Toxicological effect of Artemisinin-Based Combination Therapies plus Paracetamol in malaria patients

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A B S T R A C T

Background: Following the paucity of safety reports in the use of Artemisinin-Based Combination Therapies (ACTs) plus paracetamol, the study assessed safety potential of artemether-lumefantrine (ALP), artesunate-amodiaquine (AAP), artesunate-mefloquine (AMP), artesunate-sulphadoxine-pyrimethamine (ASPP) and dihydroartemisinin-piperaquine (DHPP) combination with paracetamol in malaria patients.
Methods: ACTs and paracetamol were administered concomitantly in conventional doses/regimen to randomly selected patients. Blood samples were collected from the ante-cubital vein before and after completion of therapies. Toxicity markers such as weights, glucose, lipids, renal electrolytes, liver enzymes and haematological indices were assessed using standard protocols.
Results: The total numbers of participants were 57 patients. Male to female ratio was 1:1.1. Mean body weight and ages were 59.19 ± 1.39 kg and 42.86 ± 1.32 years respectively. The mean temperatures prior to and after therapy were 37.49 ± 1.02 °C and 37.50 ± 0.17 °C respectively. Mean parasitaemia before the commencement of therapy was 6282 ± 21.01 parasites/µl. Out of thirty-seven toxicological indices evaluated, twenty–four were significantly altered by ACTs plus paracetamol ($P < 0.05$). Increased serum toxicity markers due to the drug combinations were glucose (AAP, AMP), urea (ALP, ASPP), bicarbonate ion (ALP, AAP, AMP, ASPP), chloride ion (ALP, AAP, AMP), creatinine (ALP, AAP, AMP, ASPP), alkaline phosphatase (ALP, AAP), aspartate aminotransferase and alanine aminotransferase (ALP, AAP, AMP, ASPP, DHPP), total protein (AMP, DHPP) and albumin (AMP, DHPP). Decreased serum toxicity markers due to the drugs were glucose (ALP, ASPP, DHPP), urea (AMP), bicarbonate ion (DHPP), chloride ion (ASPP, DHPP), creatinine (DHPP), alkaline phosphatase (AMP, ASPP, DHPP), total protein (ALP, AAP, and ASPP) and albumin (ALP, AAP, ASPP). Altered haematological indices were white blood cells, red blood cells, mean cell haemoglobin and platelets.
Conclusion: Since ACTs plus paracetamol altered human system, discrete selection is essential in managing uncomplicated malaria most especially in patients with co-morbid conditions.

1. Introduction

Following the introduction of Artemisinin-Based Combination Therapies [1], many countries have adopted their use in the treatment of uncomplicated malaria [2–4]. Paracetamol (acetaminophen) is often added to antimalarial medications to reduce fever during malaria attacks. This agent is widely used either alone or as a combination for the treatment of mild and moderate pain [5,6]. Unlike other Non-Steroidal Anti-inflammatory Drugs, paracetamol has a weak anti-inflammatory property and its actions are due to the central nervous system activity [6]. Current evidence suggests that paracetamol may interfere with

prostaglandin synthesis [7]. There are documented toxic potential when used alone in high doses [6]. Paracetamol has been reported to be commonly used among patients in the environment [8,9]. The use of ACTs alone as new agents [1] may be tolerable but combining them with paracetamol may increase the toxic potential. Several shortcomings have been observed in the adverse event reports when ACTs were used alone in different patients [3,4,10]. In all of these past studies, paracetamol was excluded, few ACTs were studied, and limited organ/-system were explored [3,4,10] and effects of paracetamol has been studied in malaria parasitaemia [11] and there is little or no information available concerning toxicity of ACTs plus paracetamol administered

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concomitantly. The study therefore assessed the toxic effects of five commonly used ACTs plus paracetamol in patients that had uncomplicated malaria with special focus on major organs such as liver, kidney, blood elements and electrolytes.

2. Materials and methods

The study was carried out in the University of Benin Teaching Hospital, Benin City, Nigeria which is a tertiary health institution located in the South-South region with a population of about 1,676,000 individuals. Prior to the study, ethical approval (*EC/FP/015/05*) was obtained from the ethics committee of the institution. The commonly used branded ACTs and paracetamol were purchased directly from reputable pharmacy shops that registered with the Ministry of Health, Benin City, Nigeria. Sample size of the recruited patients was got from standard schedule as specified [12,13,2]. Patients that met the inclusion criteria such as those above 18 years of age with *Plasmodium falciparum* parasite count of between 1000 and 10,000 parasites/µl of blood, axillary temperature of $\geq 37.5\ ^{\circ}C$, those that had not taken anti-malarials, paracetamol, herbal or related supplement for treatment, no history of hypersensitivity to ACTs and paracetamol, absence of symptoms of complicated malaria as described [14,3,4]. The patients were systematically randomized by selecting an average sample size of 600 patients that reported with uncomplicated malaria per month as reported previously [2,12]. This was divided by 40 which represented an average population size that reported with adverse drug reaction in the use of antimalarials [13,2]. This resulted in a sampling factor of 15. That is every fifteenth patient was selected. This resulted in a total of 70 patients. Out of the 70 patients recruited, 50 filled the consent document. Every ten recruited patients were allotted to each group as ALP, AAP, AMP, ASPP and DHPP. That is every ten patients per group (n = 10). The ACTs and paracetamol were given freely to all the participants to be administered orally according to the three days (Days 0, 1 and 2) recommended dosing regimen [1,3,4,6]. That is ACTs were administered twice daily (Every 12 h for 3 days according to [1] regimen) and paracetamol was administered three times daily (Every 8 h for 3 days according to [5,6]).

Insecticide Treated Nets (ITNs) were also given freely to participants as incentives before and after the completion of therapy. About 5–7 milliliters of blood were collected from the ante-cubital vein of each patient. Blood samples collected in Day 0 before commencement of therapy were for the control group. Other test group samples were collected from the patients that used ACTs and paracetamol concurrently after completion of therapy in day 3. All the blood sample collection resulted in six groups; meaning the pre-drug administration samples (control) in Day 0 and post-drug administration samples for the test groups in Day 3 as ALP, AAP, AMP, ASPP and DHPP). Collected blood samples were withdrawn into plain and lithium heparin bottles for biochemical and haematological assays respectively. These samples were assayed for common toxicity markers according to standard specifications [15]. Samples collected in plain bottles were allowed to clot at room temperature prior to centrifugation using the Hettich centrifuge (Rototix 32A, Germany) at 4000 rpm for 10 min. The sera samples were withdrawn carefully into plain containers using sterile syringes. Standard diagnostic kits specified in Randox 2015 www.randox.com were used. Automated Clinical System analyzer (VIS-7220 G, Biotech Engineering Management Company Limited, UK; ISE 4000 SFRI, France) was used to assay for the essential biochemical parameters as: Pancreatic index (serum Glucose), Renal indices (creatinine, sodium ion, potassium ion, urea, bicarbonate), Liver indices (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, conjugated bilirubin, total bilirubin, albumin and total protein) and serum lipids (Total cholesterol, low-density lipoprotein, high-density lipoprotein and total triglycerides). Heamatological indices were assayed using the automated haematological multichannel analyzer (*ERMA PCE 210*, Japan)

3. Data analysis

The data were analyzed using GraphPrism Version 6, San Diego, USA. They were computed as mean variants. Inferential statistics were applied using Analysis of Variance, Tukey's and Fisher's *post hoc* tests. *P*-values equal to or less than 0.05 was regarded as significant.
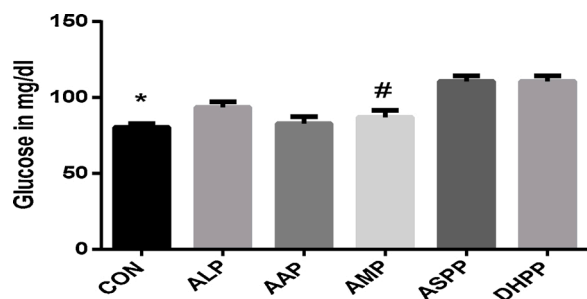
4. Results

Table 1 showed the baseline parameters of participants that met the inclusion criteria. The total numbers of participants were 57. Male to female ratio 1:1.1. Mean body weight and ages were 59.19 ± 1.39 kg and 42.86 ± 1.32 years respectively. The mean temperatures were $37.49 \pm 1.02\ ^{\circ}C$, and $37.50 \pm 0.17\ ^{\circ}C$ in pre and post-drug administration respectively. Parasite counts were 6282 ± 21.01 parasites/µl. Out of the thirty-seven toxicological indices evaluated, twenty–four were significantly altered by ACTs plus paracetamol ($P < 0.05$) compared with control. Body weights and serum lipids did not change significantly. AAP and AMP significantly increased serum glucose but ALP, ASPP and DHPP decreased serum glucose in Fig. 1. AMP decreased serum urea significantly but increased this due to ALP and ASPP as in Table 3. Serum bicarbonate decreased significantly due to DHPP but increased due to ALP, AAP, AMP and ASPP. Serum chloride ion decreased significantly due to ASPP and DHPP but increased due to ALP, AAP and AMP as in Table 3. Creatinine decreased significantly due to DHPP but increased due to ALP, AAP, AMP and ASPP as seen in Table 3. Alkaline phosphatase increased significantly due to ALP and AAP but decreased due to AMP, ASPP and DHPP. Aspartate aminotransferase and alanine aminotransferase increased significantly due to all the ACTs plus paracetamol combinations. Total protein was significantly increased due to AMP, DHPP but decreased due to ALP, AAP, and ASPP. Albumin decreased significantly due to ALP, AAP and ASPP but increased due to AMP and DHPP as in Table 4. Table 5 showed alteration in haematological indices as follows: White blood cells were significantly decreased due to all the ACTs plus paracetamol combinations. All the combinations significantly decreased lymphocytes except AAP. The percentage of lymphocytes decreased due to ALP, AMP, ASPP and DHPP but increased due to AAP. Monocytes increased significantly due to AAP and DHPP, but decreased due to ALP, AMP and ASPP. Percentage monocytes increased significantly due to AAP, AMP, ASPP and DHPP but decreased due to ALP. Percentage granulocytes decreased due to AAP and DHPP but increased due to ALP, AMP, ASPP. Red blood cells increased significantly due to ALP, AAP AMP and DHPP but decreased due to ASPP. Mean cell haemoglobin decreased due to ALP but increased due to DHPP. Mean cell haemoglobin concentration increased due to DHPP but decreased due to ALP and ASPP. Platelet increased due to ALP but decreased due to AAP, AMP, ASPP and DHPP. Platelet concentration transmittance increased due to ALP but decreased due to AAP, AMP, ASPP and DHPP. Mean platelet volume decreased due to ALP, AMP, DHPP but increased due to AAP and ASPP. Platelet diameter weight increased due to AAP and ASPP but decreased due to ALP, AMP and DHPP. None of the participants reported with severe adverse effect during and after therapy. All the patients tolerated the doses except for in combinations such as AAP, AMP, ASPP, DHPP. The common adverse

Table 1
Baseline parameters.

Sex	Male: 27, Female: 30. Total participants 57
Ages	42.86 ± 1.32 years (Mean ± SD)
Weight	59.19 ± 1.39 kg (Mean ± SD)
Blood Pressure	Systolic 119.8 ± 1.95 mmHg (Mean ± SD)
	Diastolic 79.45 ± 1.10 mmHg (Mean ± SD)
Parasitaemia	6282 ± 21.10 parasite/µl (Mean ± SEM)
Temperature	Pre: 37.49 ± 0.12 °C (Mean ± SD), Post: 37.50 ± 0.17 °C (Mean ± SD)

Baseline parameters of participants.

Fig. 1. Effects of ACTs and Paracetamol on Glucose profile of participants. Results are written in mean ± SEM of two replicates. Bars with * are significantly (p < 0.05) different relative to ALP, AAP, DHPP, while bars with # are significantly (p < 0.05) different relative to ALP, ASPP.

effects presented as intolerable were restlessness, dizziness, and body weakness as also reported in [4]. These categories of patients were counseled and the intolerabilities resolved at the completion of therapy

5. Discussion

In this study, alteration of hematological and biochemical parameters as essential tool in assessing the toxic fate of drugs has been proven since none of the patients presented with severe adverse effects. It gives further credence to treating uncomplicated malaria [16]. The use of paracetamol may have influenced the adverse effects of ACTs as previously reported [3,4] in the various organs/systems assessed for toxicity. Literatures on the effect of paracetamol in antimalarial combination therapies are rare. Earlier studies [10,17] had showed comparison between two or more ACTs but in this study the adverse effects of the five commonly used ACTs were compared in therapeutic doses. Therefore information about the toxic potential of ACTs has been broadened in Fig. 1 and Tables 1–4. This study has also validated the safety potential in the use of paracetamol in conventional doses with ACTs in humans [1] thus obviating the limitations in animals. Since the weights were not significantly altered during the period of therapy, there is still need for caution in the use of paracetamol with ACTs in individual with slim and heavy weight values because changes in parameter may occur in repeated exposures or switching from one ACTs to another. Finding in this study seems contrary to [18] reports of increase in body weight after oral administration of artemether/lumefantrine in rats but the work of Nwanjo and Oze [19], Izunya et al. [20] agreed with our information which states that artesunate administration alone had no significant effect on body weight of guinea pigs. Although long term effect and higher doses may be hazardous as in repeated dosing in regions where there are higher frequencies of malaria [14,1]. The primary concern in this study is the possible interactions which may be antagonistic or additive or synergistic irrespective of the altered parameters. The result in the study showed that serum glucose significantly dropped in ALP, ASPP and DHPP combination as shown in Fig. 1. This suggests that combinations may be safe in hyperglycemic patients. Increasing effect was observed more with ASPP and DHPP showed that there may be

greater adverse synergy in ACTs and paracetamol combination. This also suggests that ASPP and DHPP may be avoided in diabetic patients. The reducing effect of serum glucose level due to AAP and AMP in this study is similar to earlier report when amodiaquine and sulphadoxine/pyrimethamine were used singly [21] in both cases. This effect may further potentiate hypoglycemic symptoms that may be seen in complicated malaria. There could also be variation in the alteration of the integrity of the pancreatic beta cells [6] which might lead to greater utilization of glucose. This finding shown in Fig. 1 suggests that AAP and AMP combinations can be prescribed to patients that are already diabetic. This reduction of serum glucose values as seen with some ACTs and combination with paracetamol may also resolve major microvascular complications [22–24] in existing diabetic patients. This may be achieved through mechanistic pathway that can be linked to a common upstream event and overproduction of superoxide by electron transport chain [25]. In addition, these observed ACTs and their combination with paracetamol as in Fig. 1 may also interfere with the intermediates such as fructose-6-phosphate, dihydroxyacetone phosphate identified as substrates/activators in reported pathways [26], thus leading to reduction in the expression of glucose. The activation of these pathways by the combinations can lead to the secondary production of reactive oxygen species which can result in rebound effect [26]. There is need for caution in patients with ionic derangement as seen with bicarbonate and chloride ions in Table 3. The variation could be associated with the subjective adverse effects [3,4]. Combination such as ALP, AAP, AMP and ASPP as in Table 3 can be withdrawn from patients that had cardiovascular disease being characterized by elevated creatinine as a risk factor in cardiovascular death, myocardial infarction, and stroke [27]. Significantly increased urea and creatinine levels due to some combinations can pose possess a risk to the kidneys. Some of these agents should be avoided in patients with hyper-uremia. Increased levels can further impose the generation of reactive oxygen species that can lead to further consequences. Also there can be endogenous antioxidant and reactive oxygen scavengers that have been shown to be effective in animals for protecting kidney [28], although this may be hard to translate to humans [28]. Meanwhile, malaria pathogens may have induced oxidative stress on the kidney indirectly potentiating the sum effect of some ACTs and their combination with paracetamol. This phenomenon is in line with a lot of experiments showing the increased oxidative stress and decreased antioxidant defense mechanism, antioxidant enzyme systems in kidney due to infection [29–32] can also be seen in malaria infection. Selection of malarial patients provides a clear evidence of how the adverse effects of ACTs with paracetamol would have been potentiated by malaria pathogen as suggested by earlier authors [71]. The lipids were not significantly affected as seen in Table 2; therefore the use of ACTs with paracetamol may be safe in co-existing cardiovascular diseases associated with altered serum lipids. Care should be taken in patient that may be dose sensitive; because it has been documented that lipids have altered correlation with cardiovascular diseases [33]. It has been documented that other lipid disorders such as low levels of high-density lipoprotein cholesterol (HDL-C), high triglycerides and high lipoprotein levels are also independently associated with cardiovascular morbidity and mortality [34–36]. The increase observed with ALT and AST represents toxic potential of the combination as seen in Table 4. Meanwhile these extrinsic insults may be related to individual agents in the combination. Hepatic toxicity of artesunate and dihydroartemisinin has been reported by [37]. The increased in AST and ALT due to all ACTs and paracetamol may be due to artemisinin components. The effect in the increase in AST and ALT could also be due to the potentiating effect of the non-artemisinin derivatives and paracetamol. These showed a potential risk to the liver which is similar to Aniefiok et al. [38] reporting that artemisinn only elevated the activities of serum aspartate amino transferase, alanine amino transferase and serum alkaline phosphatase significantly at four dose levels. Chronic administrations of the drugs may cause more negative changes than short duration administration as seen with the combinations. The

Table 2
Effect of ACTs plus Paracetamol on serum lipids.

ACTs + P	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
CON	181.4 ± 2.87	101.2 ± 2.25	49.60 ± 0.86	101.8 ± 3.93
ALP	174.0 ± 13.46	109.0 ± 14.60	45.20 ± 3.94	88.80 ± 9.93
AAP	178.2 ± 3.30	106.4 ± 4.03	50.40 ± 3.27	96.40 ± 8.52
AMP	174.0 ± 6.75	116.8 ± 2.23	41.40 ± 2.77	106.0 ± 6.89
ASPP	180.6 ± 8.18	103.0 ± 2.70	41.00 ± 2.49	119.8 ± 6.66
DHPP	166.8 ± 9.50	100.4 ± 6.13	44.40 ± 2.84	102.2 ± 6.40

TC: Total Cholesterol, TG: Total triglycerides, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein. n = 10. TC, TG, HDL, LDL: There was no significant difference in all the serum lipids (P > 0.05).

Table 3
Effect of ACTs plus paracetamol on renal indices.

ACTs + P	Urea (mg/dl)	Na$^+$ (mMol/L)	K$^+$ (mMol/L)	HCO$_3^-$ (mMol/L)	Cl$^-$ (mMol/L)	Creatinine(mg/dl)
CON	27.20 ± 1.37	138.8 ± 0.13	3.90 ± 0.00	22.40 ± 0.16	102.6 ± 0.16	0.68 ± 0.01
ALP	32.60 ± 0.62	138.8 ± 0.45	4.04 ± 0.07	23.20 ± 0.25	102.8 ± 0.49	0.74 ± 0.02
AAP	27.90 ± 0.81	139.4 ± 0.27	4.04 ± 0.09	22.40 ± 0.27	103.8 ± 0.74	0.76 ± 0.03
AMP	26.30 ± 1.71*	139.6 ± 0.27	4.18 ± 0.08	22.60 ± 0.58	105.2 ± 0.33*	0.72 ± 0.04
ASPP	32.50 ± 1.95	138.4 ± 0.27	4.04 ± 0.00	23.60 ± 0.58	101.8 ± 0.80	0.74 ± 0.02
DHPP	27.60 ± 1.54	138.6 ± 0.54	3.94 ± 0.16	20.40 ± 0.16*	101.4 ± 0.50#	0.60 ± 0.00*

Urea: (*$P < 0.05$) Vs ALP, ASPP. Bicarbonate: (*$P < 0.05$) Vs CON, ALP, AAP, AMP, ASPP. Chloride: (*$P < 0.05$) Vs CON, ALP, ASPP. (#$P < 0.05$) Vs ALP. Creatinine: (*$P < 0.05$) Vs CON, ALP, AAP, AMP, ASPP.

Table 4
Effects of ACTs plus paracetamol on liver indices.

ACTs + P	ALP (U/L)	AST (U/L)	ALT (U/L)	CB(mg/dl)	TP (mg/dl)	ALB (g/dl).	TB (mg/dl)
CON	172.8 ± 3.09	31.20 ± 0.49*	29.60 ± 1.02#	0.34 ± 1.01	8.24 ± 0.06	4.16 ± 0.06	0.67 ± 0.01*
ALP	214.8 ± 13.6*	56.80 ± 3.50	49.00 ± 4.47	0.36 ± 0.02	7.76 ± 0.12	4.10 ± 0.15	0.68 ± 0.01
AAP	183.0 ± 24.9	51.40 ± 4.19	35.60 ± 3.09	0.36 ± 0.03*	7.92 ± 0.03	4.02 ± 0.05	0.52 ± 0.05
AMP	153.0 ± 13.04	40.80 ± 5.31	33.60 ± 5.95	0.34 ± 0.04	8.44 ± 0.12	4.26 ± 0.09	0.52 ± 0.07
ASPP	168.0 ± 9.04	53.60 ± 6.70	41.60 ± 5.75	0.32 ± 0.03	7.54 ± 0.18#	3.64 ± 0.09*	0.60 ± 0.03
DHPP	145.8 ± 14.58	47.40 ± 1.78	54.60 ± 5.38*	0.34 ± 0.03	8.48 ± 0.21*	4.40 ± 0.13	0.62 ± 0.04

Alkaline phoshatase (ALP) (*$P < 0.05$) Vs AMP, DHPP. Aspartate aminotransferase (AST) (*$P < 0.05$) Vs ALP, AAP, ASPP, Alanine aminotransferase (ALT) (*$P < 0.05$) Vs CON, AMP. (#$P < 0.05$) Vs ALP. Conjugated bilirubin (CB) (*$P < 0.05$) Vs ASP, Total Protein (TP) (*$P < 0.05$) Vs ALP, AAP, ASPP. (#$P < 0.05$) Vs CON, AMP. Albumin (ALB): *$P < 0.05$ Vs CON, ALP, AMP, ASPP. Total Bilirubin (TB) (*$P < 0.05$) Vs ALP, AMP. n=10.

overall hepatic finding is in line with the previous reports that artemisinin caused elevation in the serum levels of hepatic enzymes [19,37–40]. Utoh-Nedusa et al. [17] had also reported that dihydroartemisinin had no effect on serum level of Alanine amino transferase, serum alkaline phosphatase and serum Aspartate amino transferase activities (ALT, ALP, AST). Transaminases are important useful markers for hepatocellular damage [41] and specifically ALT has hepatocellular damage because they are predominantly produced within the liver cells [19]. It therefore means that paracetamol should be avoided in patients with liver disease or in patient that present no pyrexia and body pain as symptoms of malaria. Meanwhile, necrosis associated with paracetamol hepatotoxicity has been a well recognized issue [6,42]. Elevations of AST and ALT should not be allowed to occur because they are reflections of liver cell damage by free radicals generated due to artemisinin derivatives. Although these free radicals are also known to be responsible for their antimalarials actions [19]. Hematological alterations that are thought to characterize malaria may be related to the overt biochemical changes caused by the drug combination. They may have been significantly affected due to the contributory effect of the parasite. Anemia may be common with DHPP and is believed to occur due to hemolysis of parasitized and non-parasitized RBCs, peripheral removal/sequestration of RBCs, and ineffective erythropoiesis due to high circulating Tissue Necrotic Factor (TNF) [43,70]. Recent evidence points to frequent mild adverse events caused by amodiaquine also the combination of amodiaquine and artesunate revealed the common adverse events of hepatotoxicity and leucopenia [44]. This had which justified some aspects of this study. People with mutant genotypes of CYP2C8, CYP1A1 and CYP1B1 have also been found to have immunogenic adverse reactions due to amodiaquine [45–47]. The reduction in some of the heametological indices as seen in the study could be due to the effect of amodiaquine and related analogues in the combination. It can be deduced that the overall haematological effects may have been influenced by paracetamol alone or in combination with ACTs as in Table 5. Slight increase in percentage of lymphocytes observed during short duration administration of the drugs may be due to infection of the cells by the malaria parasites. The use of ACTs with paracetamol as in Table 4 can interfere with cell differentiation and proliferation. These can further alter erythrocyte production, resulting in anemia leucopenia and thrombocytopenia (pancytopenia). Although other mean red blood cell indices such as Hb, HCT, MCV and

RDW [48] were not significantly altered. Therefore caution is of essence in dose sensitive patients that may report with acute uncomplicated malaria. Significant changes in differential White Blood Cell Count (lymphocytes, neutrophils, and eosinophils) count and RBC indices [48] in Table 4 could be due to the pathogen and drugs. Changes observed in the levels of platelets and monocytes may also be related to the level of pathogen. These effects could contribute to the adverse subjective response resport seen previously [4]. The artemisinins may differ in the routes of administration but they are metabolized to active metabolite dihydroartemisinin [45]. Artemisinins had caused an interruption in erythropoiesis in rats even with single or short-course exposures [49]. This major metabolite had a dose-dependent interference with hematopoiesis and angiogenesis [50]. It can be deduced from this study that artemisinin derivatives such as artemether, artesunate may have interfered with haematological indices due to this common metabolite. Nosten and White [51] had also reported that artemisinin and its derivatives on their own has low toxicological effects and that any toxicity observed in artemisinin combination treatment may be due to the partner agents such as amodiaquine, lumefantrine, mefloquine, and piperaquine. Amodiaqune may induce leukopenia or agranulocytosis when used repeatedly or prophylactically [52]. It can be observed from this study that amodiaquine may have potentiated the effect observed in artesunate-amodiaquine combination with paracetamol. Inhibition of erythropoiesis may have contributed to reduction in RBC as seen with ASPP. It could be that either drug in the combination existed as a higher dose or the acted synergistically thus resulting in a drop in RBC values. It has been estimated that acute uncomplicated *P. falciparum* malaria [53,54] induces a slight net increase in total leukocyte counts resulting from a large increase in neutrophils and a proportionally smaller decrease in lymphocytes as seen in the combinations. Platelet counts were decreased by DHPP could be linked to parasitemia and leukocyte counts as reported by [53,54]. In a related report, leukocyte counts in persons with *P. falciparum* or *P. vivax* malaria and uninfected persons (essentially adults from Myanmar and Peru) showed that leukocyte counts in persons with *P. falciparum* malaria were greater than those in persons with *P. vivax* malaria and healthy subjects [55]. Although no difference was detected in leukocyte and neutrophil counts between persons with *P. vivax* or those with *P. falciparum* malaria [56]. Malaria is the most common cause of severe anemia in endemic areas [70]. It may also worsen due to hemolysis of parasitized and nonparasitized RBCs,

Table 5
Effects of ACTs plus paracetamol on haematological indices.

ACTs + P	WBC	LY	MO	GR	LYY	MOO	GRR	RBC	MCH	MCHC	PLT	PCT	MPV	PDW
CON	4.92 ± 0.09	1.28 ± 0.03	0.46 ± 0.02	3.26 ± 0.10	26.20 ± 0.57	10.02 ± 0.15	64.14 ± 1.00	4.62 ± 0.09	30.88 ± 0.13	34.10 ± 0.17	226.6 ± 9.21	0.18 ± 0.00	8.32 ± 0.09	11.18 ± 0.60
ALP	4.76 ± 0.21	0.78 ± 0.11#	0.42 ± 0.01	3.52 ± 0.12*	16.64 ± 1.55*	8.16 ± 0.32*	73.14 ± 1.19#	9.04 ± 0.17#	28.70 ± 0.06*	33.44 ± 0.36	267.6 ± 32.28*	0.22 ± 0.02*	7.58 ± 0.35*	10.54 ± 0.57
AAP	4.82 ± 0.40	1.46 ± 0.20	0.60 ± 0.07	2.62 ± 0.14	28.32 ± 2.18	11.74 ± 0.34	57.78 ± 2.69#	4.83 ± 0.88#	30.32 ± 1.03	34.20 ± 0.42	143.8 ± 9.41	0.13 ± 0.01	9.04 ± 0.18	12.62 ± 0.46
AMP	3.24 ± 0.09*	0.58 ± 0.05*	0.32 ± 0.01*	2.34 ± 0.05	17.00 ± 1.04#	11.24 ± 0.40	72.44 ± 0.92	8.22 ± 0.21	30.64 ± 1.59	34.58 ± 0.63	197.0 ± 3.54	0.16 ± 0.01	8.16 ± 0.53	10.66 ± 1.13
ASPP	4.12 ± 0.23	1.02 ± 0.13	0.42 ± 0.06	2.70 ± 0.23	24.80 ± 3.03	10.20 ± 0.19	65.00 ± 4.17	4.48 ± 0.16	30.14 ± 1.16	33.24 ± 0.40*	188.6 ± 12.29	0.17 ± 0.01	9.32 ± 0.16	15.02 ± 0.59*
DHPP	4.56 ± 0.59	1.16 ± 0.19	0.56 ± 0.10	2.82 ± 0.36	25.62 ± 2.16	12.08 ± 1.02	62.30 ± 2.91	7.01 ± 0.23	33.28 ± 1.16	35.16 ± 0.58	176.0 ± 38.82	0.13 ± 0.03	7.46 ± 0.58#	9.12 ± 1.43

WBC: (*$P < 0.05$) Vs ALP, AAP. LY: (*$P < 0.05$) Vs CON, AAP, DHP. GR: (*$P < 0.05$) Vs AAP DHP. MO: (*$P < 0.05$) Vs CON, AAP, AMP, ASPP, DHPP. (#$P < 0.05$) Vs CON, AAP, DHPP. MOO: (*$P < 0.05$) Vs AAP, AMP, DHPP. GRR: (*$P < 0.05$) Vs AAP, DHPP. (#$P < 0.05$) Vs AMP. RBC: (*$P < 0.05$) Vs CON, AAP, AMP, ASPP, DHPP. (#$P < 0.05$) Vs CON, ALP, AMP, ASPP, DHPP. WBC×10³µl, LY×10³µl, MO×10³µl, GR×10³µl, LYY%, MO%, GRR%, RBC×10⁶µl, MCH pg, MCHC g/dl, PLT×10³µl, PCT%, MPV fl, PDW%.

WBC: White blood cells, LY: Lymphocytes, MO: Monocytes, GR: Granulocytes, RBC: Red blood cells, MCH: Mean cell haemoglobin, MCHC: Mean cell haemoglobin concentration, PLT: Platelets, PCT: Platelet concentration transmittance, MPV: Mean platelet volume, PDW: Platelet diameter weight, volume. n = 10.

peripheralremoval/sequestration of RBCs, and ineffective erythropoiesis due to high circulating Tissue Necrotic Factor (TNF) (Abdalla and Pasvol 2004). As observed elsewhere [57], the mean red blood cell indices (Hb, MCV, MCH, MCHC and RDW) of patients with acute uncomplicated malaria were normal. This could probably have been because uncomplicated malaria is associated with milder biochemical changes which can be linked to low production of cytokines, less endothelial cell activation, milder changes in the coagulation profile, less sequestration and less hemolysis as opposed to complicated malaria. Monocytes have been reported to act against the malaria parasite through several mechanisms; phagocytosis of malaria pigment by monocytes/ macrophages, and less frequently by neutrophils, has been observed in peripheral blood cells and bone marrow of patients with malaria [58–60]. Lower Hb concentration has been associated with malaria parasitaemia in young children [61–64] that had symptomatic malaria cases and persons with patent parasitaemia [54,65] than in malaria negative subjects. Even when plasmodium infection is thought to lower erythrocyte counts and Hb levels by inducing haemolysis, decreased erythropoiesis and increased clearance of both parasitized and non-infected erythrocytes, findings from the study revealed no significant effect of the parasite density on the mean RBC count [66]. reported that human neutrophils *in vitro* are capable of extracting *P. falciparum* parasites from RBCs, leaving the RBCs intact and the removal of intra-erythrocytic parasites occurs naturally *in vivo* when the host immune system can act. Findings from the study revealed that thrombocytopaenia with malaria parasitaemia and a significant relationship exists between the platelet counts and the WBC counts [67]. Slight increase in lymphocyte count observed during short duration administration of AAP may be influenced by the presence of malaria parasite in the blood stream. Ovuakporage [68] had reported that malaria parasite could reduce Red blood cell count, Packed Cell Volume (PCV) and Haemoglobin concentration (Hb). The study suggests that ALP may be used in patients that are already anaemic. The conventional doses per kilogram body weight recommended [1,69] suggest a good safety range and the challenges can easily be resolved when caution is built-in during co-morbid conditions. Since single-sample centre study and few sample size are the limitations in this study, therefore multicentre and large sample sizes are recommended to support the generalization of the findings.

6. Conclusion

ACTs and their combination with paracetamol altered organ and system indices as indicators for toxicity potential. It is therefore recommended that careful choice of ACTs and paracetamol should be adopted in the treatment of diagnosed uncomplicated malaria cases associated with co-morbidities. As a follow up, similar studies are recommended in different regions where malaria is endemic to validate the claims.

Significance

The study can help in discrete selection of the combinations in managing uncomplicated malaria in existing co-morbid conditions.

Data availability

No data was used for the research described in the article.
Data will be made available on request.

Conflict of Interest

The authors declare no conflict of interest.

Declaration of Competing Interest

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