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Research Article

Effect of some Artemisinin and Combination Therapy Regimens with and without Concomitant Administration of Phospholipids on the Levels of Plasma Aminotransferases and Bilirubin in Nigerian Male Subjects

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Background: Previous studies in animal have shown that high doses of artemisinin caused injury to liver cells. Presently artemisinin and its derivatives such as artesunate (ART), and it's combination therapy (ACT) has been adopted as the frontline drug for treatment of uncomplicated malaria in Nigeria without considering the effect it has on some major organs of the body.

Objective: The objective of this study was to evaluate the effect of ART when administered as a monotherapeutic agent and in combination as ACT on the plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin (BIL) and the effect of concomitant administration of a hepatotonic phospholipid (PL) on such effects.

Methodology: The prepared plasma samples were analyzed using the end point calorimetric method for each parameter as explained in the Randox kits manual.

Results: Co-administration of ART with amodiaquine (AMQ), mefloquine (MFQ) and sulphadoxine/pyremethamine (SP) respectively, on the 4th day of the studies increased the mean plasma concentration of AST to 80.70%, 108.0% and 75.0% against 59% for ART alone; ALT increased to 104%, 33.0% and 43.30% against 25.05% for ART alone; total bilirubin (TBIL) increased to 80.0%, 78.88% and 98.91% against 17.6% for ART alone. The co-administration and post-administration of ART and the ACTs with 900mg and 1800mg daily dose of PL respectively reduced the levels of the AST, ALT and BIL by 65.0% and 97.0% of the increased values respectively on 4th day.

Discussion: The results suggest that ART as a monotherapeutic agent has injurious effect on the liver, and this effect is aggravated when ART is used in ACTs, however, the co-administration with phospholipids cushions the adverse effects.

Key words: Artesunate; Artemisinin Combination Therapy (ACT); Aminotransferases; Bilirubin; Phospholipids

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1. Introduction

The disease malaria is a major health and developmental challenge for many of the poorest countries in the world. Estimates of the annual incidence of malaria vary widely, according to the estimate of the world malaria report, 2011, there were 216 million episodes of malaria in 2010, of which approximately 81%, or 174 million cases were in the African region. In 2008, there were 247 million cases of malaria and nearly one million deaths mostly among children below the age of five in Africa. In Africa, a child dies every 45 seconds of malaria. About 30% of children under the age of five in Nigeria die from malaria every year. It is responsible for many medical complications such as low birth weight in infants, sudden abortion and stillbirths in pregnant women. (Joda et al, 2005; Verhoeff et al, 1999; Lindsay et al, 2010; World Malaria Report, 2011). It is estimated that 60% of the clinical malaria cases and over 80% of all deaths occur in Sub-Saharan Africa. Other highly affected regions are South East Asia and South America, (WHO, 2010; Aguwa, 1996).

Malaria is a dual-host hematoprotozoan parasitic infection transmitted by certain species of the infected female anopheline mosquitoes. Transmission depends on climatic conditions that may affect the abundance and survival of mosquitoes. In many places transmission is seasonal, with the peak during and just after the rainy season (Coker et al, 2001). *Plasmodium*, a unicellular eukaryotic cell of the phylum protozoa is the fatal parasite responsible for malaria disease. In the human body, the parasite multiply in the liver, red blood cells, the brain, lungs, kidneys, placenta and other tissues, (David and Peter, 2004; Adams et al, 2002; Alkawe et al, 1988).

Early diagnosis and treatment of malaria reduce the disease and prevent death.

Control measures of malaria include preventive (which involves control of the vector of the malaria parasite by indoor spraying with residual insecticides, sleeping under long lasting insecticide-impregnated nets (LLINs), the maintenance of good sanitary conditions, and treatment of the disease by the use of drugs), (Habluetzel et al, 1999). Drug treatment of malaria has so far not been a complete success story because of the complexity of the life circle of the protozoa (*Plasmodium)*, both in human and in the mosquito vector, and the development of resistance by *Plasmodium* (Foote et al, 1990). Chloroquine (CQ) resistance was first documented in South East Asia in the late 1950 and had spread to Africa by the end of the 1970s. Sulphadoxine-pyremethamine (SP) has been used as a replacement for CQ, but its effectiveness is now also seriously impaired by resistance, (Joac et al, 1997; Nzila et al, 2000). The emergence of resistance to chloroquine and other antimalarials has led to the search and development of newer drugs including artemisinin and its derivatives (Sanjeev et al, 2004).

Artemisinin, the endoperoxide sesquiterpene lactone is the active constituent of the plant *Artemisia annua* which is a common weed in Southern China. Since the isolation of artemisinin in 1971, a water soluble ester called artesunate and two oil soluble preparations artemether and arteether have been developed, (Hsu, 2006; Liao, 2009; Tu, 2004; Liy and Wy, 1998). Inhibition of protein synthesis (Hong and Meshnick, 1994) in *Plasmodium* and generation of organic free radicals are the basic mechanisms of action of artemisinin. It also causes morphological changes in the ribosome, endoplasmic recticulum and membrane structure of the plasmodium (Meshnick et al, 1996; Cumming et al, 1997, Robert and Maunier, 1998; Meshnick, 2002). Artemisinin and its derivatives have a short elimination half life and this confers the theoretical advantage that selection for drug resistant parasite is less likely. However, to avoid the higher risk of recrudescence when used in monotherapeutic regimen, artememisinins are used in combination with other antimalarials with relatively longer elimination half life. This combination is known as artemisnin combination therapy (ACT), and it has been adopted as the front line drugs for the treatment of uncomplicated malaria in Nigeria (Olliaro and Tailor, 2004; Davis et al, 2005).

Phospholipids are a class of lipids that are a major component of all cells membrane since they can form lipid bilayers. Clinically PL is proven to be safe and beneficial for the brain, liver, fluid circulation and intestinal tract motility. It is best valued for its support to liver recovery following toxic chemical or viral damage, (Kidd, 2002; Tallon, 2008)

Artemisinins are considered to have high safety margins, however, they may be toxic under certain conditions such as in high doses, (Udobre *et al*., 2009), and when used in combination as ACTs on renal functions, (Etim et al, 2012).

Some enzymes act as indicators of disease states. Enzyme levels in the intracellular fluids such as blood form an integral part of diagnosis. Increase in the level of enzyme in plasma is indicative of cell damage. Liver disease is the most important cause of increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity. In hepatocellular injury or necrosis, and in inflammatory condition, there is usually leakage of cytoplasmic enzymes into the systemic circulation and as such the level of the enzymes in plasma is higher (Hasper and Jones, 2011; Giannini et al, 2003). Some drugs such as pyridoxine lower the plasma level of ALT (Tiestz, 1982). The present study is aimed at investigating the effects of artesunate as a monotherapeutic agent and in combination as ACTs on the liver functions and the influence of phospholipids on such effects.

2. Materials and Methods

2.1 Ethical Approval

The study was conducted between October and December 2012 at the Health Centre of the University of Uyo, after approval by the Ethics Committee of the University. The ethics approval number is **UU/HC/EC/vol.2/168**, of 3rd June, 2012.

2.2 Recruitment of Subjects

One Hundred and four male volunteer subjects were identified through personal contact from among students, and staff of the University of Uyo community. Written informed consent of participation in the study was sought and obtained from all volunteers. They were physically fit with no history of malaria infection or antimalarial treatment or the use of any other medication in the past two weeks before commencement of the studies. Volunteers who took any form of tobacco or cigarette were also excluded. They were also screened and found free from any chronic liver and kidney diseases, by analyzing the levels of creatinine and alanine aminotransferase using the calorimetric method as explained in the Randox manual for these substances. Volunteers with plasma creatinine levels above 120µmol/L were excluded from the studies because of suspected renal problems and those with ALT above 40 IUL^{-1} were also excluded for liver insufficiency (Marshall and Bangert, 2009). Vital signs: body temperature, body weight not less than 55kg, and blood pressure of each volunteer was recorded before drug administration.

2.3 Drugs/Chemicals

The drug products used in these studies were: Artesunate 100 mg tablets (manufactured by Bliss GVS Pharma India); Larimal containing Artesunate 50 mg and 153.1 mg Amodiaquine base manufactured by Ipca Laboratories Ltd, Mumbai India; Artequine tablets, each containing 200 mg of artesunate and 250 mg of mefloquine base manufactured by Mepha Ltd, Switzerland, and Amala Plus tablet each containing 100 mg of Artesunate and 500 mg sulfadoxine – 25 mg pyrimethamine, manufactured by Elbe Pharma Ltd. India, Essential forte containing 300 mg of phospholipid, manufactured by Avensis Pharma Germany. All the drug products were obtained direct from the manufacturer's representative here in Uyo and they were all less than one year from the date of manufacture. The chemicals were freshly prepared as explained in the Randox manual for each of the substances analysed.

2.4 Study Design

An open single centre study was carried out involving all the subjects. After an overnight fast and a light breakfast at 8.00 am, the subjects were divided into twelve groups with eight volunteers in each group. All the subjects were to abstain from any other medication, alcohol and cigarettes within the period of the studies. They were also to eat similar types of snacks and food within the period of the studies.

2.5 Administration of Drugs to Volunteers

Each member in the groups received adult doses of the following drugs, as shown in table one and swallowed it with 500 ml of water. The dosing regimen administered was as recommended by the manufacturers and is in agreement with WHO guidelines for the treatment of uncomplicated malaria (WHO, 2010). The dosing information is tabulated a shown in **Table 1**.

Table 1: Administration of Drugs to Volunteers

Group	Trade Name Of Drugs	Artesunate (mg)	Partner Drug In ACT	No. Of Phospholipid Cap Three times daily
A	Artesunate	100	-	-
B	Artesunate	100	-	1
C	Artesunate	100	-	2
D	Larimal	200	Amodiaquine 600mg	-
E	Larimal	200	Amodiaquine 600mg	1
F	Larimal	200	Amodiaquine 600mg	2
G	Artequine	200	Mefioquine 250mg	-
H	Artequine	200	Mefloquine 250mg	1
I	Artequine	200	Mefloquine 250mg	2
J	Amala Plus	200	Sulfadoxine 1500g and Pyrimethamine 75mg	-
K	Amala Plus	200	Sulfadoxine 1500g and Pyrimethamine 75mg	1
L	Amala Plus	200	Sulfadoxine 1500g and Pyrimethamine 75mg	2
M	Water	-	-	-

The Phospholipid capsules were taken as stated daily for seven days
Group M served as control and took only water 500ml with no drugs

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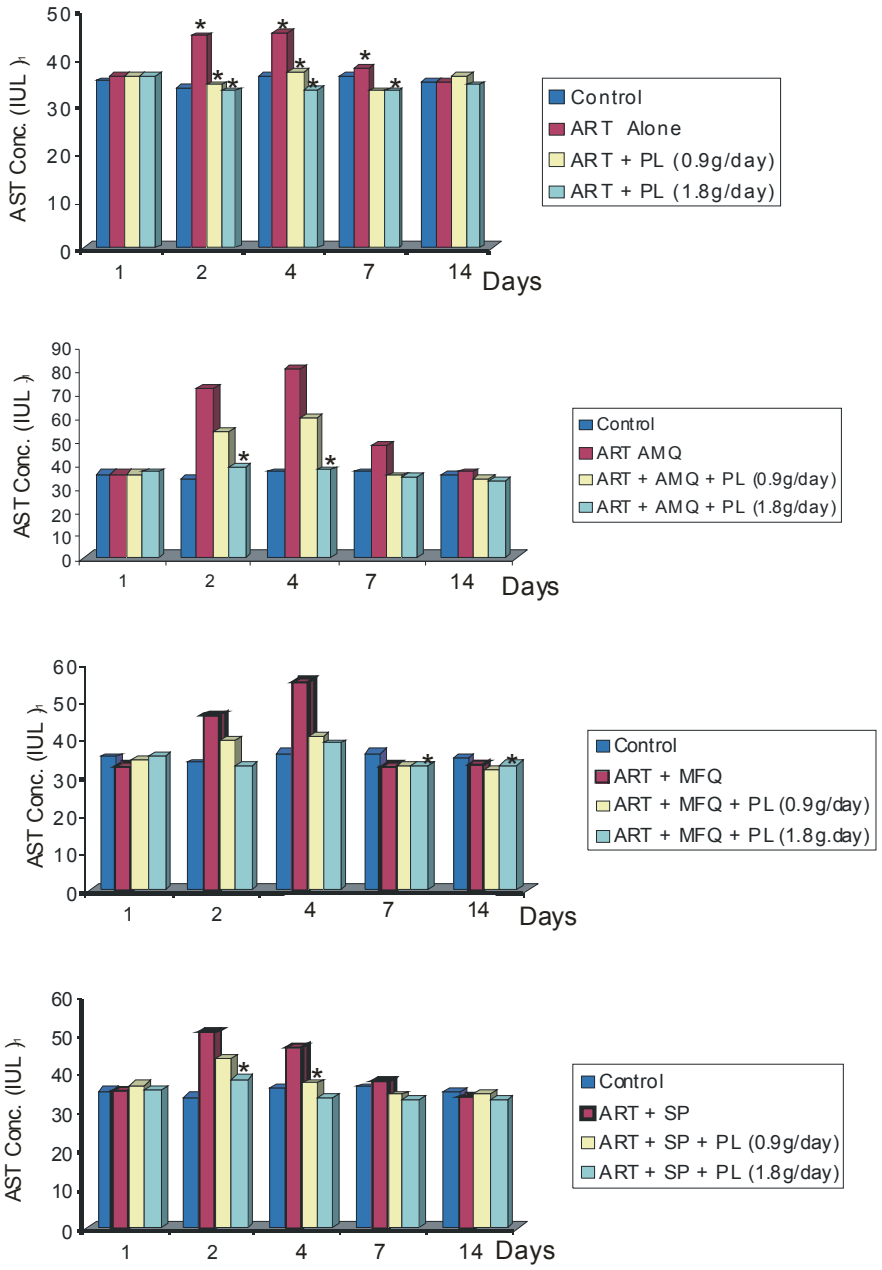

Figure 1: Effect of half and therapeutic doses of phospholipids (PL) on the levels of Plasma Aspartate Aminotrasferase (AST) during co-administration with (a) ART (b) ART + AMQ (c) ART + MFQ (d) ART + SP). Data are expressed as mean. n = 8
* Significant at $p<0.05$.

2.6 Collection of Blood and Preparation of Plasma

The *in vivo* procedure used in the study was a WHO *in vivo* 7 days standard field test (WHO, 1973). 5.0 ml of blood was collected from each volunteer subject before drug administration on the first day. After administration of the drugs, 5.0 ml of blood was collected from each of the volunteer between 8 and 10 am on the 2nd, 4th, 7th and 14th days. The blood was put in heparinized tubes and allowed to stand for 5 minutes for equilibration at room temperature.

The fresh blood was put in a clean centrifuge tube and spun at 5,000 rpm for 20 min in a centrifuge machine (MSE England) to separate the plasma from the cells. The plasma was aspirated, transferred into a specimen bottle, and stored at -15 °C for a period not exceeding 24 hrs.

2.7 Quantitative Determination of AST, ALT and BIL

The prepared plasma samples obtained from the blood of volunteers were analyzed. The levels of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were measured using the end point calorimetric

method as explained in randox enzyme kits (Reitman and Frankel, 1957). The absorbance of the test solution was measured using an UV-vis-NIR spectrophotometer with a wavelength of 540 nm at 37 °C against sample blank. Enzyme activity is given in International Units per Liter (IUL^{-1}).

Bilirubin was measured using the end point colorimetric method as explained in Randox kits based on that described by Jendrassik and Grof 1938. Total bilirubin (TBIL) was measured at a wavelength of 580nm at 25 °C against sample blank, while direct bilirubin (DBIL) was estimated at wavelength of 550 °C at 25 °C against sample blank. Amount of bilirubin was measured in µmol/l.

2.8 Statistical Analysis

The values obtained were expressed as percentage increase (+) or decrease (-) of the mean values of the blank. The data obtained were expressed as mean ± SD. Students t-test was used to assess statistical significance, values of $P < 0.05$ were considered to be significant.

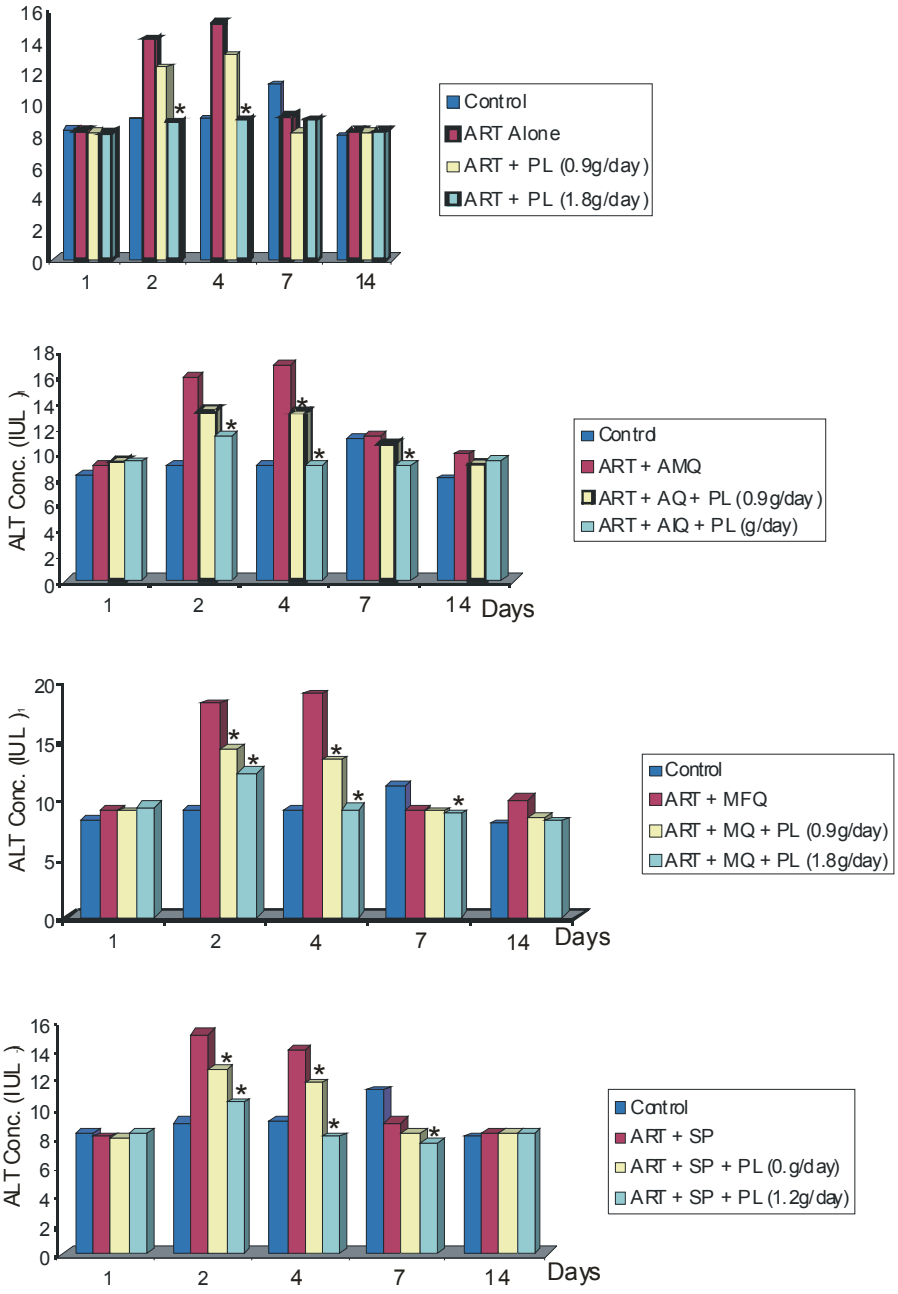

Figure 2: Effect of half (0.9g/day) and therapeutic doses (1.8g/day) of phospholipids (PL) on the levels of plasma Alanine Aminotransferase (ALT) during co-administration with (a) Artesunate (ART) (b) ART with amodiaquine (AMQ) (c) ART with mefloquien MFQ (d) ART with sulfadoxine – pyrimethamine (SP). Data are expressed as mean . n = 8
*Significant at $p<0.05$.

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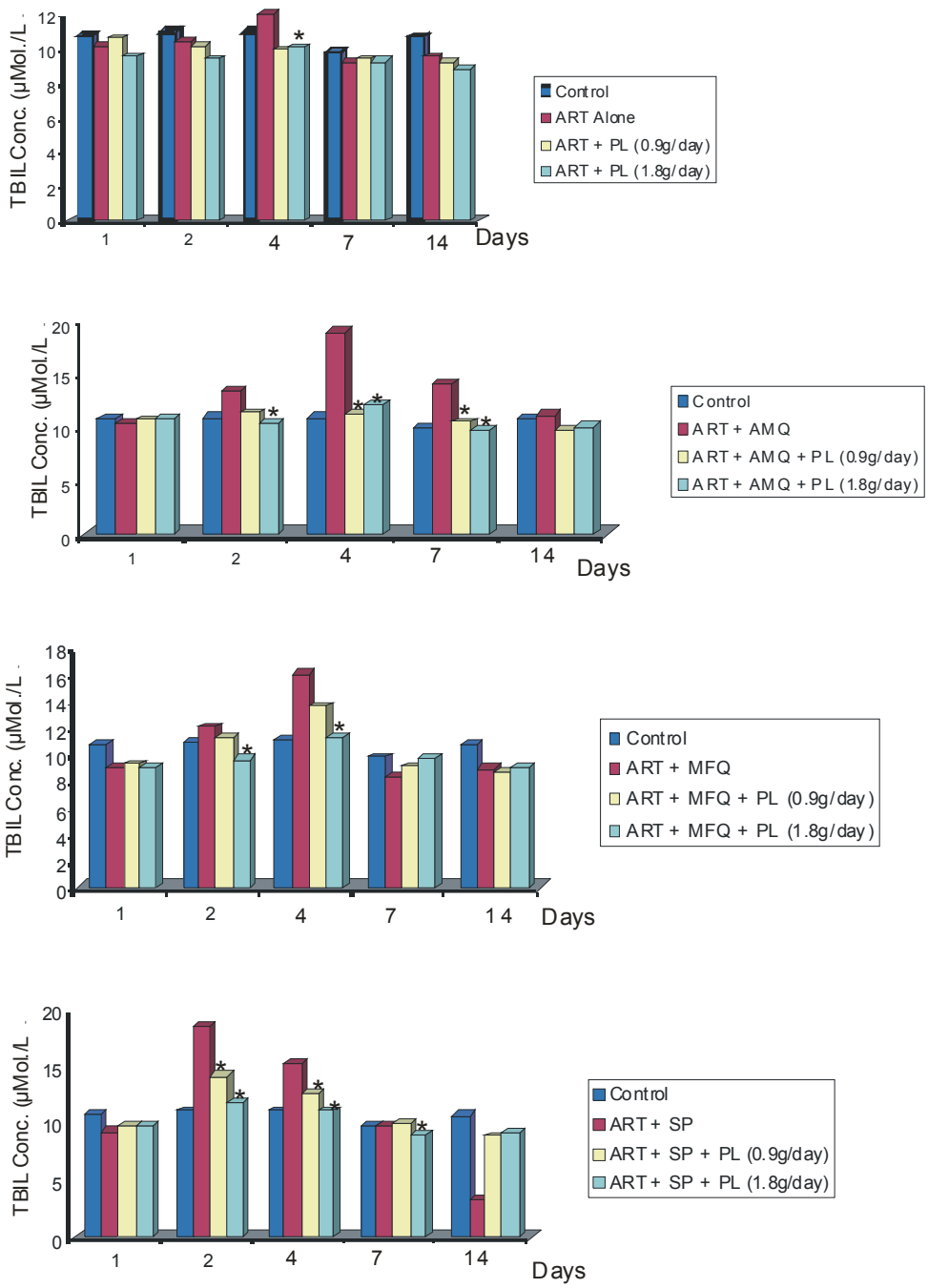

Figure 3: Effect of half (0.9g/day) and therapeutic doses (1.8g/day) of phospholipids (PL) on the level of total bilirubin (TBIL) in plasma during co-administration with (a) Artesunate (ART) (b) ART with amodiaquine (AMQ) (c) ART with mefloquine(MFQ) (d) ART with sulfadoxine – pyrimethamine (SP). Data are expressed as mean. n = 8
* Significant at $p<0.05$

3. Results and Discussion

Since healthy volunteers were used for the studies, the increase or decrease in the various parameters were expressed as percentage, relative to the blank samples. The antimalarial ART and ACT administered were normal therapeutic doses as are used in the treatment of malaria. The phospholipids were administered as therapeutic doses 1800 mg/24hrs and half therapeutic doses 900 mg/24 hrs. **Table 2** (Supporting Information) shows the effect of ART alone, and ART with AMQ, MFQ and SP, respectively, on the levels of plasma AST. On the 2nd and 4th days of the studies the levels of AST with ART alone was about 25% higher than the mean of the control values. When the ACTs were used the increase was between 43% to 118% of the control. This value is statistically significant ($P < 0.05$). This elevated activity is an indication of liver dysfunction (Murray et al, 1990). The co-administration of ART and the ACTs with half therapeutic doses, and therapeutic doses of phospholipids caused a dose dependent decrease in the concentration of AST figure 1: (a-d).

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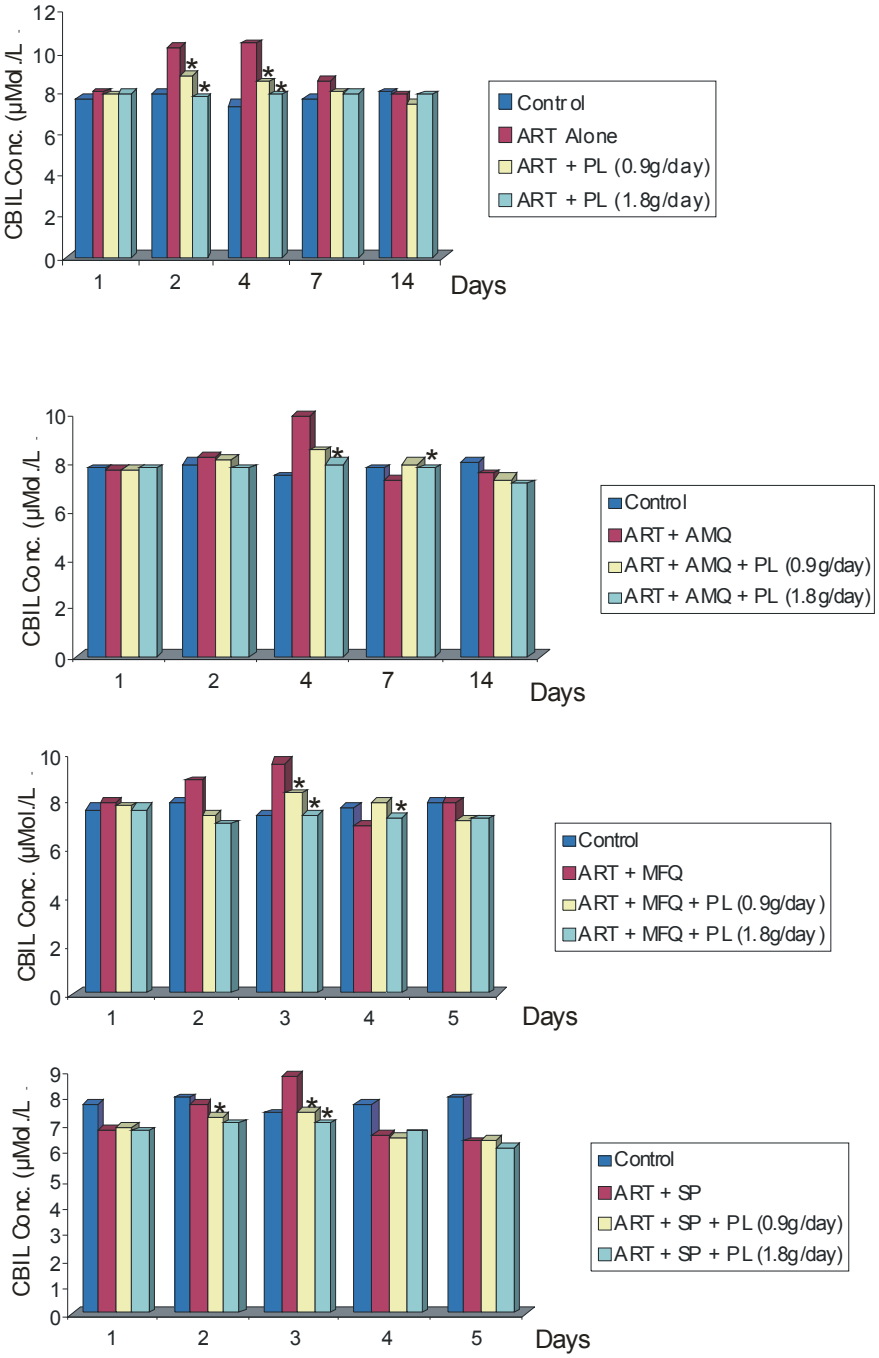

Figure 4: Effect of half dose(0.9g/day) and therapeutic dose (1.8g/day) of phospholipids (PL) on the level of conjugated bilirubin (CBIL) in plasma during co-administration with (a) Artesunate (ART) (b) ART with amodiaquine (AMQ) (c) ART with mefloquine (MFQ) (d) ART with sulfadoxine – pyrimethamine (SP). Data are expressed as mean. n = 8
* Significant at $p<0.05$ relative to control

Neither artesunate as a monotherapy drug nor its combination therapy with other antimalarials which were studied affected the concentrations of the liver enzymes AST and ALT beyond the normal physiological levels of 5 to 40 IUL^{-1}. However, the effect they had on each group of volunteers was converted to percentage change increase or decrease relative to the blank value at the start of the studies and any difference between ±4.5% for AST, ±3.6% for ALT, ±4.2% for CBIL and ± 6.0% for TBIL were taken as normal physiological change or variation between individuals. Volunteers who took only artesunate had an average increase in ALT levels of 59% on the 4th day of measurement. On this same day, volunteers administered with artesunate plus MFQ had the highest average increase of 108%, followed by AMQ 80.70%, but SP was 75% on the second day, (**Table 3**; Supporting Information). All these values were within normal physiological levels,

but the increase were statistically significant. For normal subjects, the use of the ACTs is quite in order, but for patients with liver problems or the elderly, the drug combination should be administered with caution and liver function test should be performed before drug administration. Also the combination could be co-administered with PL since it causes a dose depended decrease in the level of plasma ALT (**Figure 2 a –d**).

Normal physiological variation of TBIL was within ±6.0% of the mean value of control. ART alone caused a mean increase in TBIL of 17.60% while in combination with AMQ the mean increase was 80.0% on the 4th day of the studies. On the same day, the increase caused by MFQ combination was almost the same with that of AMQ 78.88%. The combination with SP showed an abnormally high value of 98.91% on the second day of measurement (**Table 4**; Supporting Information).

The normal physiological level of conjugated bilirubin is up to 4.3 $\mu mol.L^{-1}$ and that of total bilirubin is up to 17 $\mu mol.L^{-1}$ (Marshall and Bangert, 2009). Artesunate alone and with its partner drugs in ACTs all caused an increase in the levels of both conjugated and total bilirubin above the normal physiological levels. The mean values of conjugated bilirubin for most of the groups in the studies were at the upper end of the normal range. Artesunate as a monotherapeutic agent caused an increase of 2.45 $\mu mol.L^{-1}$ (28%) in the level of conjugated bilirubin on the 4th day of measurement. On this same day, the administration of ART plus MFQ caused a marginal increase of 31.52% while with AMQ an increase of 36.63% was observed. A combination of artesunate with SP caused a significant increase of 70.87% in the level of conjugated bilirubin (**Table 5**; Supporting Information).

High levels of CBIL indicates that bile is not being properly excreted, therefore an obstruction may be present in the bile duct or gall bladder. High levels of unconjugated bilirubin indicates that too much haemoglobin is being destroyed or that the liver is not actively treating the haemoglobin it is receiving (Craziadei, 2011). From these studies, the combination of artesunate with sulfadoxine-pyrimethamine caused a significant increase in the concentration of both conjugated and total bilirubin. This value is of clinical importance when deciding on a combination regimen for patients who are anemic or those with liver problems. However, the coadministration of artesunate with folic acid reduces these effects (Udobre et al, 2009).

4. Conclusion

From the results of these studies, we conclude that the administration of therapeutic doses of artesunate as a monotherapeutic agent may be toxic to the liver by causing the elevation in the plasma levels of hepatic enzymes and bilirubin. The co-administration of therapeutic doses of ART with its partner drugs as ACT aggravates these effects by increasing the levels of these hepato-biochemicals. However, the co-administration or post-administration of the ACTs with therapeutic doses of phospholipids cushions the effect (**Figure 1-4**).

Conflict of Interest declaration

The authors declare no conflict of interest

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