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IMPLICATION OF FOLIC ACID SUPPLEMENTATION ON KIDNEY OXIDATIVE STRESS AND ION HOMEOSTASIS IN ARTEMISININ COMBINATION THERAPY INDUCED METABOLIC ALTERATION

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ABSTRACT

Report on the toxicity of acute administration of ACTs in malarial infection appears to be contradictory and only very few studies has reported on the long term use effect of the drug on kidney function and the protective role of folic acid when co-administered. In this study 56 male wistar rat assigned to 4 groups of 14 rats each were used. Group A was treated as normal control; group B and D were pre-treated with folic acid for 28 days. Leonart (2.67mg artemether/16mg lumefantrine per Kg body weight) was administered to groups C and D twice daily for 4 days starting from the 24th day after which seven rats were sacrificed from each group. Folic acid treatment was made to continue in the respective groups for another 4 days while Leonart was further administered in the respective group at a dose of 5.34 mg artemether and 32 mg lumefantrine per kg body weight for the 4 days period. The rats were then sacrificed. The serum was analysed for Na^+, K^+, Ca^{2+}, Cl^- ,HCO_3^-, creatinine and urea while the kidney homogenate was analysed for the levels of peroxidation and GSH and the activities of catalase and superoxide dismutase. Result of the study indicates that acute administration of these drugs did not alter these parameters. Chronic use of the drug raised the level of kidney peroxidation, decrease the kidney GSH, catalase activity and also increased the concentration of Na^+, K^+, Cl^+ and HCO_3^- ions. The result suggest that whereas acute use of leonart may not potentiate kidney toxicity, chronic use of the drug may lead to renal dysfunction and co- administration with folic acid may offer no protection against the disorder.

Key Words: Leonart, Oxidative stress, Kidney dysfunction, Serum electrolyte, Malarial, ACT.

INTRODUCTION

Malaria is caused by protozoan parasites of the genus Plasmodium, namely, *P. falciparum, P. vivax, P. malariae, P. ovale, and P. knowlesi.* Report indicates that patients with *P. falciparum* infection are prone to develop severe malaria in 30% of cases, which resulted in case fatality rate of 20% (Mohanty *et al*., 2003, Stremmel and Junghanss, 2008). Malaria is a disease of global public health importance. Its social and economic burden is a major obstacle to human development in many of the world's poorest countries. In heavily affected countries, malaria alone accounts for as much as 40% of public health expenditure, 30% to 50% of hospital admissions, and up to 60% of outpatient visits (WHO 2007). It has an annual

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incidence of approximately 250 million episodes and is the cause of more than a million deaths, most of them in infants, young children, and pregnant women (WHO 2008b). In Nigeria, malaria is considered to be a major public health problem. It causes more than 50% of the disease burden (Federal MoH, 2005) and almost 50% of all-cause health expenditure (WHO 2008a). Report indicates that about half of Nigeria's population is exposed to at least one episode of malaria every year (Annie, 2013). Malaria, affects almost all organ systems including the kidneys leading to both tubulointerstitial damage as well as glomerulonephritis. Malaria induce reenal lesion are commonly caused by *P. falciparum* which is seen in around 1 % of all *P. Falciparum* infected patients (Eiam-Ong and Sitprija, 1998). The incidence of acute renal failure rises to as high as 60% in patients having heavy parasitaemia (More than 10% P. falciparum infested RBCs). Acute renal failure (ARF) is one of the most common complications in adults with falciparum malaria (Eiam-Ong and Sitprija, 1998, Day *et al*., 2000). The incidence of ARF in patients with severe malaria varies widely ranging from 15% to 48% (Day *et al*., 2000, Mehta *et al*., 2001, Krishnan and Karnad, 2003, Koh *et al*., 2004, Kochar *et al*., 2006, Dondrop and Day, 2007) which resulted in a high fatality rate of over 70% in untreated patients (Dondrop and Day, 2007).

Many of the commonly used antimalarial drugs have been associated with one form of metabolic complications or the other. Findings from a study indicates that fifty per cent of chloroquine, and 70% of proguanil, are excreted by the kidneys. It is therefore recommended that doses should be lowered in patients with renal impairment. The artemisinin-derivatives, artemether, artesunate, and dihydroartemisinin, are currently the most potent anti-malarial medicines on the market. Artemisinin derivatives have been shown to produce faster relief of clinical symptoms and faster clearance of parasites from the blood than other antimalarial drugs (Adjuik 2004; WHO 2006). Artemisinin and its derivatives are generally reported as being safe and well tolerated. Artemisinin contains a 1, 2, 4-trioxane ring and the highly reactive endoperoxide group plays a key role in its anti-malarial activity (Galasso *et al*., 2007). The breaking of this bond, mediated by iron complexes, generates reactive radicals and the resultant chain of reactions damages the parasite (Galasso *et al*., 2007). Several mechanisms have been reported for the anti-malarial effect of Artemisinin and other related endoperoxides (Sen *et al*., 2010; Zhang and Gerhard, 2008; Sharif *et al*., 2006). Although Artemisinin was considered to be safe in therapeutic doses, a number of researchers have shown different side effects of the drug, as well as its synthetic or semi-synthetic derivatives in humans and some animal species. Following artemisinin administration in wistar rats, a study reported evidence of embryolethality and some evidence for morphological abnormalities induced during early pregnancy, without maternal toxicity following drug consumption. There was also in addition hormonal imbalance in progestagens and in testosterone levels (Boareto *et al*., 2008). Some other studies on artemisinin derivatives in animals have reported significant neurotoxicity (brain damage) (Price, 1999) and adverse effects on the early development of the fetus, (Nosten, 2007). Other reported adverse events include gastrointestinal (GI) disturbance (stomach upset), dizziness, tinnitus (ringing in the ears), neutropenia (low levels of white blood cells), elevated liver enzymes (a marker for liver damage), and electrocardiographic (ECG) abnormalities (changes in cardiac conduction)

Folic acid is often administered along with antimalarial drugs. Studies have provided some evidence in favor of iron supplementation after anti-malarial treatment of children with malaria anemia, (WHO, 2007, Nosten and White, 2007) but the value of treatment with folic acid remains controversial. The goal of the present study is to evaluate the effect of artemisinin combination therapy on kidney function and implication of its co-administration with folic acid on some biomarkers of renal function

MATERIALS AND METHOD

Drug

Leonart® composed of 80 mg Artemether and 480mg lumefantrine was manufactured by Bliss GVS Pharma Ltd. India.

Experimental design

Fifty six (56) male adult rats of the Wistar Kyoto strain weighing 127-133g obtained from the Animal Facility Centre Department of Physiology, University of Ibadan, Nigeria were used for the study. After a period of 14 days acclimatization, the animals were randomly assigned into 4 groups of 14 rats each labeled as:

Group A (Normal control): Administered with normal saline

Group B (Test control): Administered with folic acid

Group C (Test 1): Administered with artemisinin combination therapy.

Group D (Test 2):Pre-treated with folic acid for 24 days and then administered with artemisinin combination therapy.

All the rats were maintained on standard rat feed and clean drinking water *ad libitum* throughout the period of the experiment.

Treatment protocol

Groups B and D were first pre-treated with folic acid supplement for 28 days. On the 24th day after the commencement of treatment, leonart was administered to rats in group C and D at a therapeutic dose of 2.67mg artemether/16mg lumefantrine per Kg body weight twice daily. The administration was done for the next four days

at the respective dosage after which 7 rats in each group were sacrificed. The remaining rats were made to continue with the respective treatment. Leonart was administered to rats in group C and D at a dose of 5.34 mg artemether and 32 mg lumefantrine per kg body weight twice daily for another 4 days. All the rats were then sacrificed.

The protocol conforms to the guidelines of the National Institute of Health for laboratory animal care and use (NIH, 1985), and in accordance with the principles of good laboratory procedure (WHO, 1998) as approved by the Animal Use Ethics Committee College of Health sciences, Olabisi Onabanjo University, Ogun State, Nigeria.

Preparation of tissue homogenate.

Rats were sacrificed 24 hours after the last administration of leonart and/or supplements after an overnight fast. Blood was collected from the inferior *vena cava* of heart of the animals into plain centrifuge tubes and was allowed to stand for 1 hr. Serum was prepared by centrifugation at 15000 ×g for 15 minute in a centrifuge. The clear supernatant was used for analysis. Kidneys were quickly removed and washed in ice-cold 1.15% KCl solution, dried and weighed. The kidney samples were homogenized in 4 volumes of 5 mM phosphate buffer, pH 7.4 and centrifuged at 10,000 ×g for 15 minutes to obtain post-mitochondrial supernatant fraction. The samples were stored at –80°C until use. All procedures were carried out at temperature 0–4°C.

Assay procedure

Serum protein level was determined according to the method of Lowry *et al*., (1951), using bovine serum albumin as standard. Assay for plasma urea was carried out by the Urease- berthelot method (Weathewrburn, 1967) using the laboratory kit reagents (Randox laboratory Ltd. UK). Colorimetric method as described by Bartels and Bohmer (1972) was used in assaying for plasma creatinine using laboratory kit reagents (Randox laboratory Ltd. UK). The extent of lipid peroxidation (LPO) was estimated by the method of Buege and Aust (1978). The method involved the reaction between malondialdehyde (MDA; product of LPO) and thiobarbituric acid to form a pink precipitate, which was read at 535 nm spectrophotometrically. Superoxide dismutase (SOD) activity was measured by the nitroblue tetrazolium reduction method of McCord and Fridovich (1969); Catalase (CAT) activity was assayed by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by Aebi (1974); Reduced GSH level was assayed by measuring the rate of formation of chromophoric product in a reaction between DTNB (5,5′-dithio-bis (2-nitrobenzoic acid) and free sulfhydryl groups (such as reduced glutathione) at 412 nm according to the method of Moron *et al*. (1979).

Statistical analysis

All values were expressed as mean ± S.D. The statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) (Duncan, 1957). Test with $P< 0.05$ were considered significantly different

RESULTS

Therapeutic dose administration of leonart alone or the co- administration of leonart with folic acid did not significantly alter the concentration of any of the electrolyte determined in this study (Table 1). With continuous administration of leonart at an increased dose for the next 4 days, the serum sodium ion concentration of rats administered with leonart alone and that co-administered with leonart and folic acid were significantly ($p<0.05$) raised above the observed value in the normal control group. No significant difference ($p>0.05$) was however observed between the sodium ion concentration observed in the rats treated with leonart alone and the group co-administered with leonart and folic acid throughout the treatment period. Administration of leonart was not seeing in the study to have altered the serum potassium and calcium level both after 4 days and 8 days treatment with leonart and folic acid. Although no significant difference was seeing in the chloride ion and bicarbonate ion among the treatment groups after the initial 4 days administration of leonart, continued treatment for another 4 days raised the chloride and bicarbonate ions of the rats treated with leonart above the normal control value. A similar result was obtained with rats co-administered with both leonart and folic acid.

Acute administration of leonart and folic acid for the initial 4 days period did not significantly ($p>0.05$) alter the serum creatinine, urea and total protein concentration. After further administration of leonart at an increased dose for another 4 days, the serum creatinine and urea level of rats treated with leonart alone and that of the group co-administered with leonart and folic acid were raised above the normal control level. When compared with the obtained value after the acute dose administration, continuous administration of the drug alone and its co-administration with folic acid significantly ($p<0,05$) raised the level of chloride and bicarbonate ions.

The result of treatment on the oxidative status indices indicates that administration of leonart for the initial 4 days period had no significant effect on the kidney peroxidation status. The kidney thiobarbituric acid level, the GSH, catalase and superoxide dismutase activities were not different significantly between among the treatment groups. After further treatment at an increased dose for another 4 days, there was a significant elevation in the kidney thiobarbituric acid reactive substance level with a concomitant decrease in the GSH level of rats treated with leonart alone when compared with the corresponding value in the normal control. The observed value was not

different significantly from that of rats co- administered with leonart and folic acid. Similarly, the catalase activity was observed to decrease significantly with leonart treatment after the 8 days administration period. The kidney SOD activity was however not affected with the treatment.

Table 1. Effect of treatment on serum sodium, potassium and calcium level

Group	Treatment	Serum sodium, potassium and calcium (mmol/L)					
		After 4 days of ACT administration			After 8 days of ACT administration		
		Na	K	Ca	Na	K	Ca
A	Normal saline	142.95±9.52	35.51±1.15	6.05±0.18	131.67±7.61	37.62±3.01	6.20±0.11
B	Folic acid	147.72±8.63	36.29±2.89	5.67±0.61	140.11±10.09	36.12±2.21	6.12±0.40
C	ACT	127.80±3.47	39.61±1.24	5.82±0.24	209.62±9.21*	40.51±3.12	6.23±0.14
D	ACT + Folic acid	148.98±4.99	31.42±0.77	6.14±0.11	218.69±7.12*	33.46±4.01	6.62±0.41

Results are mean ±SEM of 7 samples; *: P<0.05 compared with the normal control group

Table 2. Effect of treatment on serum chloride and bicarbonate level

Group	Treatment	Serum chloride and bicarbonate ion (mmol/L)			
		After 4 days of ACT administration		After 8 days of ACT administration	
		Cl	HCO_3^-	Cl	HCO_3^-
A	Normal saline	78.46±6.53	19.38±1.21	76.31±4.21	21.12±3.11
B	Folic acid	78,22±0.26	21.44±1.54	79.42±6.11	21.62±0.91
C	ACT	72.55±3.91	19.72±1.09	97.61±4.32*	33.24±4.01*†
D	ACT + Folic acid	69.00±9.73	19.41±1.55	99.41±6.17*	39.16±2.11*†

Results are mean ±SEM of 7 samples; *: P<0.05 compared with the normal control group;
†: P<0.05 compared with the mean after the 4 days initial treatment.

Table 3. Effect of treatment on some serum creatinine, urea and total protein

Group	Treatment	Serum creatinine, urea and total protein (mmol/L)					
		After 4 days of ACT administration			After 8 days of ACT administration		
		Creatinine	Urea	Total protein	Creatinine	Urea	Total protein
A	Normal saline	66.29±4.15	12.50±0.38	3.65±0.35	68.19±3.09	12.69±1.09	4.01±0.97
B	Folic acid	77.23±4.97	12.36±0.17	3.57±0.21	67.74±3.13	10.91±2.10	3.91±0.16
C	ACT	72.51±5.87	12.46±0.26	3.80±0.07	89.17±1.62*†	21.40±1.19*†	3.89±0.18
D	ACT + Folic acid	76.33±4.79	12.27±0.42	3.64±0.07	97.43±4.12*†	23.67±1.07*†	4.22±0.98

Results are mean ±SEM of 7 samples ; *: P<0.05 compared with the normal control group.
†: P<0.05 compared with the mean after the 4 days initial treatment

Table 4. Effect of treatment on kidney peroxidation and antioxidant status

Group	Treatment	Peroxidation status							
		After 4 days of ACT administration				After 8 days of ACT administration			
		TBARS (nmol MDA/g tissue)	Catalase activity (µg/mg proteinx10^3	SOD activity (ng/mg protein x 10^3)	GSH (µg/g tissuex10^3)	TBARS (nmol MDA/g tissue)	Catalase activity (µg/mg proteinx10^3)	SOD activity (ng/mg protein x 10^3)	GSH (µg/g tissuex10^3)
A	Normal saline	13.3±0.36	1.70±0.56	1.34±0.13	7.67±0.26	13.37±0.52	1.68±0.132	1.29±0.14	7.89±0.11
B	Folic acid	13.51±0.81	1.67±0.06	1.31±0.26	7.70±0.07	13.11±0.64	1.68±0.12	1.31±0.31	7.74±0.16
C	ACT	13.18±0.36	1.71±0.13	1.46±0.31	7.66±0.45	16.75±0.32*†	1.61±0.63*†	1.04±0.12	5.14±0.07*†
D	ACT+ Folic acid	13.07±0.36	1.67±0.16	1.31±0.25	7.68±0.26	17.12±0.47*†	1.47±0.34*†	1.27±0.09	5.18±0.12*†

Results are mean ±SEM of 7 samples. ;*: P<0.05 compared with the normal control group.
†: P<0.05 compared with the mean after the 4 days initial treatment.

DISCUSSION

Although many authors have reported that therapeutic dose of ACT may not potentiate serious toxicity, there was no agreement among various reports cited in the literatures on the toxicity of non therapeutic dose (Olufemi *et al*., 2009, Farombi *et al*., 2001, Adaramoye *et al*., 2007). Moreover, as at the time of this study, there is no report in the literature on the implication of co-administration of ACT and folic acid on renal function, yet ACT is often prescribed along with folic acid for malarial treatment.

Findings from previous studies have implicated oxidative stress as an important factor in the development of cell injury (Adaramoye *et al*., 2007; Thamilselvan *et al*., 2009; Thanachartwet *et al*., 2013). Generally, oxidative stress is associated with a number of disorders, including atherosclerosis, ischemia/reperfusion injury, arthritis, stroke and neurodegenerative diseases, as well as with a variety of metabolic, toxic, or hypoxic conditions. (Thamilselvan *et al*., 2009). Reactive oxygen species (ROS) including hydrogen peroxide, superoxide dismutase and hydroxyl radical generated during oxidative stress causes formation of cytosolic aldehyde and peroxide products. An important procedure for assessing the degree of oxidative stress of any biological sample is to measure the level of malondialdehyde (MDA), a thiobarbituric acid reactive substance (TBARS) which is the major end product of lipid peroxidation reaction (LPO) (Draper and Hadley, 1990). Data from the present study indicates that acute dose of ACT does not increase the amount of malondialdehyde generated. However, when the dosage was increased above the therapeutic regimen and the administration period was increased, a significant increase in the level of malondialdehyde with a concomitant decrease in the level of GSH and catalase activity was observed. The increase level of MDA observed with this treatment suggests an increase in the rate of production of reactive oxygen species. This finding is similar to the observation of Olufemi *et al*. (2009) which reported an increased lipid peroxidation in the testes of rats administered with higher doses of artemether. It also comform with the report of Adaramoye *et al* (2007) who observed increased peroxidation status in the liver of rats following acute administration of arthemether but not in the kidney. When administered during malaria infection, the mechanism by which artemisinin achieve therapeutic effect is firstly through reductive cleavage of the endoperoxide bridge of Artemisinin, followed by intramolecular electronic rearrangements that produce carbon-centered radicals. Subsequent reactions, including the alkylation of proteins, lead to the death of the parasite (Zhang and Gerhard, 2008). The observed increase in the kidney MDA reported in this study thus indicates that administration of artemisinin combination causes increase generation of free radicals/ reactive oxygen species.

A balance normally exists between free radicals and antioxidants scavenging enzymes activities. As a result of such balance, only minimal amounts of oxidants needed for normal cellular function remains. The level of reduced GSH, catalase and superoxide dismutase (SOD) is a measure of endogenous antioxidant and cellular redox status of cells in higher animals (Chance *et al*., 1979). The decreased level of GSH and activities of CAT as was observed in the kidney following the chronic use of leonart further confirmed the formation of reactive oxygen species or toxic metabolites from artemisinin combination and that this effect is evident when administered at doses above therapeutic use of the drug and that co administration of the drug with folic acid does not ameliorate this changes. Our finding is similar to the observation of Adaramoye *et al*., (2007).

Over all, the result of the study suggest that the kidney is not able to protect itself from the assaults of the radical species, generated when ACT was administered at more than the therapeutic dose and that combined administration of the drug with folic acid does not also offered any significant protection against reactive oxygen specie damage to the kidney. This could have serious implication on the renal functions of the animals on long-term prophylaxis.

Serum electrolytes, urea, and creatinine are marker indices of kidney functions and renal damage has often been associated with alteration in the levels of these parameters (Yakubu *et al*., 2006). Creatinine and urea are major catabolic products of muscle, and purine metabolism respectively. They are waste products which are passed into the blood stream to be removed by the kidney. Both creatinine and BUN are sensitive biochemical indices for the evaluation of renal functions (Ogawa, 1992). Increased levels of BUN and creatinine have been implicated in kidney diseases such as acute glomerulonephritis, nephrosclerosis and tubular necrosis (Ogeturk *et al*., 2005; Pari and Murugan, 2006). In the present study, acute administration of ACT was not seen to have significantly altered the concentration of these parameters in the serum. Continuous administration of the drug was however observed to raise the creatinine and urea concentration significantly. Though, Anyasor *et al.,* (2011) also observed an alteration in creatinine level with artemisinin administration, however whereas, the report from the study indicate a decrease in the concentration of these parameters, we observed an increase serum creatinine concentration. Our report however agrees with that of Adaramoye *et al.,* (2007). In our opinion, increased oxidative stress of the kidney induce by the drug may have affected both the urea and creatinine clearance thus leading to the observed increase in the serum concentration. It is also possible that the kidney might have compromised all or part of its functional capacity of tubular excretion at this dosage regimen. This may be as a result of partial impairment on their glomerular clearances. Folic acid, we

observed in the present study, does not offered a significant protection against this defect.

Calcium, sodium and potassium are important electrolytes involved in maintenance of homeostasis. Calcium ion plays a vital role in muscle contraction and serves as an intracellular second messenger for hormones. It is also important in nerve cells for effective transfer of nerve impulses and also for blood clotting (Guyton and Hall, 2006). Based on the report of this study, neither acute nor chronic administration of ACT significantly alters the serum calcium concentration. Adequate level of potassium ions is essential for normal cell function. Many processes in the body, especially in the nervous system, muscles and renal selective reabsorption, require electrical signals for communication. The movements of these ions are critical in generation of these electrical signals (Devine *et al*., 1999).

Sodium regulates the total amount of water in the body and its transmission across cells play roles critical to body functions. The observed changes in these ions as reported in this study is similar to our earlier report of another study (Ajani *et al*., 2013) and the report of Akomolafe *et al.,* (2011). The observed increased in serum Na^+ and K^+ concentration in ACT treated animals suggest oxidative stress induced alteration of some biochemical parameters, such as an increase production of aldosterone and other mineral corticoids which may in turn affect the tubular reabsorption of of these ions or alter tubular sensitivity to the hormone (Tietz *et al*., 1994).

Findings from this study also indicate that ACT administration at dosage above therapeutic indications might have caused an imbalance in the concentration of chloride and bicarbonate ions in the rats. This result is similar to the observation of Anyasor *et al*., (2011). Chloride ion (Cl^-) is the most abundant anion in the extracellular fluid. Chloride ion concentration in the plasma generally follows those of Na^+. However, fluctuations in serum or plasma Cl^- and bicarbonate ion (HCO_3^-) may have little consequence but are often signs of an underlying disturbance in cellular fluid and acid–base homeostasis (Philips, 1994). Folic acid was also observed in this study not to offer significant protection against alteration in the serum concentration of these ions.

Both acute and chroninc administration of artemisinin combination and the co administration of the drug with folic acid appears not to affect the overall protein concentration of the serum. This observation is similar to the report of Adaramoye *et al*., (2007) but differ from the findings of De Feo *et al*., (1994) who in the study with another antimalarial drug, chloroquine reported a decrease in protein turnover following therapeutic dose of the agent in human beings .

CONCLUSION

Based on the observations from the present study, ACTs as administered presently for the treatment of malaria may be regarded safe for the kidney. Administration of the drug at an increased dose above the present therapeutic regimen may however increase the kidney oxidative stress status and also compromise kidney function. Although co-administration of the drug with ACTs may be beneficial in malaria associated anaemia, it may however offer no protection against kidney dysfunction.

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