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Comparative Effects of Two Antimalarial Drugs (P-Alaxin and Coartem) on Serum Electrolytes and Serum Enzymes in Albino Wistar Rats

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ABSTRACT

This study was set out to compare the effects of p-alaxin and that of coartem on serum electrolytes and serum enzymes. Thirty albino wistar rats weighing 200 - 220 g were randomly divided into 3 groups of 10 rats each. Group 1 served as control, group 2 animals were administered P-alaxin (16mg/kg) while group 3 animals were administered coartem (15.4mg/kg). All animals had unrestricted access to food and water throughout the duration of the experiment. Five animals were randomly selected from each group and sacrificed after 3 days of treatment with the test drugs. The remaining five animals for each group were sacrificed after 7 days of treatment with the test drugs. Na^+ was significantly higher in the coartem treated group compared to control ($P<0.01$) and P-alaxin group ($P<0.05$) after 3 days of treatment. After 7 days of treatment, Na^+ concentration significantly increased in the coartem treated group ($P<0.001$) and P-alaxin treated group ($P<0.01$) compared to control. K^+ significantly reduced in the P-alaxin ($P<0.01$) and coartem treated group ($P<0.05$) compared to control after 7 days of treatment; it also significantly increased ($P<0.001$) in the coartem group compared to p-alaxin group. Cl^- significantly ($P<0.05$) increased in P-alaxin group compared to control. After 3 days of treatment, HCO_3^- significantly ($P<0.05$) increased in P-alaxin and coartem treated group compared to control. It reduced significantly ($P<0.05$) in the coartem treated group compared to control after 7 days of treatment. Alkaline phosphatase was the only affected serum enzyme in this study. Coartem significantly ($P<0.01$) reduced alkaline phosphatase levels when administered for 7 days. We therefore conclude that both antimalarial drugs did alter serum electrolyte levels, with coartem doing most of the alterations, hence the need for caution and proper attention while undergoing malaria treatment.

Keywords: coartem, p-alaxin, serum electrolytes, serum enzymes

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INTRODUCTION

Malaria is a life threatening disease, with nearly half of the world's population being vulnerable to this infection [1]. Malaria has been reportedly linked to the death of an estimated 2-3 million people annually [2]. Incidence of malaria varies by weather, which determines whether the main carrier of malaria parasites (anopheles mosquitoes) survives or not. Tropical areas including Nigeria, have a great combination of adequate rainfall, temperature and humidity, which allows for breeding and survival of anopheles mosquitoes. Four species of *Plasmodium* exist. Of the four species of *Plasmodium* that infect humans - *P. falciparum, P. vivax, P. malariae* and *P. oval*, *Plasmodium falciparum* causes most of the severity and deaths attributable to malaria [2].

Coartem (artemether-lumefantrine; 20/120 mg per tablet) and p-alaxin (dihydroartemisinin-piperaquine; 40/320 mg per tablet) tablets are indicated for treatment of acute, uncomplicated malaria infections due to *Plasmodium falciparum* in patients $\geq$ 5 kg bodyweight [3,4]. Coartem tablets have been shown to be effective in geographical regions where resistance to chloroquine has been reported [5]. Both coartem and p-alaxin belong to the artemisinin-based combination therapy (ACT). Artemisinin and its derivatives are rapidly eliminated and have been shown to produce rapid clearance of parasitaemia and rapid resolution of symptoms [6]. When given in combination with a rapidly eliminated compound like clindamycin, a 7-day course of treatment with an artemisinin compound is required; but when given in combination with slowly eliminated antimalarials, shorter courses of treatment (3 days) are effective [3,4]. Electrolytes play an important role in many body processes, some of which are; acid-base balance (pH), nerve conduction, muscle contraction and blood clotting [7]. Electrolyte balance is especially disturbed between sodium and potassium [8]. Electrolyte imbalance may result from kidney failure, dehydration, fever and vomiting. Electrolyte imbalance may also result from intake of some drugs. In this case, the imbalance becomes a side effect of the ingested drug [7].

Nosten and White [9], had reported that if there is any toxicity observed in artemisinin combination treatments, it may be due to the non-artemisinin component as artemisinin derivatives alone may have relatively low toxicological effects. However, animal studies had shown that artemisinin and its analogues cause acute hepatotoxicity in guinea-pigs [10] and have adverse effects on biochemical parameters in rabbits [11]. Olayinka and Ore [12] also observed that therapeutic and double therapeutic doses of p-alaxin on rats induced marked renal and hepatic failure and decreased the levels of antioxidant systems. In view of the above reports, this study was therefore carried out to a ascertain and compare the effects of p-alaxin and coartem on serum electrolytes and enzymes with a view to better inform the medics of their possible alteration of these indices.

MATERIAL AND METHODS

Drug Preparation

The antimalarial drugs used for this study was p-alaxin (Bliss Gvs Pharma Limited, India) and coartem (Novartis Pharmaceuticals Corporation, Basel, Switzerland). The drugs used for this study were purchased from the University of Calabar Pharmacy. One tablet of

coartem was dissolved in 10ml of distil water to give a concentration of 14mg/ml while one tablet of p-alaxin was dissolved in 24ml of distil water to give a concentration of 15mg/ml. The drugs were dissolved at the time of administration and given to the animals immediately. The dissolved drugs were not stored.

Animal Preparation and Protocol

Thirty albino wistar rats weighing 200 - 220 g were used for this study. The animals were randomly divided into 3 groups, such that each group contained 10 animals. Group 1 served as control, group 2 served as p-alaxin treated group, while group 3 served as coartem treated group. All animals had access to food and water *ad libidum*. The animals were exposed to 12/12 light/dark cycle. Treatment of animals in the different groups commenced after 12 days of acclimatization.

Drug Administration

Drug administration began after 12 days of acclimatization. The drugs were orally administered to the test groups. The second dose for day 1 was administered 8 hours after the first dose. The drugs were subsequently administered twice daily from day 2. The dose used for this study was 16mg/kg and 15.4mg/kg, for p-alaxin and coartem respectively. Administration was facilitated by the use of a syringe and orogastric tube. The first line administration lasted for 3 days, after which 5 animals were randomly selected and sacrificed from each group, while the remaining continued to receive treatment for another 4 days. All experiments were in line with approved guidelines of the local ethics committee and were therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Sample Collection

Animals were sacrificed using chloroform as anesthesia. Blood samples were collected by cardiac puncture using a syringe and needle. Samples were collected into plain labeled sample bottles and centrifuged at 3000 rpm for 10 minutes to separate and collect serum for analysis.

Determination of Serum Electrolytes Concentration

Serum Na^+ and K^+ concentrations were determined using a flame photometer (Model 410C, Petracourt Ltd, England). Serum Cl^- concentration was determined by end point calorimetric titration following the method of Kolthoft *et al* [13]. Serum bicarbonate (HCO_3^-) concentration was measured by the modified method of Forrrester *et al* [14].

Determination of Serum Enzymes Concentration

Measurement of alkaline (ALP) phosphatase

ALP was measured by the optimized standard method recommended by the deutsche Geseiischage fur Klinishche Chemic GSCC (1972). P-nitrophenyl phosphate was

hydrolysed to phosphate and p-nitrophenol in the presence of ALP. A calculated amount of sample 0.01ml in a test tube was mixed with reagent (0.5ml) containing the substrate p-nitrophenyl phosphate and kept at room temperature. The solution was mixed, initial absorbance read after 1 minute. The reaction was allowed to stand for 3 minutes and the absorbance read again at 405nm. Alkaline phosphate activity was calculated from.

$$UL = 2760 \times \Delta A \text{ nm/minute micro}$$

Where UL = Unit of alkaline phosphatase affinity
ΔA = Change in absorbance

Measurement of aspartate (ALT) and alanine transferase (AST)

Serum AST and ALT levels were determined using endpoint colorimetric-diagnostic kit (Randox Labouratories, UK) based on Reitman and Frankel's method [15]. The pyruvate produced by transamination reaction between L-alanine and ketoglutarate reacts with 2, 4, dinitrophenyl hydrazine to give a coloured hydrazone, and was used to measure alanine aminotransferase activity. The oxaloacetate hydrazone formed with 2, 4 dinitrophenyl hydrazine was used to measure aspartate aminotransfarase (AST). Both ALT and AST were read at 540nm wavelength.

Statistical Analysis

Results are presented as mean $\pm$ standard error of mean. One way analysis of variance (ANOVA) was used to analyze results, followed by the least significant difference (LSD) procedure for significant F values. P = 0.05 was considered significant. Computer software SPSS and Excel Analyzer were used for the analysis.

RESULTS

Serum Electrolyte Concentration in the Different Experimental Groups

Serum Na^+ Concentration in the Different Experimental Groups

The mean Na^+ concentration in the control, p-alaxin treated and coartem treated group for day 3 was 168.43 $\pm$ 24.49, 224.64 $\pm$ 27.59 and 291 $\pm$ 4.29 mmol/L respectively. Na^+ concentration was significantly ($P<0.01$, $P<0.05$) higher in coartem treated group compared to control and p-alaxin treated group respectively. The mean Na^+ concentration in the control, p-alaxin treated and coartem treated group for day 7 was 146.15 $\pm$ 31.60, 263.90 $\pm$ 15.10 and 307.98 $\pm$ 22.68 mmol/L respectively. Na^+ concentration was significantly ($P<0.001$, $P<0.01$) higher in the coartem treated and p-alaxin treated group respectively compared to control. (Fig. 1).

Serum K^+ Concentration in the Different Experimental Groups

The mean K^+ concentration in the control, p-alaxin treated and coartem treated group for day 3 was 11.87 $\pm$ 0.15, 12.16 $\pm$ 0.42 and 11.51 $\pm$ 0.16 mmol/L respectively. There

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was no significant difference in K^+ concentration in day 3. After seven days, the mean K^+ concentration in the control, p-alaxin treated and coartem treated group was 14.47 ± 0.61, 11.69 ± 0.09 and 12.91 ± 0.16 mmol/L respectively. Serum concentration of K^+ was significantly (P<0.05, P<0.01) lower in coartem treated and p-alaxin treated group reaspectively, compared to control. (Fig. 2).

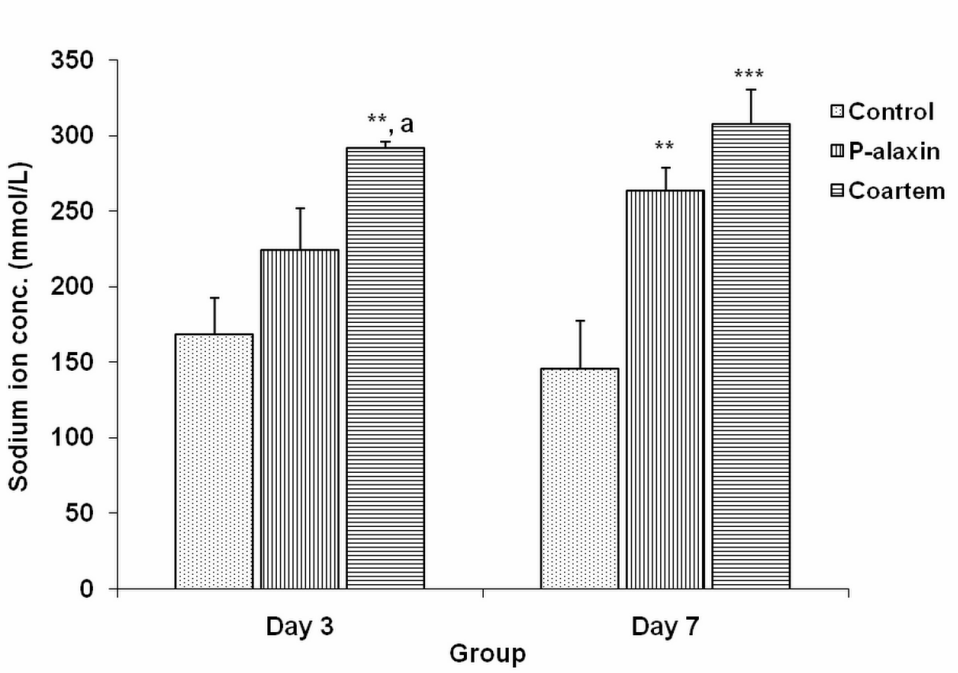

Figure 1 Comparison of sodium ion concentrations in the different experiment groups at days 3 and 7.
Values are mean ± SEM, n = 5.
****p<0.01, ***p<0.001 vs control; a = p<0.05 vs p-alaxin**

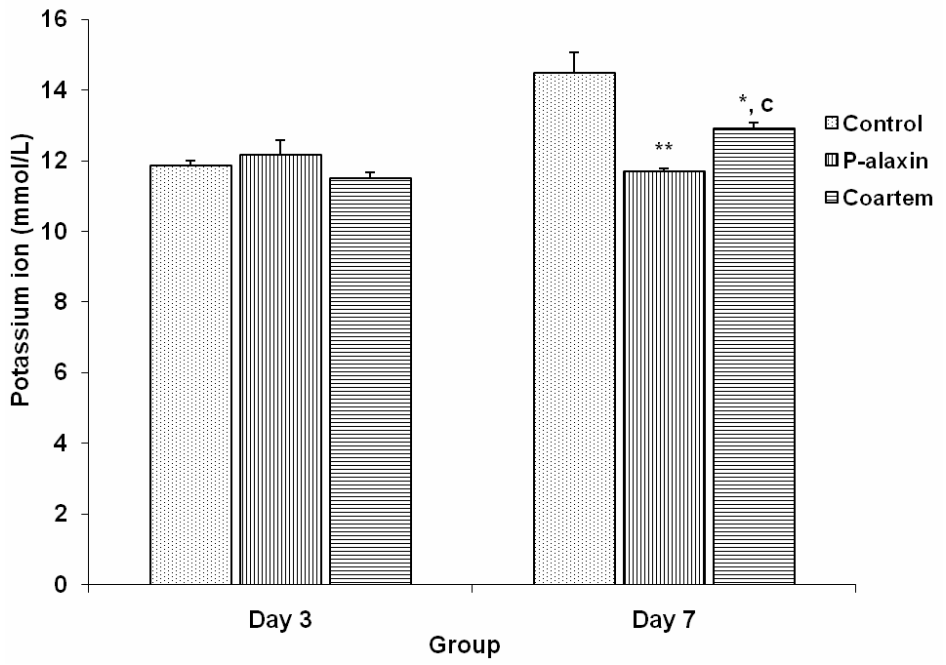

Figure 2 Comparison of potassium ion concentrations in the different experiment groups at days 3 and 7.
Values are mean ± SEM, n = 5.
***p<0.05, **p<0.01 vs control; c = p<0.001 vs p-alaxin**

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Serum Cl^- Concentration in the Different Experimental Groups

At day 3, the mean Cl^- concentration was 38.05 ± 0.51, 42.84 ± 1.11 and 42.36 ± 2.06 mmol/L for control, p-alaxin treated and coartem treated group respectively. Cl^- concentration was significantly (P<0.05) higher in the p-alaxin treated group, compared to control. At day 7, the mean Cl^- concentration was 44.62 ± 1.22, 42.48 ± 1.28 and 45.00 ± 3.66 for control, p-alaxin treated and coartem treated group respectively. There was no significant difference in Cl^- concentration on the 7th day. (Fig. 3).

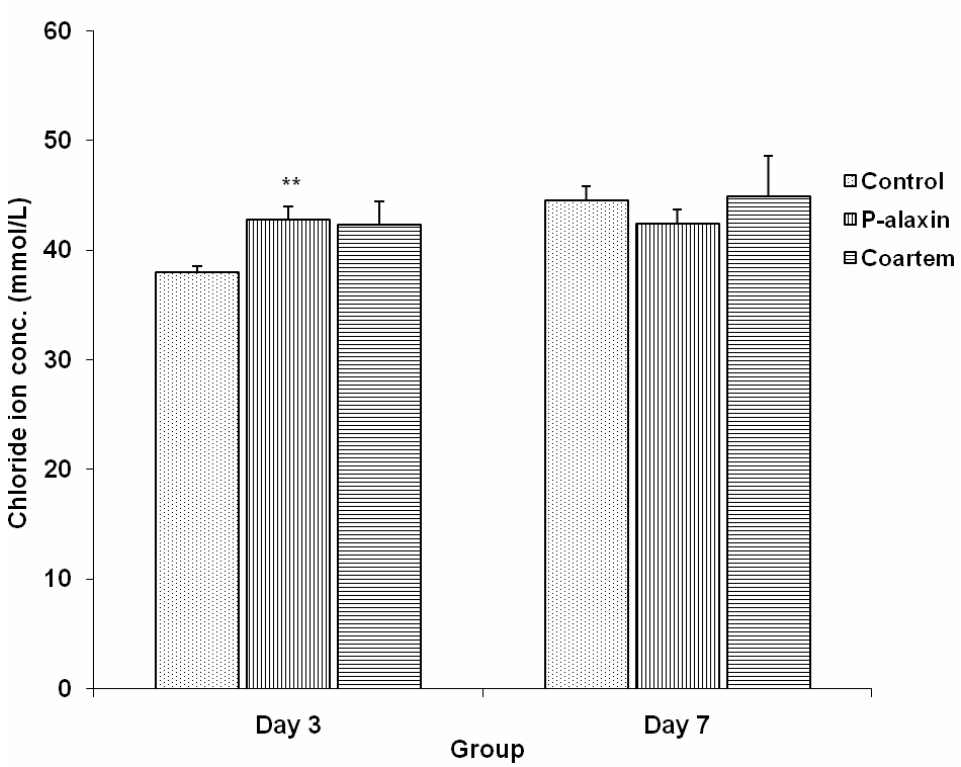

Figure 3 Comparison of chloride ion concentration in the different experiment groups at days 3 and 7. Values are mean ± SEM, n = 5. **p<0.01 vs control.

Serum HCO_3^- Concentration in the Different Experimental Groups

At day 3, the mean HCO_3^- concentration was 34.12 ± 1.01, 40.45 ± 1.33 and 43.92 ± 2.68 mmol/L for control, p-alaxin treated and coartem treated group. HCO_3^- concentration was significantly (P<0.05) higher in the p-alaxin and coartem treated groups, compared to control. On the 7th day, HCO_3^- concentration was 34.60 ± 6.59, 32.49 ± 2.19 and 24.16 ± 3.51 mmol/L for control, p-alaxin and coartem treated group respectively. HCO_3^- concentration was significantly (P<0.05) lower in the coartem treated group, compared to control. (Fig. 4).

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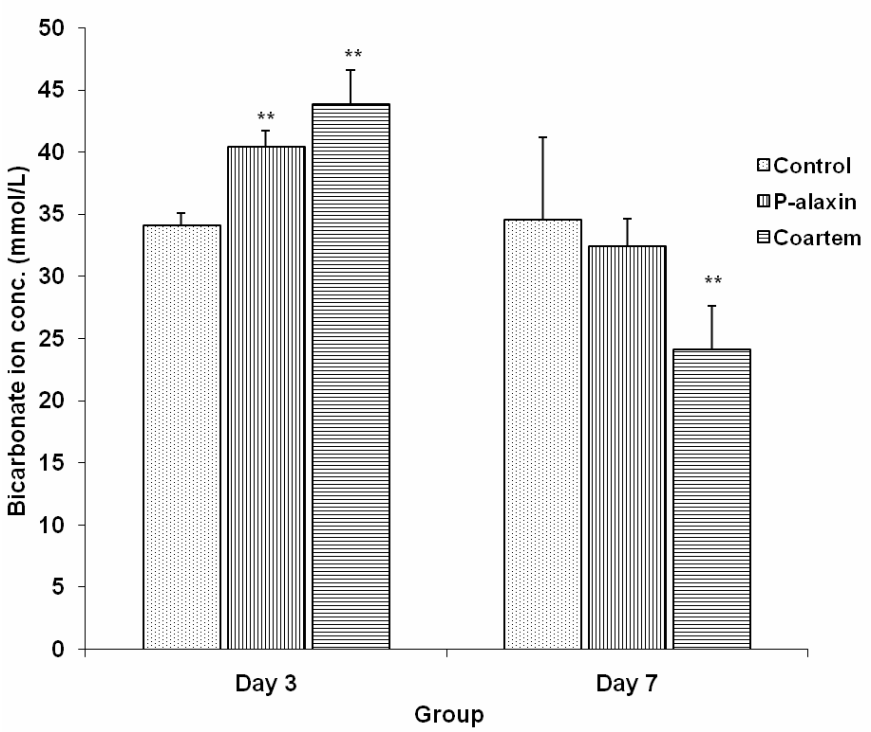

Figure 4 Comparison of bicarbonate ion concentration in the different experiment groups at days 3 and 7. Values are mean ± SEM, n = 5. **p<0.01 vs control

Serum Enzyme Concentration in the Different Experimental Groups

Mean serum enzyme (ALP, ALT and AST) concentrations in the test groups were not significantly different (at P<0.05) compared to control in the period studied. (Table 1).

Comparison of the Effect of Duration of Treatment with P-alaxin and Coartem on Serum Electrolytes and Enzyme Concentration

The concentration of K^+ in the coartem treated group on the 7th day was significantly (P<0.001) higher, compared to the 3rd day of treatment. HCO_3^- concentration in the coartem treated group on the 7th day was significantly (P<0.01) lower, compared to the 3rd day. ALP concentration in the coartem treated group on the 7th day was significantly (P<0.05) lower, compared to the 3rd day. (Table 2).

Table 1: Comparison of serum enzyme concentrations in the different experimental groups

	Day 3			Day 7		
	Control	P-alaxin	Coartem	Control	P-alaxin	Coartem
ALT (IU/L)	11.45 ± 1.42	13.64 ± 2.08	16.20 ± 2.15	17.12 ± 0.90	13.66 ± 1.80	13.87 ± 1.71
AST (IU/L)	3.82 ± 0.19	4.66 ± 0.53	5.52 ± 1.15	12.68 ± 3.29	12.98 ± 4.52	6.84 ± 1.78
ALP (IU/L)	5.53 ± 0.79	5.91 ± 0.25	5.35 ± 0.43	3.60 ± 0.76	4.54 ± 0.63	3.09 ± 0.33
AST:ALT	0.35 ±0.03	0.39 ± 0.09	0.38 ± 0.12	0.72 ± 0.17	1.16 ± 0.51	0.51 ± 0.13

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Table 2: Comparison of the effect of duration of treatment with p-alaxin and coartem on serum electrolytes and enzyme concentration

	Day 3			Day 7		
	Control	P-alaxin	Coartem	Control	P-alaxin	Coartem
Na^+ (mmol/L)	168.43 ± 24.49	224.64 ± 27.59	291.94 ± 4.29	146.15 ± 31.60	263.90 ± 15.10	307.98 ± 22.68
K^+ (mmol/L)	11.87 ± 0.15	12.16 ± 0.42	11.51 ± 0.16	14.47 ± 0.61	11.69 ± 0.09	12.91*** ± 0.16
Cl^- (mmol/L)	38.05 ± 0.51	42.84 ± 1.11	42.36 ± 2.06	44.62 ± 1.22	42.48 ± 1.28	45.00 ± 3.66
HCO_3^- (mmol/L)	34.12 ± 1.01	40.45 ± 1.33	43.92 ± 2.68	34.60 ± 6.59	32.49 ± 2.19	24.16 ± 3.51**
ALT (IU/L)	11.45 ± 1.42	13.64 ± 2.08	16.20 ± 2.15	17.12 ± 0.90	13.66 ± 1.80	13.87 ± 1.71
AST (IU/L)	3.82 ± 0.19	4.66 ± 0.53	5.52 ± 1.15	12.68 ± 3.29	12.98 ± 4.52	6.84 ± 1.78
ALP (IU/L)	5.53 ± 0.79	5.91 ± 0.25	5.35 ± 0.43	3.60 ± 0.76	4.54 ± 0.63	3.09 ± 0.33**
AST:ALT	0.35 ±0.03	0.39 ± 0.09	0.38 ± 0.12	0.72 ± 0.17	1.16 ± 0.51	0.51 ± 0.13

Values are expressed as mean ± SEM, n = 5.
**P<0.01, ***P<0.001 vs coartem day 3

DISCUSSION

P-alaxin and coartem have been indicated for treatment of acute, uncomplicated malaria in most countries, Nigeria inclusive [16]. In the course of treatment however, it is important to ascertain the effect of the drug material on physiological indices.

Changes in serum electrolyte concentrations are known to affect body fluid distribution, blood pressure, cardiovascular functions and acid base balance. P-alaxin and coartem significantly increased Na^+ concentration in serum after 3 days and 7 days of administration, with coartem almost doubling the normal value (Fig. 1). Our findings correlate well with the report of Akomolafe *et al* [17], who reported that coartem increased urinary output of dilute urine. It can therefore be deduced that the hypernatremia observed in the treated animals was as a result of increased urinary output. Serum K^+ concentration was not significantly altered after 3 days of treatment with the test drugs. After 7 days of treatment, p-alaxin and coartem significantly ($P<0.01$, $P<0.05$ respectively) reduced serum K^+ concentration, compared to control. Coartem produced a significantly ($P<0.001$) lower K^+ concentration compared to p-alaxin (Fig. 2). The decrease in K^+ concentration observed in this study was however not low enough to cause detrimental effects.

Both p-alaxin and coartem increased serum Cl^- concentration in this study. The increase was significant ($P<0.01$) in the p-alaxin group when compared to control. The increase in Cl^- concentration caused by coartem was however not significant when compared to control (Fig. 3). P-alaxin and coartem significantly ($P<0.01$) increased serum HCO_3^- concentration, compared to control after 3 days of administration. Continuous administration of p-alaxin for up to 7 days reduced the serum HCO_3^- concentration to levels lower than control, though the difference was not significant; while continuous administration of coartem significantly ($P<0.01$) reduced serum HCO_3^- concentration,

compared to control. The serum concentration of bicarbonate to a large extent gives an idea of the pH of blood, owing to the fact that the bicarbonate buffer system is the most important amongst blood buffers when blood pH is considered [18]. The reduction in the serum HCO_3^- concentration implies that the blood pH was lowered. This reduction could be linked to either respiratory excretion through hyperventilation or increased urinary excretion of bicarbonate [19]. In this study, there was no hyperventilation observed in the experimental animals. Therefore, the renal route of excretion was the likely mechanism of loss of bicarbonate ions observed in the coartem treated group after 7 days of administration.

Although administration of p-alaxin and coartem to the respective groups altered serum enzyme concentrations, the alterations were not significant enough to cause adverse effects (Table 1). Of the three serum enzymes (AST, ALT and ALP) measured, alkaline phosphatase (ALP) in the coartem treated group reduced significantly ($P<0.01$) on the 7th day, compared to the 3rd day (Table 2). Since ALP is produced by the mucosal cells that line the biliary system of the liver, the free flow of bile through the liver and down into the biliary tract and gallbladder are responsible for maintaining the proper level of this enzyme in the blood. Its enhanced elimination on the 7th day of coartem administration reflects the decreased bicarbonate ion concentration on the 7th day (Fig. 4) already mentioned.

CONCLUSION

The results obtained from this study is suggestive of the fact that treatment of malaria with p-alaxin or coartem for 3 days, predisposes an individual to hypernatremia, while a seven days treatment predisposes to hypokalemia and disturbances in acid - base balance. Most of the changes observed were severe in the coartem treated group than the p-alaxin treated group. It is therefore important to take proper medical history of patients before prescription of these drugs to prevent hazards secondary to acid - base imbalance.

REFERENCES

[1] Mishra SK, Mohapatra S, Mohanty S, Patel NC, Mohapatra DN. J Indian Acad Clin Med 2002;3:141-47.
[2] Uzuegbu UE. Asian J Med Sci 2011;3(2):50-51.
[3] World Health Organization (WHO) Guidelines for the treatment of malaria. Geneva, 2010. 2nd Edition, pp 19-21. Available at http://whqlibdoc.who.int/ publications/ 2010 / 9789241547925_eng.pdf.
[4] Juma EA, Obonyo CO, Akhwale WS, Ogutu BR. Malaria J 2008;7:262.
[5] World Health Organization. Antimalarial drug combination therapy. Report of WHO technical consultation. WHO/CDS/RBM/2001.35. Geneva: WHO, 2001.
[6] Song JP, Duong S, Suou S, Thou T, Ses S, Sim Y, Tan B, Li GQ. Natl Med J China 2003;83:1099-1100.
[7] Rao GM. Indian J Med Sci 1992;46(10):301-303.
[8] Lisa Michael. Electrolyte imbalance in diabetes. Live strong foundation 2011. Accessed July 20, 2010. Available: ttp://www.livestrong.com/article/353569.
[9] Nostenand F, White NJ. Am J Trop Med Hyg 2007;77:181-192.
[10] Nwanjo HU, Oze G. Internet J Toxicol 2007;4:1-8.

[11] Ngokere AA, Ngokere TC, Ikwudinma AP. J Exp Clin Anat 2004;2:11-16.
[12] Olayinka ET, Ore A. J Pharm Biol Sci 2013;5(4):43-53.
[13] KolthoffI M, Coetzee JF. J Am Chem Soc 1957;79:870-874.
[14] Forrester RL, Watagi LJ, Silvermman DA, Pierre KJ. Clin Chem 1976; 22:243.36.
[15] Reitman, Frankel. Determination of Alanine Aspartate Aminotransferases. Using End point Colorimetric Diagnostic Kit (Randox Laboratory UK). 1952.
[16] Medical Letter on Drugs and Therapeutics. 2009;51(1321): 75-76.
[17] Akomolafe RO, Adeoshun IO, Fakunle JB, Iwalewa EO, Ayoka AO, Ajayi OE, Odeleye OM, Akanji BO. African J Biotechnol 2011;10(20):4226-4233.
[18] Fischer EW. Hydrogen Ion concentration-Anion-Cation (acid base) balance. In: Textbook of Veterinary Clinical Pathology, Medway W, Prier JE and Wilkinson JS (ed). 1969.
[19] Shirley DG, Capasso G, Unwin R. J Renal Physiology. In: R. Johnson and Feehally, (ed), Principles of Clinical Nephrology (2nd ed), Mosby International, Philadelphia. 2003.