.77±3.02
	CtA200	81.44±21.77	30.54±2.08*	82.80±2.76	41.71±3.30	39.88±0.87^	80.30±5.06
	CtA400	65.84±11.52	31.50±2.79	92.00±4.22*	ND	ND	ND
	CtA800	122.86±5.37	42.69±4.88*	96.60±2.76*	37.99±0.47	40.71±0.84^	77.00±1.27
	CtB200	57.34±8.97	27.28±2.15	76.36±6.95	ND	ND	ND
	CtB400	61.66±13.07	32.63±6.75	77.28±7.30	36.91±0.26	43.49±0.46^	83.14±0.49
	CtB800	96.33±3.67	34.35±2.83	93.84±5.52*	ND	ND	ND
Prophylactic	DW	69.49±20.48	33.23±2.85	101.20±5.75	45.29±2.86	35.05±1.91	81.62±1.32
	PY	27.01±4.38	23.84±1.16	80.04±5.52	33.68±4.94	47.13±1.43	83.63±3.11^
	CtA200	29.66±9.96	19.09±4.45	80.04±2.76	ND	ND	ND
	CtA400	26.77±3.49	21.23±0.68	81.88±4.22	92.41±42.75	63.36±13.62	86.48±5.75^
	CtA800	46.01±17.07*	26.49±6.33	101.20±4.22	54.13±1.35	59.17±2.03	90.14±2.03^
	CtB200	41.16±15.30*	22.96±3.74	78.20±4.22	ND	ND	ND
	CtB400	29.78±5.82	19.99±4.22	80.96±4.22	34.37±0.67	55.99±0.68	90.38±0.73^
	CtB800	61.21±18.38*	28.38±3.27	95.68±4.22*	67.53±0.55	49.57±0.53	99.68±0.47
Treated/ Unparasitized	DW	135.88±0.79	33.20±7.26	109.91±2.07	29.77±1.57	11.41±1.68	90.16±4.22
	CQ	124.95±21.95	28.89±0.94	66.24±2.76*	49.03±10.26	9.87±1.68*	61.64±6.95
	PY	140.48±17.093^	36.24±3.90	75.44±3.19	80.24±29.26	16.09±2.02	69.92±4.22
	CtA200	92.28±46.43*	19.33±2.66	78.20±5.75	32.84±6.09^	9.79±1.92*	62.56±3.19
	CtA400	124.77±27.56*	35.89±2.15^	84.64±3.19	85.82±38.86	13.10±5.11^	75.44±4.22
	CtA800	146.76±26.66^	35.15±11.48	100.28±4.22	48.09±11.82	17.82±4.63	83.72±4.22*
	CtB200	143.27±23.38^	29.01±9.19*	80.04±2.76	32.79±3.61^	11.20±0.42^	72.68±4.22
	CtB400	154.97±15.21^	33.97±6.38^	71.76±2.76*	82.80±21.82	13.28±2.96^	80.96±4.22*
	CtB800	153.01±1.33	52.43±0.68	91.95±1.64	36.41±7.81	15.08±6.05	99.06±2.81^

N.B: Values are represented as mean of replicates ± standard deviation. (P<0.05)^* was considered significant to negative control using Dunnett's multiple post hoc test.

Key: AST (Aspartate Aminotransferase), ALT (Alanine Transaminase), ALP (Alkaline Phosphatase), DW (Distilled water), CQ (Chloroquine**)**, PY (Pyrimethamine), CtA (Cocktail treatment A), CtB (Cocktail treatment), ND (Not determined).

Table 4 Assessment of biochemical markers of oxidative stress post treatment

Tests	Treatments mg/kg	Oxidative stress post antimalarial evaluation (Curative Day 9, Suppressive Day 8, Prophylactic Day 12, Treated/Unparasitized Day 6)			Oxidative stress on Day 25		
		SOD /min/mgprotein	CAT /min/mgprotein	MDA nmol/ml	SOD /min/mgprotein	CAT /min/mgprotein	MDA nmol/ml
Curative	DW	302.89±11.18	3.72±0.19	1.58±0.05	238.54±0.53	3.80±0.04	2.82±0.05
	CQ	215.09±4.44*	2.74±0.06	0.77±0.03	141.57±22.29	2.44±0.15	3.75±0.84
	CtA200	193.68±1.41	1.51±0.03	1.16±0.04	ND	ND	ND
	CtA400	292.17±9.12*	3.01±0.07	0.91±0.05	ND	ND	ND
	CtA800	198.47±6.99	1.39±0.06	1.18±0.05	ND	ND	ND
	CtB200	164.86±2.01	3.45±0.07*	1.24±0.05	ND	ND	ND
	CtB400	265.77±3.62*	2.65±0.67	0.75±0.04	125.03±81.67	5.00±1.41	3.08±0.69
	CtB800	222.28±5.37	1.80±0.01	0.72±0.03	262.13±7.11	8.06±0.11	2.51±0.17
Suppressive	DW	280.14±2.60	3.33±0.06	1.92±0.08	128.49±23.37	4.50±0.09	2.56±0.58
	CQ	291.46±3.76*	2.27±0.04	0.77±0.08	125.96±9.15*	2.21±0.06	2.00±0.05
	CtA200	234.87±5.37	2.79±0.06	1.38±0.06	250.09±2.92	7.28±0.68	1.32±0.90
	CtA400	249.49±5.83*	3.18±0.04*	0.93±0.04	ND	ND	ND
	CtA800	178.54±3.22	1.74±0.02	1.49±0.03	67.98±1.36	3.37±0.04	2.15±0.13
	CtB200	237.49±4.97	1.49±0.05	0.85±0.06	ND	ND	ND
	CtB400	218.43±4.51	1.74±004	1.18±0.05	152.15±4.01	4.12±0.15*	2.82±0.05^
	CtB800	177.00±3.28	1.78±0.03	1.50±0.04	ND	ND	ND
Prophylactic	DW	213.78±1.77	2.84±0.05	1.17±0.08	90.50±1.54	2.67±0.01	2.74±0.09
	PY	235.22±5.18^	1.29±0.06	1.53±0.04	180.54±3.77	3.49±0.34	2.96±0.39^
	CtA200	230.89±2.85^	3.08±0.04^	1.27±0.04^	ND	ND	ND
	CtA400	279.19±4.47	3.48±0.06	1.39±0.08	144.51±1.28	4.28±0.16	3.32±0.04
	CtA800	198.62±4.91*	2.84±0.04	1.43±0.06^	153.02±2.08	6.27±0.08	2.03±0.04
	CtB200	140.93±0.52	3.23±0.06^	0.94±0.04	ND	ND	ND
	CtB400	215.06±1.75^	3.49±0.04	1.26±0.05^	182.47±133.95	3.58±0.38	1.62±0.21
	CtB800	219.12±5.72^	3.86±0.07	1.18±0.05^	165.55±7.28	4.27±0.11	3.16±0.14^
Treated/ Unparasitized	DW	216.73±0.43	3.76±0.05	1.87±0.17	106.21±14.70	2.71±0.38	1.37±0.16
	CQ	193.24±0.65*	2.57±0.13	1.87±0.07	158.07±0.29	4.89±0.08	0.87±0.01
	PY	139.90±2.23	1.35±0.3	2.75±0.17	176.56±0.43	1.86±0.02	1.93±0.06
	CtA200	287.01±0.35	5.59±0.02	1.90±0.10^	203.37±1.57	3.91±0.11	1.19±0.11*
	CtA400	206.82±0.44*	3.57±0.12*	2.01±0.09^	355.54±15.97	7.58±0.55	1.85±0.06
	CtA800	222.00±1.25^	3.77±0.04^	2.33±0.08	175.88±0.16	1.96±0.09	1.97±0.15
	CtB200	242.40±1.59^	3.31±0.06*	1.73±0.08*	281.54±2.65	9.68±0.33	1.74±0.07
	CtB400	268.19±0.44	5.18±0.04	2.03±0.07^	188.34±4.45	2.84±0.001^	1.48±0.07^
	CtB800	324.39±1.54	7.37±0.13	1.68±0.04*	176.39±0.87	1.80±0.04	1.90±0.10

N.B: Values are represented as mean of replicates ± standard deviation. (P<0.05)^* was considered significant to negative control using Dunnett's multiple post hoc test.

Key: SOD (Superoxide Dismutase), CAT (Catalase), MDA (Malondialdehyde), DW (Distilled water), CQ (Chloroquine), PY (Pyrimethamine), CtA (Cocktail treatment A), CtB (Cocktail treatment B), ND (Not determined).

Histopathological findings

Animals' body weights post treatments

Loss of body weight which was statistically significant (p<0.05), was observed in the groups treated with the cocktail extracts. The CQ and PY groups showed a gradual increase in weight. The treated/unparasitized groups also presented a statistically significant (p<0.05) weight increase (Table 5).

Animals' organ weights post exposure

Compared to negative control, organ weight changes post antimalarial tests showed that treatments with CtA and CtB significantly ($p<0.05$) increased liver, spleen, kidney, heart and brain weights in majority of the groups. A significant ($p<0.05$) increase in organ weights was also observed in majority of the unparasitized/treated groups. Relative organ weights post treatments in mice is here presented in (Tables 6, 7).

Table 5 Animals' body weight post treatments

Tests	Treatments	Day 0	Day 7	Day 14	Day 21	Day 25
Curative	DW	24.0	20.5±0.9	20.0±0.0	19.0±0.0	17.0±0.0
	CQ	19.0±0.0	19.2±0.2*	20.25±0.8^	22.5±1.2	26.0±1.7
	CtA 200	21.0±0.0	20.5±0.5*	18.0±0.0	16.0±0.0*	ND
	CtA 400	22.6±0.4	22.8.0.9^	21.0±0.0^	10.0±0.0	ND
	CtA 800	26.0±0.0	25.7±0.3	24.0±1.0	20.0±2.0^	ND
	CtB 200	21.0±0.0	19.3±0.7*	17.0±0.0	14.0±0.0	ND
	CtB 400	22.6±0.4	23.8±.03^	21.0±0.7^	22.7±1.3	22.0±5.0
	CtB 800	26.0±0.0	24.5±0.5	27.5±0.5	28.0±1.0	29.0±2.0
Suppressive	DW	21.0±0.0	21.5±0.0	21.0±0.0	21.0	20.0
	CQ	20.0±0.0	21.8±0.8^	23.3±0.3^	25.3±0.3	28.7±0.3
	CtA 200	20.0±0.0	20.5±0.3*	18.3±0.7*	17.7±2.4	16.0±6.0
	CtA 400	21.0±0.0	20.6±0.4*	18.3±1.2*	18.0*	ND
	CtA 800	23.0±0.0	22.3±0.3^	21.0±0.0*	20.0±0.0*	19*
	CtB 200	20.0±0.0	21.3±1.3*	18.0±0.0*	12.0±2.0	ND
	CtB 400	21.0±0.0	21.0±1.0*	19.7±0.9*	19.0±1.0*	19*
	CtB 800	23.0±0.0	23.2±0.0^	23.0±0.0^	16.0	ND
Prophylactic	DW	25.0±0.0	24.7±0.3	24.0	21	25.0±0.0
	PY	19.0±0.0	19.6±0.0	20.4±0.0	21.4±1.2^	24.2±1.5*
	CtA 200	19.0±0.0	18.4±0.2	17.0±0.0	ND	ND
	CtA 400	22.0±0.0	20.4±0.9	21.4±1.3*	20.8±1.0*	19.7±2.0
	CtA 800	22.0±0.0	21.2±0.7*	21.8±1.1*	20.0±0.0*	18.0
	CtB 200	19.0±0.0	19.2±0.2	18.3±0.3	14.0	ND
	CtB 400	22.0±0.0	20.8±0.9	19.7±0.8	20*	18.0
	CtB 800	22.0±0.0	20.8±0.2	20.2±0.7	20.3±1.3*	25.0*
Treated/ Unparasitized	DW	20.0±0.0	21.3±0.0	24.0±0.4	23.5±0.5	24.5±0.5
	CQ	24.0±0.0	22.5±0.9^	24.8±0.6^	24.0±0.4^	25.5±0.9^
	PY	20.0±0.0	19.5±0.3*	21.5±0.6*	22.0±0.4*	23.0±0.6*
	CtA 200	24.0±0.0	23.3±0.9^	23.5±1.3*	24.3±0.9^	25.5±0.6^
	CtA 400	25.0±0.0	23.8±1.3^	23.5±0.6*	24.3±0.9^	25.0±0.8^
	CtA 800	26.0±0.0	24.0±1.6^	24.3±0.5^	24.3±0.9^	27.5±1.3^
	CtB 200	21.0±0.0	20.3±0.9*	22.5±1.3*	23.0±0.8*	24.0±0.8*
	CtB 400	22.0±0.0	20.5±1.3*	21.8±1.5*	24.0±1.4^	25.3±0.9^
	CtB 800	23.0±0.0	21.8±1.5^	27.5±1.0^	26.5±0.6^	24.8±0.5^

N.B: Values are represented as mean of replicates ± standard deviation. ($P<0.05$)^* was considered significant to negative control using Dunnett's multiple post hoc test.

Table 6 Relative organ weights of treated animals post antimalarial treatments

Tests	Treatments mg/kg	Relative organ weights post antimalarial evaluation (Curative Day 9, Suppressive Day 8, Prophylactic Day 12, Treated/Unparasitized Day 6)				
		LIVER	SPLEEN	KIDNEY	HEART	BRAIN
Curative	DW	5.69±0.01	1.05±0.01	0.65±0.02	0.35±0.01	1.19±0.01
	CQ	4.07±0.01*	0.45±0.01*	0.64±0.03	0.36±0.01	1.71±0.01^
	CtA200	4.50±0.15*	0.52±0.01*	0.65±0.01	0.70±0.02^	1.67±0.01^
	CtA400	4.20±0.09*	0.65±0.01*	0.67±0.29	0.56±0.02^	1.64±0.02^
	CtA800	4.76±0.02*	0.76±0.02*	0.94±0.01	0.59±0.01^	1.76±0.01^
	CtB200	5.58±0.04	1.23±0.03^	0.79±0.02	0.50±0.01^	1.65±0.02^
	CtB400	6.04±0.02^	0.91±0.02*	0.65±0.03	0.66±0.02^	2.04±0.02^
	CtB800	5.90±0.01	1.18±0.01^	0.82±0.01	0.61±0.01^	2.00±0.02^
Suppressive	DW	4.48±0.01	0.71±0.01	0.66±0.02	0.44±0.03	1.80±0.01
	CQ	3.11±0.06*	0.71±0.02	0.64±0.02	0.64±0.02^	1.80±0.01*
	CtA200	4.00±0.01*	0.39±0.01*	0.33±0.01*	0.55±0.02^	1.84±0.02
	CtA400	3.44±0.03*	0.81±0.02^	0.33±0.01	0.55±0.02	1.84±0.02^
	CtA800	3.55±0.02*	1.33±0.02*	0.50±0.00*	0.43±0.01	1.11±0.02*
	CtB200	3.12±0.06*	0.85±0.03^	0.45±0.02*	0.40±0.01	1.44±0.02*
	CtB400	3.70±0.01*	0.62±0.01*	0.28±0.02*	0.48±0.01	1.57±0.01*
	CtB800	3.73±0.02*	1.08±0.02^	0.58±0.02*	0.32±0.02*	1.43±0.01*
Prophylactic	DW	3.24±0.06	0.73±0.01	0.77±0.01	0.45±0.01	1.83±0.01
	PY	4.63±0.02^	0.82±0.01^	0.64±0.02*	0.45±0.02	2.00±0.02^
	CtA200	5.72±0.02^	0.74±0.01^	0.84±0.01	0.66±0.02^	1.85±0.01
	CtA400	4.61±0.02^	0.98±0.04^	0.82±0.01	0.56±0.01^	1.73±0.01^
	CtA800	6.13±0.09^	0.87±0.01^	1.09±0.04^	0.62±0.01^	1.71±0.02^
	CtB200	4.92±0.01^	0.92±0.01^	0.86±0.01^	0.56±0.01^	1.83±0.01
	CtB400	4.77±0.01^	0.95±0.01^	1.07±0.01^	0.58±0.01^	1.72±0.01^
	CtB800	6.56±0.01^	0.92±0.01^	1.08±0.02^	0.57±0.01^	1.84±0.01
Treated/ Unparasitized	DW	5.29±0.09	0.88±0.01	0.77±0.00	0.47±0.01	1.76±0.03
	CQ	6.28±0.01*	1.03±0.01	0.79±0.01	0.44±0.02	2.08±0.01
	PY	5.86±0.01*	1.05±0.02	0.75±0.01	0.62±0.01^	1.62±0.01
	CtA200	5.95±0.01*	1.08±0.01^	0.77±0.01	0.41±0.01*	1.57±0.07
	CtA400	5.73±0.02*	0.67±0.01*	0.68±0.00*	0.47±0.01	1.50±0.01
	CtA800	5.25±0.05	0.83±0.01	0.82±0.10^	0.38±0.01*	1.96±0.02
	CtB200	5.04±0.02*	0.93±0.01	0.57±0.01*	0.50±0.01	1.44±0.35
	CtB400	5.08±0.02	0.78±0.14*	0.70±0.01*	0.39±0.01*	1.40±0.01
	CtB800	5.78±0.01*	1.10±0.01^	0.57±0.01*	0.42±0.01	1.47±0.01

N.B: Values are represented as mean of replicates ± standard deviation. (P<0.05)^* was considered significant to negative control using Dunnett's multiple post hoc test.

Table 7 Relative organ weights of treated animals on day 25

Tests	Treatments mg/kg	Relative organ weights on day 25				
		LIVER	SPLEEN	KIDNEY	HEART	BRAIN
Curative	DW	5.95±0.02	1.93±0.02	0.94±0.01	0.57±0.01	2.03±0.09
	CQ	4.68±0.00*	0.97±0.08*	0.72±0.00*	0.46±0.01*	1.31±0.00*
	CtA200	ND	ND	ND	ND	ND
	CtA400	ND	ND	ND	ND	ND
	CtA800	ND	ND	ND	ND	ND
	CtB200	ND	ND	ND	ND	ND
	CtB400	4.56±0.00*	3.03±0.02^	0.67±0.00*	0.52±0.01*	1.48±0.01*
	CtB800	5.67±0.01*	3.48±0.02^	0.83±0.01*	0.70±0.00^	1.33±0.01*
Suppressive	DW	6.73±0.09	2.04±0.02	0.80±0.01	0.59±0.01	1.69±0.01
	CQ	3.63±0.02*	0.67±0.01*	0.63±0.02*	0.39±0.01*	1.11±0.01*
	CtA200	6.03±0.02*	2.66±0.02*	0.86±0.01^	0.54±0.01*	1.39±0.00*
	CtA400	ND	ND	ND	ND	ND
	CtA800	8.11±0.01^	1.79±0.01*	0.89±0.01^	0.73±0.02^	1.88±0.01^
	CtB200	ND	ND	ND	ND	ND
	CtB400	5.84±0.01*	3.46±0.03^	0.74±0.01*	0.49±0.01*	1.89±0.01^
	CtB800	ND	ND	ND	ND	ND
Prophylactic	DW	6.59±0.14	3.63±0.06	0.81±0.01	0.52±0.00	1.81±0.01
	PY	7.26±0.15^	2.16±0.02*	0.67±0.00*	0.45±0.02*	1.58±0.01*
	CtA200	ND	ND	ND	ND	ND
	CtA400	7.16±0.03^	3.44±0.03	0.70±0.01*	0.55±0.01	1.24±0.02*
	CtA800	7.56±0.01^	1.81±0.01*	0.88±0.01^	0.78±0.01^	2.13±0.03^
	CtB200	ND	ND	ND	ND	ND
	CtB400	7.17±0.07^	2.28±0.01*	0.73±0.02*	0.61±0.00^	2.17±0.01^
	CtB800	7.93±0.02^	3.47±0.12	0.72±0.01*	0.53±0.01	1.63±0.03*
Treated/ Unparasitized	DW	4.33±0.01	0.27±0.01	0.87±0.02	0.65±0.02	1.66±0.01
	CQ	3.90±0.01*	0.33±0.11	1.05±0.01^	0.50±0.01*	1.44±0.02*
	PY	4.47±0.09	0.35±0.02	0.64±0.02*	0.40±0.00*	0.86±0.01*
	CtA200	4.25±0.01	0.72±0.01^	0.73±0.02*	0.39±0.01*	1.70±0.01^
	CtA400	3.57±0.05*	0.76±0.07^	0.65±0.03*	0.45±0.02*	1.39±0.01*
	CtA800	3.47±0.01*	2.14±0.02^	0.66±0.03*	0.53±0.01*	1.35±0.03*
	CtB200	5.26±0.02^	0.76±0.02^	0.62±0.00*	0.420.01*	1.38±0.01*
	CtB400	4.42±0.02	0.86±0.02^	0.76±0.01*	0.45±0.02*	1.34±0.02*
	CtB800	5.06±0.03^	2.48±0.12^	0.57±0.01*	0.46±0.01*	1.64±0.02

N.B: Values are represented as mean of replicates ± standard deviation. (P<0.05)^* was considered significant to negative control using Dunnett's multiple post hoc test.

Results from histopathological examination of animals' vital organs

Heart and spleen morphology was normal in all treatments. However, plates 1–10 are the photomicrographs of the lesions seen in kidney, liver and brain of mice post treatment. Degrees of histopathological changes in the organs is shown in (Table 8). Histopathological findings in the kidney showed blood vessels at the renal cortical interstitium are moderately congested in curative groups on day 9. There was diffuse tubular degeneration, and many tubules have proteinaceous casts in their lumina in curative groups on day 25. There were multiple tubular degeneration and necrosis foci in prophylactic groups on day 25. Also noticed were foci of severe interstitial congestion in treated/unparasitized groups on day 25. Histology of the liver immediately after exposures and in those exposed till day 25 showed multiple foci of tubular degeneration and necrosis, foci of severe interstitial congestion; diffuse hydropic degeneneration of hepatocytes, periportal cellular infiltration, severe diffuse vacuolar degeneration

and necrosis of hepatocytes. Noticeably, liver in the CQ25 mg/kg suppressive groups examined 8 days post-exposure showed a moderate portal congestion with moderate diffuse hydropic degeneration of hepatocytes. Also, the prophylactic groups examined on day 25, PY5 mg/kg group showed moderate periportal cellular infiltration compared to control group with no lesions. Histology of the brain showed increased capillary density on the cortical parenchyma, diffuse spongiosis, and reactive neurons in curative groups on day 9. But in treated/unparasitized groups on day 25, there was mild diffuse spongiosis and gliosis of the parenchyma in.

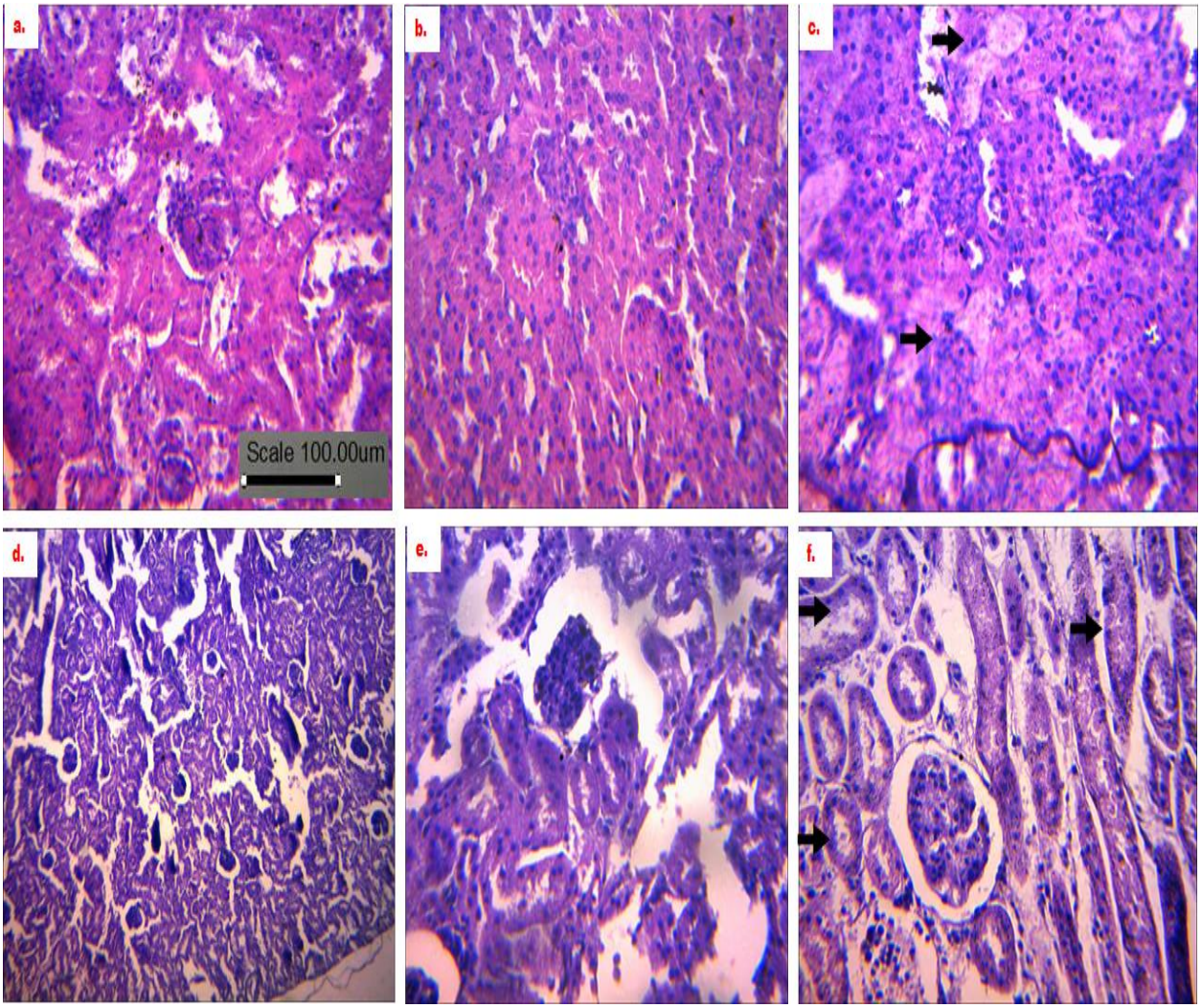


Plate 1 Photomicrographs of transverse sections of kidney for curative groups on day 9 (a-c); and curative groups on day 25 (d-f). MAG. X 400 (a) DW: No visible lesions seen. (b) CQ25 mg/kg: No visible lesions seen. (c) CtB800 mg/kg: The blood vessels at the renal cortical interstitium are moderately congested (arrows). (d) DW: No visible lesions seen. (e) CQ25 mg/kg: No visible lesions seen. (f) CtB800 mg/kg: There is a diffuse tubular degeneration. Many tubules have proteinaceous casts in their lumina (arrows).

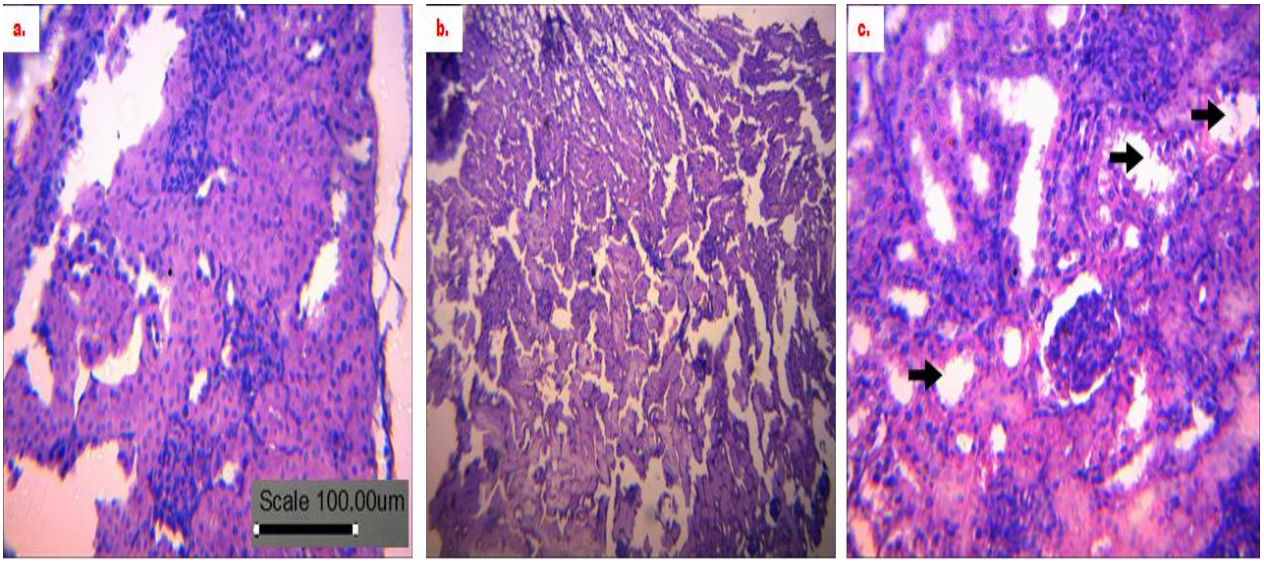


Plate 2 (a-c) Photomicrographs of transverse sections of kidney for prophylactic groups on day 25. MAG. X 400. (a) DW: No visible lesions seen. (b) PY5 mg/kg: No visible lesions seen. (c) CtA800 mg/kg: There are multiple foci of tubular degeneration and necrosis (arrows).

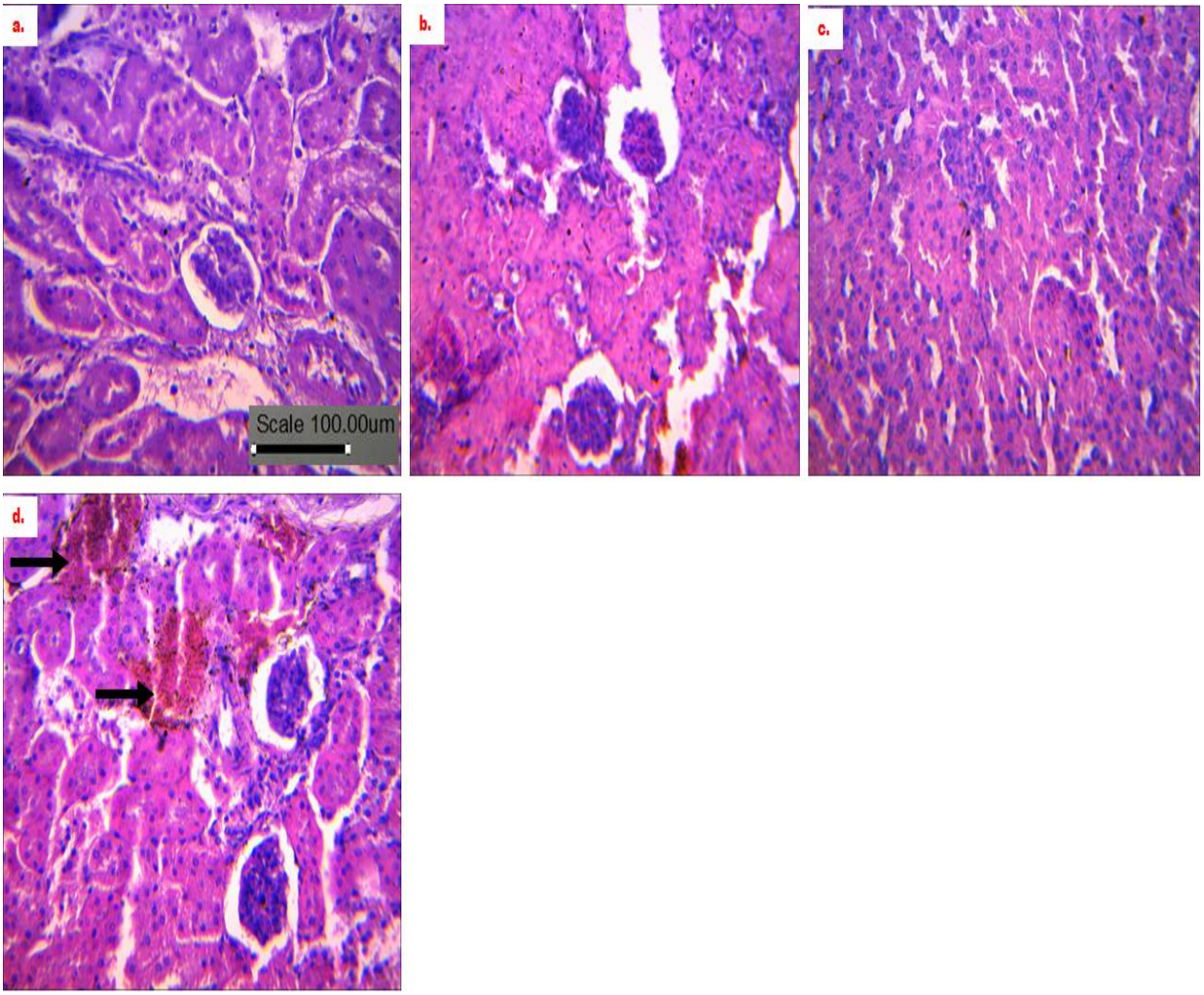


Plate 3 (a-d) Photomicrographs of transverse sections of kidney for treated/unparasitized groups on day 25. MAG. X 400. (a) DW: No visible lesions seen. (b) CQ25 mg/kg: No visible lesions seen. (c) PY5 mg/kg: No visible lesions seen. (d) CtB400 mg/kg: There are foci of severe interstitial congestion (arrows).

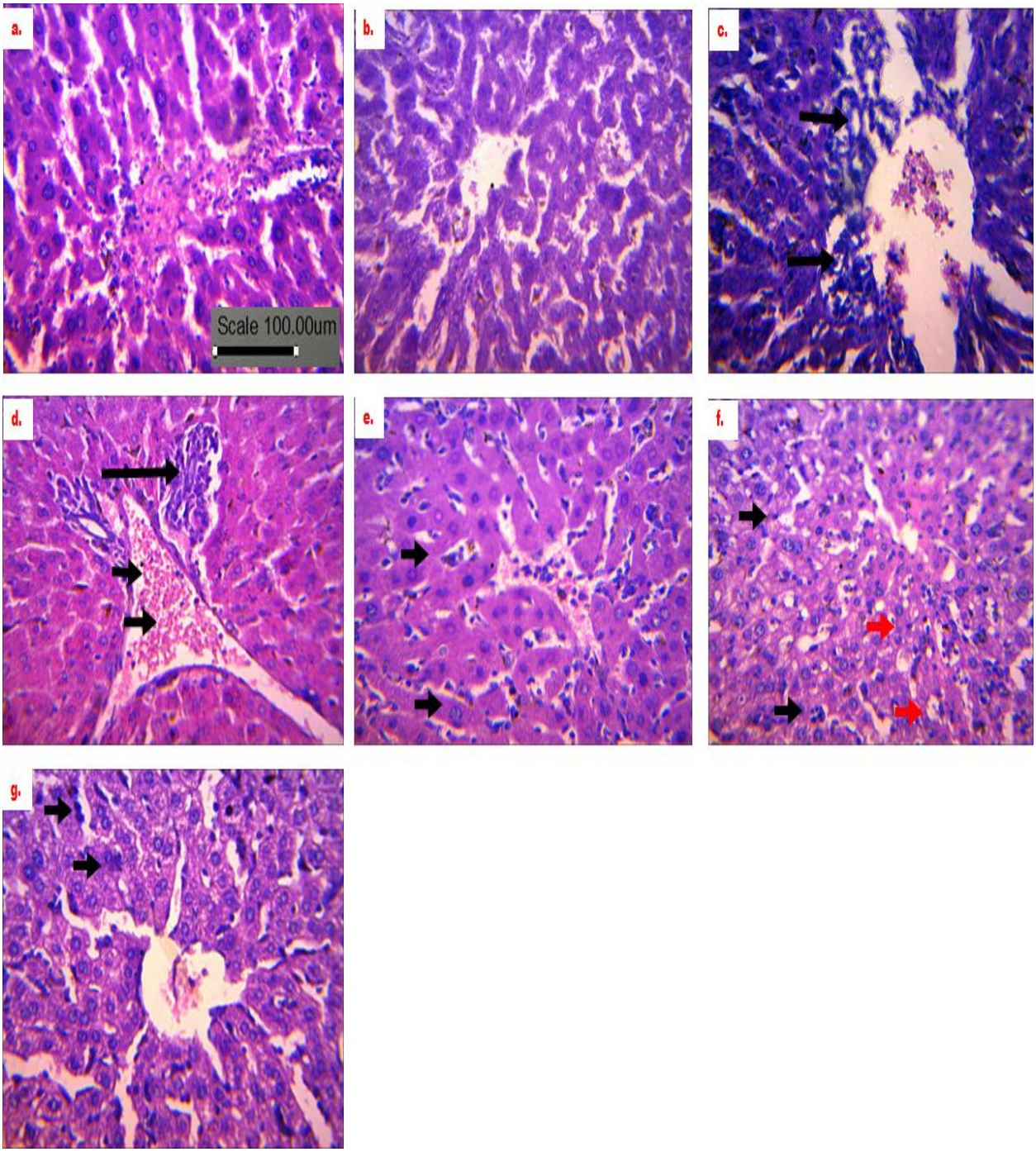


Plate 4 (a-g) Photomicrographs of sections of the liver for curative groups on day 9. (MAG. X 400). (a) DW: No visible lesions seen. (b) CQ25 mg/kg: No visible lesions seen. (c) CtA400 mg/kg: There is a mild periportal cellular infiltration (arrows). (d) CtA800 mg/kg: There is a mild portal congestion, (shorter arrows) with periportal cellular infiltration (long arrows). (e) CtB200 mg/kg: There is a moderate periportal and diffuse sinusoidal cellular infiltration (arrows). (f) CtB400 mg/kg: There is a moderate periportal cellular infiltration (red arrows). There is a mild diffuse vacuolar degeneration (black arrows). (g) CtB800 mg/kg: There is a very mild diffuse hydropic degeneneration of hepatocytes (arrows).

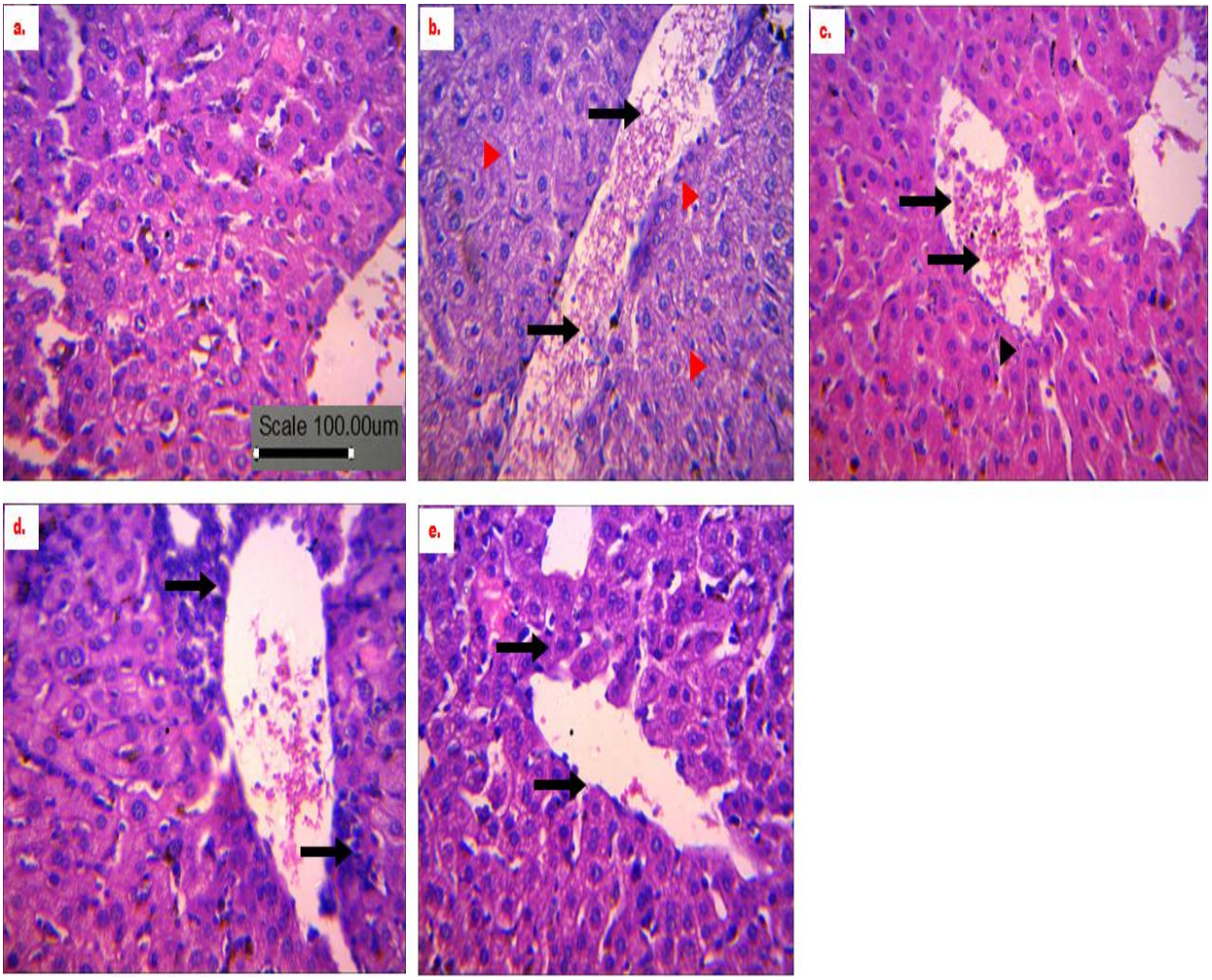


Plate 5 (a-e) Photomicrographs of sections of the liver for suppressive groups on day 8. MAG. X 400. (a) DW: No visible lesions seen. (b) CQ25 mg/kg: There is a moderate portal congestion (arrows), with moderate diffuse hydropic degeneration of hepatocytes (arrowheads). (c) CtB200 mg/kg: There is a mild congestion of the portal vessels. (d) CtB400 mg/kg: There is a mild periportal cellular infiltration. (e) CtB800 mg/kg: There is a very mild periportal cellular infiltration.

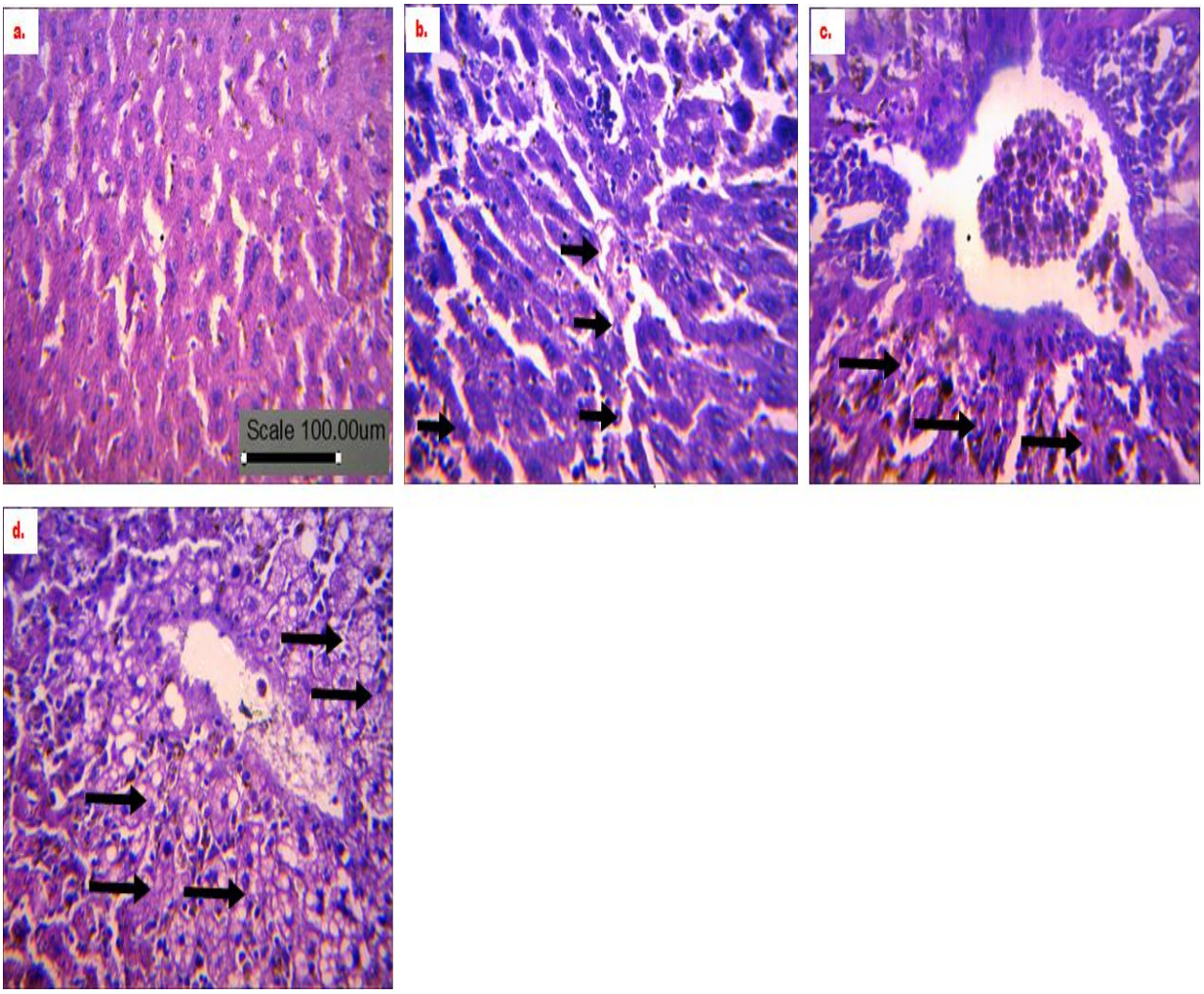


Plate 6 (a-d) Photomicrographs of sections of the liver for prophylactic groups on day 25. MAG. X 400. (a) DW: No visible lesions seen. (b) PY5 mg/kg: There is a mild to moderate periportal cellular infiltration (arrows). (c) CtA400 mg/kg: There is a severe diffuse vacuolar degeneration of hepatocytes, with severe periportal cellular infiltration. The sinusoids are severely congested (arrows). (d) CtA800 mg/kg: There is a severe diffuse vacuolar degeneration and necrosis of hepatocytes, (arrows). There is a diffuse severe cellular infiltration.

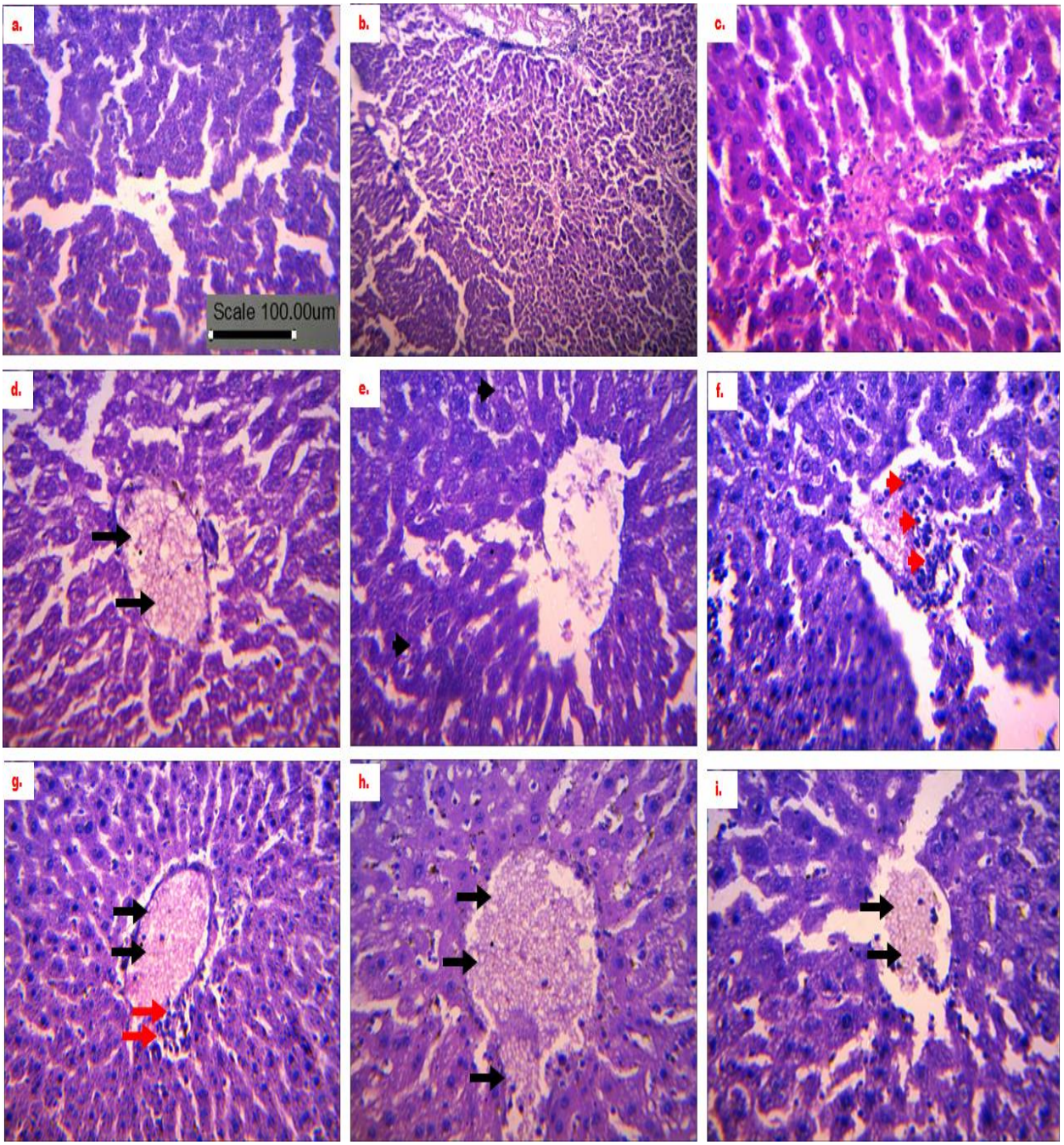


Plate 7 (a-i) Photomicrographs of sections of the liver for treated/unparasitized groups on day 6. MAG. X 400. (a) DW: No visible lesions seen. (b) PY5 mg/kg: No visible lesions seen. (c) CQ25 mg/kg: No visible lesions seen. (d) CtA200 mg/kg: There is a moderate portal and central venous congestion (arrows). (e) CtA400 mg/kg: There is a mild diffuse vacuolar degeneration of hepatocytes (arrowheads). (f) CtA800 mg/kg: There is a mild portal congestion, with a mild periportal cellular infiltration (arrows). (g) CtB200 mg/kg: There is a mild to moderate portal and central venous congestion (black arrows). There is a very mild periportal cellular infiltration (red arrows). (h) CtB400 mg/kg: There is a mild to moderate portal and central venous congestion (arrows). (i) CtB800 mg/kg: There is a mild portal congestion (arrows).

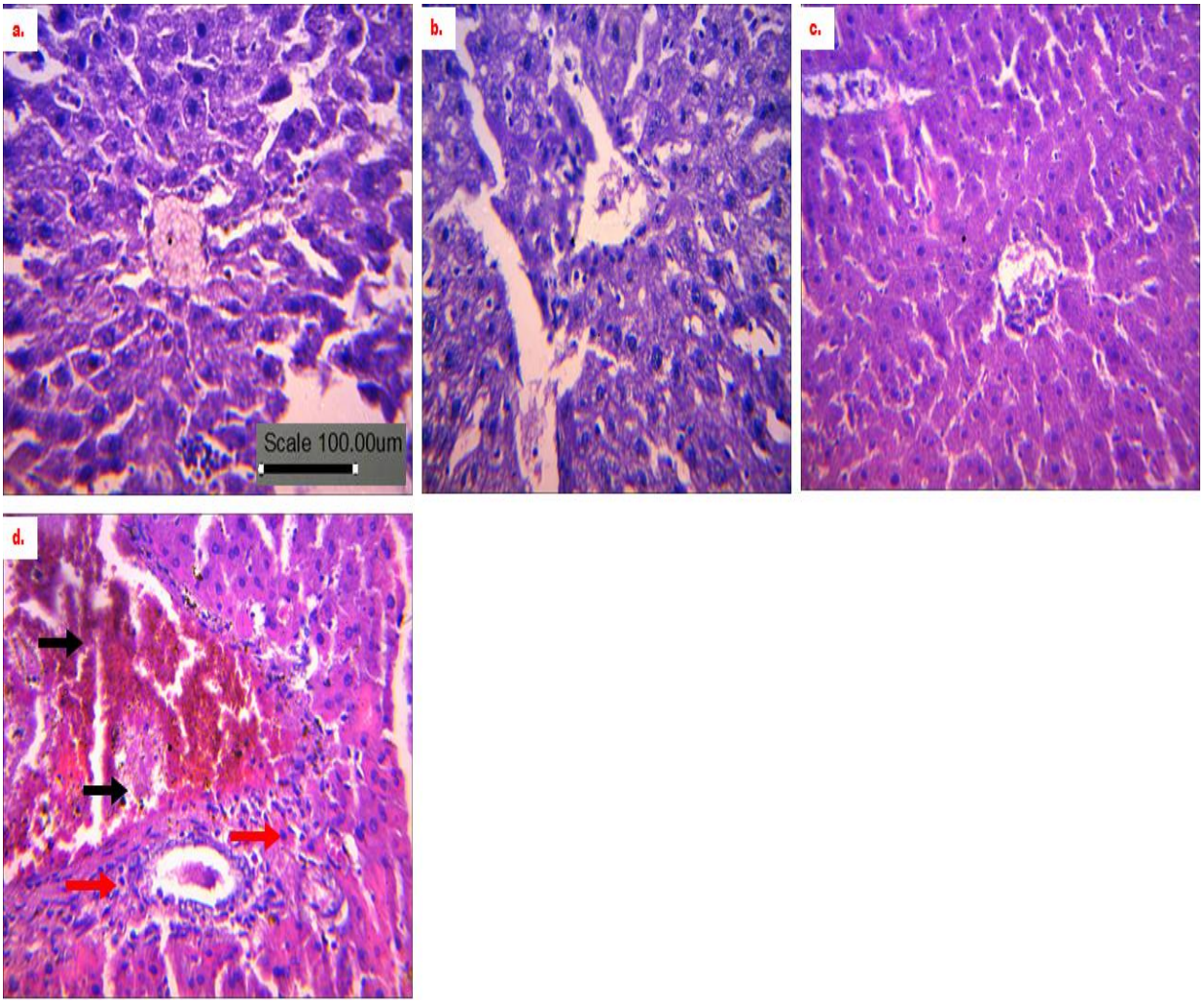


Plate 8 (a-d) Photomicrographs of sections of the liver for treated/unparasitized groups on day 25. MAG. X 400. (a) DW: No visible lesions seen. (b) PY5 mg/kg: No visible lesions seen. MAG. X 400. (c) CQ25 mg/kg: No visible lesions seen. (d) CtB800 mg/kg: There is a severe portal congestion (black arrows), with moderate periportal cellular infiltration (red arrows).

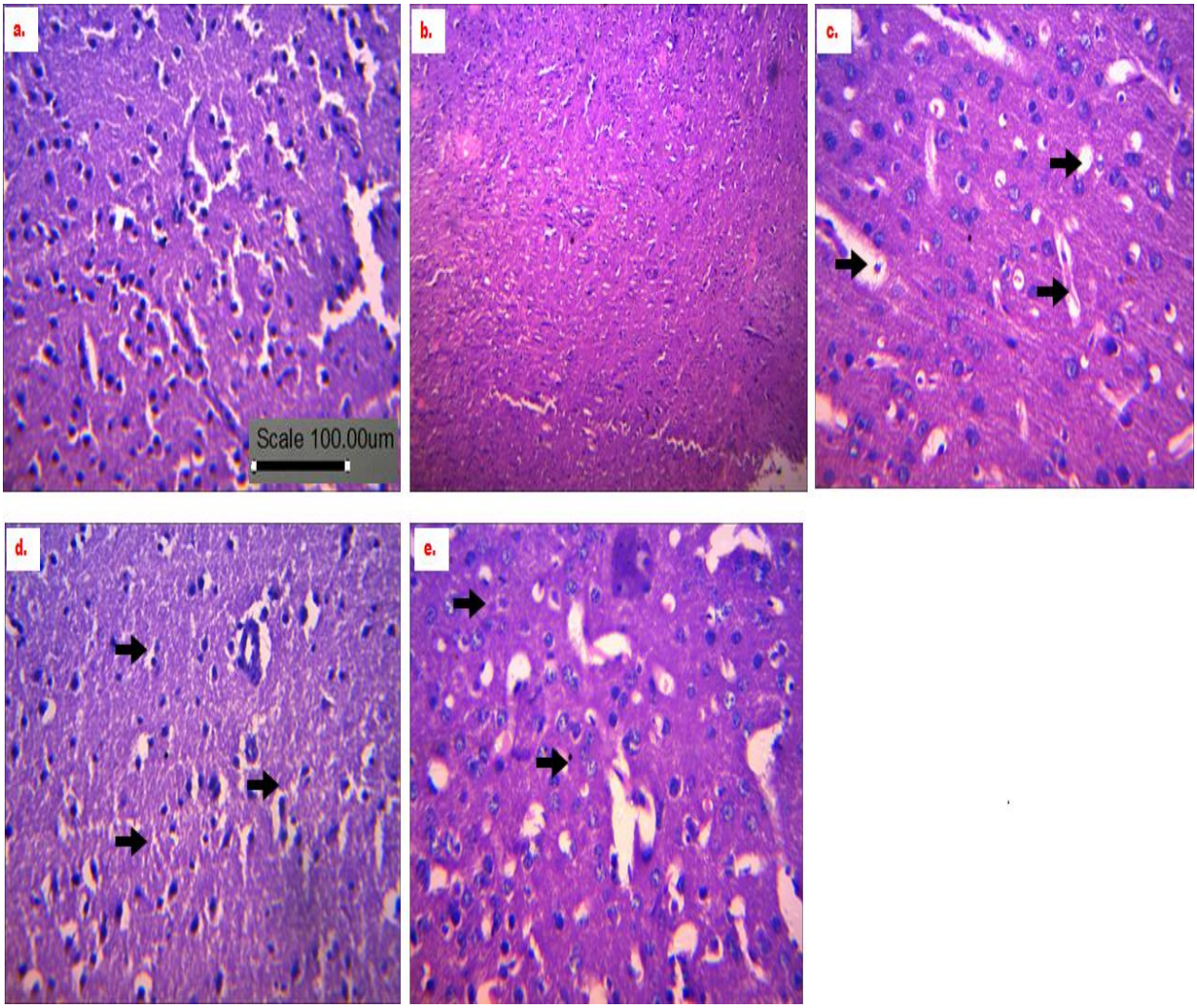


Plate 9 (a-e) Photomicrographs of sagittal sections of the brain for curative groups on day 9. (MAG. X 400). (a) DW: No visible lesions seen. (b) CQ25 mg/kg: No visible lesions seen. (c) CtA400 mg/kg: There is an increased capillary density on the cortical parenchyma (arrows). (d) CtB400 mg/kg: There is a mild diffuse spongiosis (arrows). (e) CtB800 mg/kg: Many of the neuronal cells have open faced nuclei (reactive neurons) (arrows).

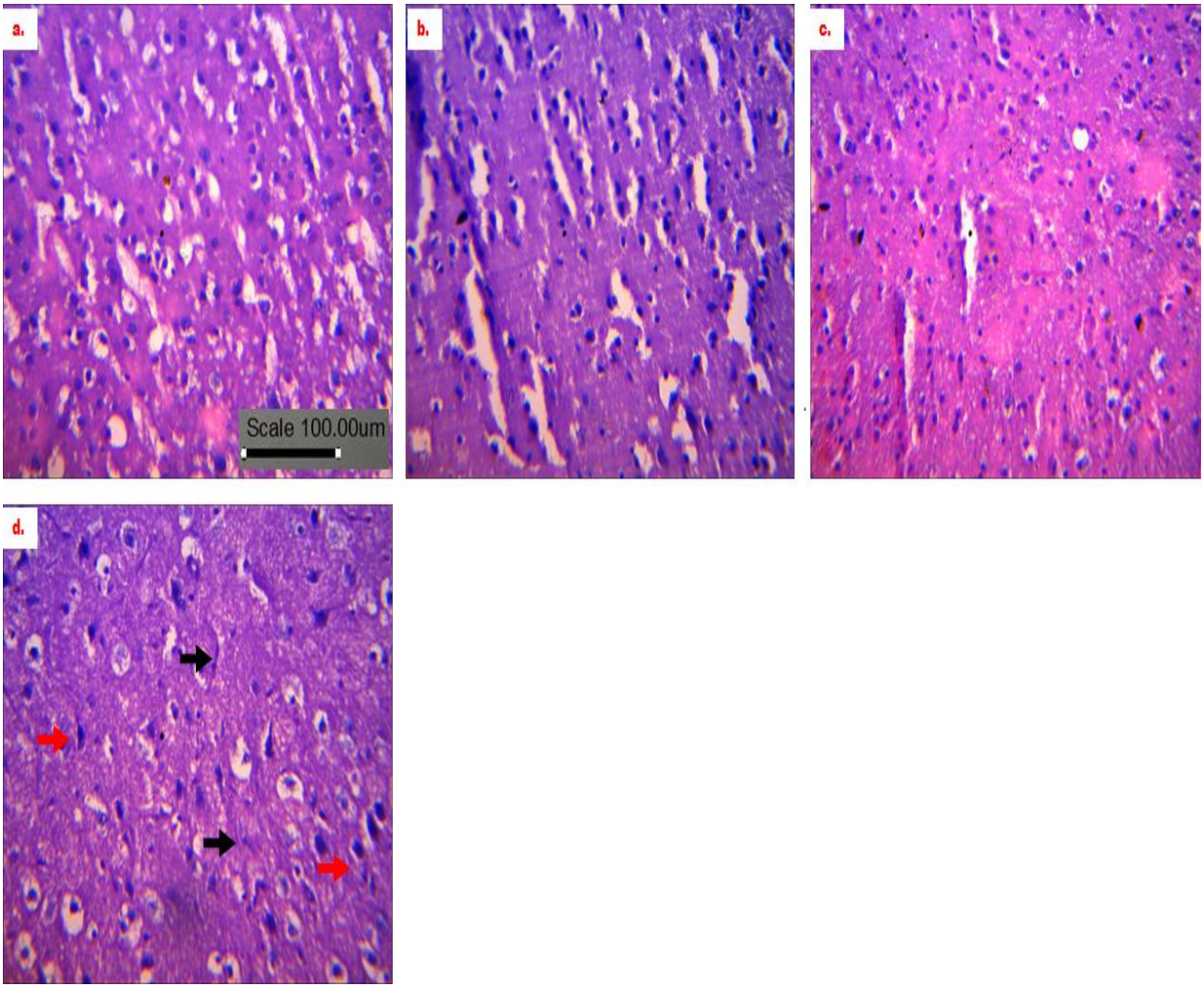


Plate 10 (a-d) Photomicrographs of sagittal sections of the brain for treated/unparasitized groups on day 25. MAG. X 400. (a) DW: No visible lesions seen. (b) PY5 mg/kg: No visible lesions seen. (c) CQ25 mg/kg: No visible lesions seen. (d) CtB800 mg/kg: There is a mild diffuse spongiosis (black arrows) and gliosis (red arrows) of the parenchyma.

Table 8 Histopathological changes in the organs of treated animals

Tests Treatments mg/kg		Degree of histopathological changes (Curative Day 9, Suppressive Day 8, Prophylactic Day 12, Treated/Unparasitized Day 6)			Degree of histopathological changes on Day 25		
		LIVER	KIDNEY	BRAIN	LIVER	KIDNEY	BRAIN
Curative	DW	-	-	-	-	-	-
	CQ	-	-		-	-	
	CtA200	-	-	-	ND	ND	-
	CtA400	++	-		ND	ND	
	CtA800	++	+++	-	ND	ND	ND
	CtB200	+++	-		ND	ND	
	CtB400	+++	-	++	-	-	ND
	CtB800	+	-		-	+++	
Suppressive	DW	-	-	-	-	-	ND
	CQ	+++	-		-	-	
	CtA200	-	-	-	-		ND
	CtA400	-	-		ND	ND	

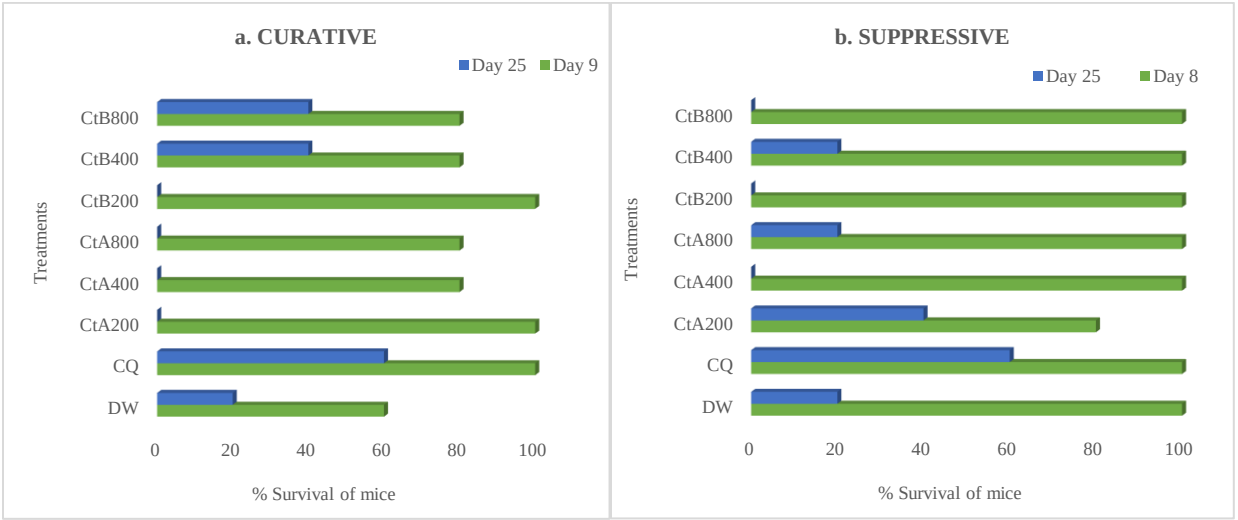
	CtA800	-	-	++		-	-
	CtB200	++	-		ND	ND	
	CtB400	++	-	++++		-	-
	CtB800	+	-		ND	ND	
Prophylactic	DW	-	-	-	-	-	-
	PY	-	-		+++	-	
	CtA200	-	-	-	ND	ND	-
	CtA400	-	-		++++	-	
	CtA800	-	-	-	++++	-	-
	CtB200	-	-		ND	ND	
	CtB400	-	-	-	-		ND
	CtB800	-	-		-	++++	-
Treated/Unparasitized	DW	-	-	-	-	-	
	CQ	-	-		-	-	-
	PY	-	-	-	-	-	
	CtA200	+++	-		-	-	ND
	CtA400	++		-	-	-	
	CtA800	++	-		-	-	ND
	CtB200	+++	-	-	-	-	
	CtB400	+++	-		-	++++	-
	CtB800	++	-		++++	-	

N.B: No lesions (-), very mild (+), mild (++), moderate (+++), and severe (++++)

Abbreviations: ND (Not determined), DW (Distilled water), CQ (Chloroquine), PY (Pyrimethamine), CtA (Cocktail treatment A), CtB (Cocktail treatment B).

Percentage survival of treated mice

Figure 2 (a-d) shows a graphical representation of the percentage survival of mice after treatment. The results showed that the survival rate was reduced in the antimalarial curative, suppressive and prophylactic studies, especially in the prolonged exposures. Overall, survival rate reached 0 % in some of the CtA and CtB treated groups in the sub-chronic exposure studies, and 20 % in the parasitized groups administered distilled water. In the treated/unparasitized groups, survival rate of the animals post administration was 100 % in all the control and treated groups.



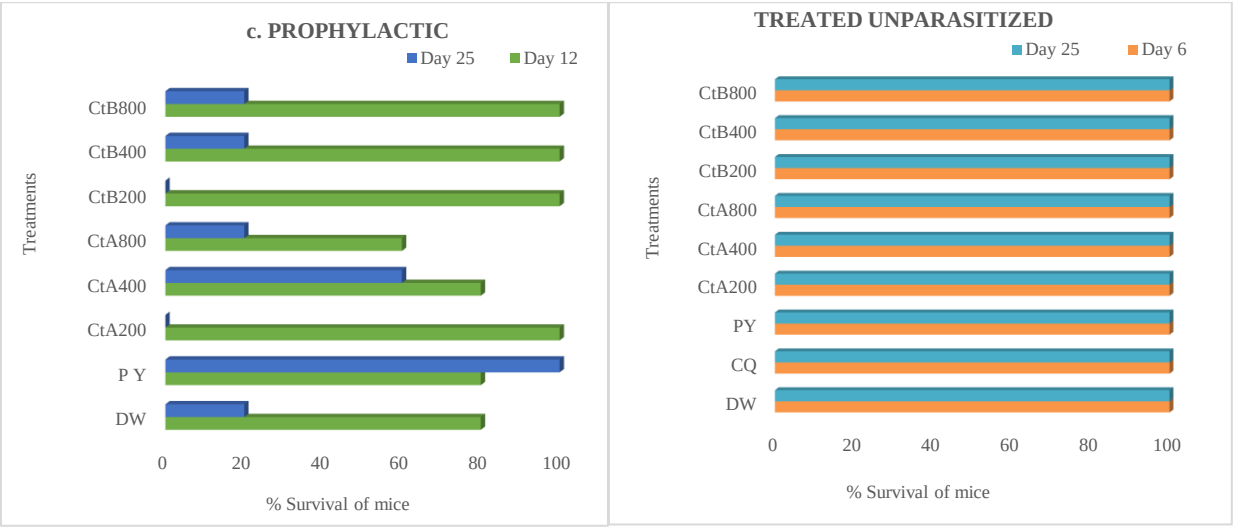


Figure 2 (a-d) Percentage survival of mice post treatment with CtA and CtB

4. DISCUSSION

The antiplasmodial activity of CtA and CtB plant cocktails Omagha et al., (2021a) could be attributed to the presence of some of the phytochemicals present in the plants used in their combinations (Omagha et al., 2020). However, some scientific evidences available from toxicological studies have reported some phytochemicals to be potentially toxic thus affecting their safe use (Mensah et al., 2019; Bode and Dong, 2015). Pharmacological screening in this study provided scientific evidences that implicated CtA and CtB as toxic antimalarials as locally prepared and used. However, findings also showed that the abnormalities observed may be transient and not true toxicities.

The findings will therefore benefit: (1) The scientific community and researchers conducting and disseminating findings from ethnopharmacological studies; (2) Pharmaceutical and drug manufacturing companies who may use scientific evidences herein as a springboard for new phytotherapies to treat malaria; (3) Regulatory and monitoring agencies taking steps to protect the health of consumers by ensuring acceptable standards of quality, safety, efficacy; (4) Individuals seeking information on herbal antimalarial treatment options; and (5) Traditional medicine practitioners who prepare and prescribe medicinal plants to locally meet their demands of malaria treatments.

Haematological indices including WBC, RBC, PCV and Hb levels are common biomarkers of malarial infection and frequently monitored as indicators of drug efficacy against plasmodial infection. Ideal plant remedies are expected to prevent malaria-associated decrease in haematological parameters reported to be associated with rise in parasitemia Langhorne et al., (2002), Kitua et al., (1997) and anemia (Alimba et al., 2016; Onyeyilli et al., 1998). In post antimalarial evaluation of Cocktail treatment, A and B, a reduction was observed in haematological parameters assessed immediately after treatments. However, levels were increased in the groups observed on day 25. The findings suggest that CtA and CtB caused anaemia and immunological defects, which resolved shortly after treatment was stopped. This indicates that the haematological abnormalities were transient, translating a short-term physiological adaptation to antimalarial administration of CtA and CtB rather than true toxicity.

This observation is in line with findings from a study carried out by Moronkeji et al., (2019) which reported that rats administered polyherbal cocktail comprising *Mangifera indica* + *Carica papaya* + *Citrus limon* recovered from anemia with a significant increase in their haematocrit values. The liver is the organ of metabolism and it is responsible for degrading majority of ingested drugs. The impairment of this very important organ in the body needs to be detected early and managed properly. It is therefore important to explore the possible deleterious effects of herbal remedies on the liver. In post antimalarial treatment in this study, findings from liver function tests implicated CtA and CtB as phytotherapies that induced significant changes in liver enzymes aspartate aminotransferase, alanine transaminases and alkaline phosphatase.

Although levels were significantly reduced in most of the treated groups, increases observed in some groups that received higher doses suggest liver disorders, including hepatocellular damage due to high level of transaminase and cholestasis caused by increased level of alkaline phosphatase associated with treatments (Onyesom and Onyemakonor, 2011). In malaria infection, the increase in these enzymes which are necessary for normal cellular functions Giannini et al., (2005) could be as a result of their leakage from the liver into the general circulation as a result of damage to liver cells during the pre-erythrocytic stage of the life cycle of malaria parasite. This has been traced to severity of the disease (Ogbadoyi and Tsado, 2009). Increased activities of these

enzymes have previously been reported to be indicative of damage to organs such as liver, kidney and heart (Adebayo et al., 2018; Viriyavejakul et al., 2014).

The pathophysiology of malaria involves oxidative stress arising from two main sources. The first has to do with the high metabolic rate of the rapidly growing and multiplying parasite within the erythrocyte. The second concerns the host's immune response to the infection (Postma and Jakobsen, 1996). Plasmodia digest hemoglobin which results in the production of heme. Heme triggers the production of reactive oxygen species which are implicated in the pathophysiology of malaria (Sies, 1991). In this study, concentrations of SOD, CAT and MDA were significantly lowered in the curative and suppressive groups. However, increased levels in the prophylactic and treated/unparasitized groups indicate cocktail extracts induced oxidative stress on the host which could result in the development of anemia Kremsner et al., (2004) and apoptosis (Guha et al., 2006).

High enzyme activities after the acute exposure in this study were observed to be returning to normal levels within weeks of treatments. This suggests the role of CtA and CtB as source of antioxidants as levels decreased in an attempt to counteract the oxidant stress induced in host cells to abate the infection (Sohail et al., 2007). The transient toxicities observed in this study is in line with previous reports that antioxidant enzyme activities may be altered after an acute exposure and then return to normal levels after the initial stress has stabilized (Almroth et al., 2008). Body weight loss prevention is also another parameter to confirm the antimalarial activity of new drugs. A potent antimalarial is expected to prevent body weight loss in malaria infected mice.

In this study, the significantly reduced body weight observed in the groups treated with the cocktail extracts is not in line with the increases observed in the CQ, PY groups and in the treated/unparasitized groups, suggesting that the cocktail extracts did not prevent weight loss following antimalarial exposures with CtA and CtB. This observation is in line with reports that body weight loss is a characteristic of *Plasmodium berghei* infected mice resulting from appetite loss, metabolic disturbance, and hypoglycemic effect of the parasite (Deharo et al., 2001). Malaria infection has been reported to induce acute injuries to vital organs, the most pronounced being on the spleen, liver and kidney of the infected host (Vineet and Upma, 2014). Therefore, this study also reports the histopathology of heart, spleen, liver, kidney and brain post treatment. The findings provide information on the type of lesions resulting from antimalarial administration of these plant-based cocktail extracts in mice.

Overall, histopathological investigation showed that though the heart and spleen morphologies were normal in all the treatments, pathological damage was seen in the kidney, liver and brain. This indicates that CtA and CtB exerted multiple organ toxicities which were mostly observed in groups administered higher doses of the treatments. In line with Viriyavejakul et al., (2014), the host liver was the primary organ affected by treatment. Antimalarial treatment with CtA and CtB in this study recorded remarkable lesions in the liver at all dose levels. Previous studies have reported hepatotoxicity posed by plant-based cocktail remedies Okpe et al., (2016) in malaria chemotherapy. According to Anyasor et al., (2011), the invasion and development of the malaria parasite in the liver during the life cycle may be responsible for liver dysfunction caused by organ congestion, cellular inflammation, and sinusoidal blockage. The kidney is a major excretory and osmoregulatory organ in mammals. This therefore makes it a target for toxic chemicals by concentrating xenobiotics and their metabolites to high levels resulting in immediate organ failure or delayed malfunctioning (Lash, 1994).

Noteworthy lesions induced in the kidney post antimalarial therapy with CtA and CtB in this study suggest kidney dysfunction. This was mostly expressed in the highest-dose groups. The findings agree with previous studies that demonstrated plant-based treatment-induced nephrotoxicity (Adebiyi and Abatan, 2013). Furthermore, increased capillary density on the cortical parenchyma, diffused spongiosis and gliosis of the parenchyma seen at the different dose levels post treatment suggest CtA and CtB caused treatment-induced brain injury indicating possible neurotoxicity. Malaria complications can develop rapidly and progress to death within hours or days (World Health Organization, 2000). Antimalarial treatments seek to improve survival rate to hundred percent. The treatment with CtA and CtB carried out in this study reduced survival rate of the animals in all three antimalarial test groups, especially in the prolonged exposures. Four groups in the curative, 3 groups in the suppressive, and 2 groups in the prophylactic models administered the cocktail extracts did not survive the course of the of the 25 days exposure studies.

This suggests that the plant extracts may not have completely cleared all parasitemia from the mice. It also suggests that there may be high toxicity-related adverse effects. Overall, though animals treated with the reference drugs CQ and PY survived better than the cocktail extracts treated groups, administration of CtA and CtB as antimalarials in this study showed that survival time is greater than that of control. Therefore, in line with Mengiste et al., (2014), the findings reveal CtA and CtB as active antimalarial agents. The 100 % survival rate in the treated/unparasitized groups post administration showed that CtA and CtB herbal recipies are not lethal to the animals at the doses administered.

The mortalities observed in the antimalarial test groups are likely due to the effect of the parasite. This is in line with Adeyemo-Salami et al., (2014) who also noted that mortality in antimalarial treatment with plant extracts are likely as a result of parasite-related effects on the host. These findings highlight the importance for further studies on mechanisms demonstrating pathways of these treatments to understand these toxicities. Important as well, further studies would help develop appropriate countermeasures to protect and better serve the growing population depending on these antimalarial remedies.

5. CONCLUSION

Safety investigations of polyherbal antimalarials are necessitated by rise in adverse effects associated with herbal remedies. Pharmacological screening showed that treatment with *Enantia chlorantha* + *Cymbopogon citratus* + *Curcuma longa* (CtA), and *Enantia chlorantha* + *Alstonia boonei* + *Carica papaya* + *Magnifera indica* (CtB) at high concentrations induced clinico-biochemical and pathological changes that could be transient. This has been demonstrated in the haematological and some biochemical parameters that resolved within weeks post treatment. Considering that the toxicities were mostly transient, and were similar to those associated with conventional antimalarials, cautious use of CtA and CtB is therefore safe and recommended for malaria treatment. The findings also underscore the need to move them up the development process for their standardization for optimal use towards achieving Sustainable Development Goal 3.b which targets an end to malaria epidemic by 2030. To protect and better serve public health, further scientific attention is required to identify the most effective method of preparing and using CtA and CtB against malaria, so as to: Determine their safest doses in line with WHO guidelines; identify countermeasures to associated toxicity risks; and create awareness for the best options of these antimalarial-active polyherbal remedies for their wider applicability. There is therefore an urgent need to put in place adequate strategies to address the research needs for the development of these phytotherapies revealed as antimalarial-active cocktails as effective, safe and locally available antimalarial drugs.

Ethical approval

The Ethics Committee at the Nigerian Institute of Medical Research Institutional Review Board (NIMR IRB) reviewed the use of animals in this study and granted approval (assigned number IRB/17/036). The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH publication #85-23, revised in 1985).

Author's contributions

RO conceptualized the idea, drafted the proposal. Under ETI, CGA, AOO, EOA, WAO's supervision, RO carried out the research work, wrote and coordinated editing of the manuscript. ETI, CGA, AOO, EOA, WAO provided guidiance, contacts for resources, and contributed to reviewing of the manuscript. RO edited and implemented the changes in the final draft. All authors approved the manuscript for publication.

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Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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