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Original Research Article

Toxicological evaluation of five brands of Artemether-Lumefantrine drugs in male albino Wistar rats

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[1]*Etim, O.E., [1]Ndem, J. I., [1]Ewere, E.G. and [1]Bassey, U.E.

[1]Department of Biochemistry, University of Uyo, P.M.B. 1017, Uyo, Nigeria

*Corresponding Author Email: betobong123@yahoo.com

Tel.: +234-8037928151

Comparative effects of five different brands of Artemether-Lumefantrine antimalaria drugs on liver and kidney function parameters and haematological indices in male albino *Wistar* rats were investigated. The medications were Coartem, Lumartem, Combiart, Amatem forte and Famter. Thirty (30) male albino Wistar rats with average weight of 155g were used. They were divided into six groups of five rats each. Therapeutic doses of artemether-lumefantrine were administered orally for three days at 8mg/kg body weight (bw). The results showed that significant (P<0.05) decrease occurred in total bilirubin in all the treated groups, and in aspartate aminotransferase (AST) in groups treated with Amatem forte and Famter when compared with control. Significant (P<0.05) decrease occurred in red blood cell (RBC) count, haemoglobin concentration (HGB), haematocrit (HCT) in groups treated with Coartem and Lumartem while mean cell volume (MCV) and mean cell haemoglobin (MCH) decreased significantly (P<0.05) in groups treated with Coartem alone. Significant (P<0.05) decrease also occurred in chlorine alone in all the treated groups except in group treated with Coartem. Some brands of medications appeared not to be deleterious to tissues except Coartem and Lumartem which seem to cause haematological derangements. Hence, caution in their usage is advised.

Key words: Artemether-Lumefantrine combination, haematological adverse effect, kidney function, liver function.

INTRODUCTION

Malaria transmission is a global health problem. In 2013, estimated 198 million cases of malaria occurred and the disease led to 548,000 deaths (WHO, 2014). There are four major species of plasmodium namely: *Plasmodium falciparum, P. malariae, P. vivax and P. ovale*. *Plasmodium falciparum* is the deadliest and is responsible for about 80% of all malaria cases (WHO, 2014). The parasite spends most of its life cycle in the red blood cells of human. The female anopheles' mosquito transmits the parasite by first ingesting them when feeding on an infected person's blood and then injecting them when biting another person (Agrawal et al., 2005).

Malaria is the second most common cause of infectious disease-related death in the world after tuberculosis (WHO, 2000). Each attack may last about 5 to 15 days often incapacitating the victim. In highly endemic areas, most cases of severe malaria occur among children aged between six months to five years with the highest mortality in those between one and three years of age. Another risk group in endemic areas is pregnant women who become susceptible to severe infection due to diminished cellular and humoral immunity during pregnancy (Anorlu et al*.,* 2001; Okwa, 2003; Adefioye et al., 2007; Uneke, 2008).

The development of drug resistance has caused the evolution of the use of different therapies for the treatment of the ailment. Available evidence show that traditional and herbal medicine have been used for the treatment of the ailment from time immemorial and have continued to play a significant role in the general provision of good health to people over the world (Farombi, 2003).

There are several families of approved drugs used in treating and preventing malaria parasite. Artemether-Lumefantrine was the first fixed-dose artemisinin-based combination therapy (ACT) recommended and pre-qualified by the World Health Organization (WHO) for the treatment of uncomplicated falciparum malaria (WHO, 2008). It has been shown to be effective in areas with multi-drug resistance to *P. falciparum* such as sub-Saharan Africa and south-east Asia. It is currently recommended as first-line of treatment for uncomplicated falciparum malaria in several countries.

The emergence of the ineffectiveness of antimalarial drugs in combating malaria led to the additional studies which produced newer and more effective anti-malaria drugs, artemesinin-based combination therapies (ACTs). WHO has recommended ACTs to be the first-line therapy for *Plasmodium falciparum* malaria worldwide (WHO, 2010). Artemisinin combination therapies are effective because the artemisinin component kill the majority of parasites at the start of the treatment, while the more slowly eliminated partner drug clears the remaining parasites (White, 1999). Several fixed-dose ACTs are now available containing an artemisinin component and a partner drug which has a long half-life, such as lumefantrine, mefloquine, amodiaquine, sulfadoxine/pyrimethamine etc.

However, Artemether- Lumefantrine combination being the commonest and most available and affordable of the ACTs has become the choice of the ACT in this study. Five different brands of ACT manufactured by five different companies were administered to the experimental animals. Furthermore, it has been observed that patients complain of specific adverse effect attributed to certain brands of artemether-lumefantrine combination and this informed the rational for the present study. The effect of administration of five different brands of artemether-lumefantrine combination was evaluated with emphasis on the liver, kidney and blood.

The five brands of artemether/lumefantrine used in this study include Coartem, Combiart, Amatem forte, Lumartem and Famter.

MATERIALS AND METHODS

Drugs Preparation

The standard antimalarial drugs (ACT) used were obtained from Leadson's Pharmacy, in Uyo, Nigeria. The drugs were five different brands of artemether- lumefantrine combination which include: Coartem, Lumartem, Combiart, Amatem forte and Famter. Therapeutic doses of artemether-lumefantrine were administered orally for three days at 8mg/kg body weight (bw).

Experimental Animals

Thirty male albino Wistar rats with average weight of 155g (weight range of between 145g to 165g) were used for the study. They were divided into six groups of five rats each. The animals were obtained from University of Nigeria, Nsukka. The animals were fed with rat pellets produced by Grand Cereals Ltd, Onitsha, Nigeria. The rats were maintained under standard conditions. Ethical approval for the study was sought and obtained from the Ethical committee of the Faculty of Basic Medical Sciences, University of Uyo, Nigeria prior to the commencement of the study.

Experimental Design

Group 1 served as control and was fed with normal rat pellets and water *ad libitum.* Groups 2 to 6 were treated with therapeutic doses i.e. 8mg/kg bw of Coartem, Lumartem, Combiart, Amatem forte, and Famter respectively.

Animal Sacrifice and Preparation of Sera for Analysis

On the third day, animals in both experimental and control groups were placed in a glass jar containing cotton wool dipped into chloroform for general anaesthesia. The animals were dissected, and using a sterile needle and syringe, blood samples were obtained, by cardiac puncture and transferred into non–heparinized sample tubes. After coagulation, the blood samples were spun at 2000 rpm for 10 minutes using a bench top centrifuge (MSE Minor, England). The sera were carefully transferred into clean sample tubes and stored in refrigerator at 4^0C for further analysis.

Biochemical Analyses

The serum activity of AST was determined by Kinetic Method (Young, 1990) while the serum activity of ALT was determined by colorimetric endpoint method described by Young et al., (1975). The method of Doumas et al., (1979) was adopted to assay for total and direct serum bilirubin. The serum concentrations of creatinine and urea were estimated by spectrophotometric method described by Henry, (1974) and Wybenga et al., (1971) respectively.

The serum Potassium, sodium and Chloride were estimated using automated ion selective electrode machine (Lanwing electrolyte analyzer LWE60D, Germany) while the serum concentration of bicarbonate was estimated according to the spectrophotometric method of Young, 1990.

Haematological Analyses

Haematological analyses were conducted using Sysmex Automated Haematology Analyser Model KX-21N (America). The parameters analysed include Red blood cell count (RBC), White blood cell count (WBC), Haemoglobin concentration (HGB), Hematocrit (HCT) or packed cell volume (PCV), Platelets (PLT), Mean cell volume (MCV),

Table 1. Mean levels of Serum AST, ALT and Bilirubin in male albino Wistar rats treated with five different brands of Artemeter-Lumefantrine drugs

Group	Direct Bilirubin (µmol/l)	AST (IU/L)	ALT (IU/L)	Total Bilirubin (µmol/l)
Control	2.050±0.273	198.313±8.143	41.866±2.496	12.272±0.300
Coartem	1.804±0.250	194.229±6.899	39.336±1.313	7.030±0.309*
Lumartem	5.002±0.089*	184.644±6.875*	44.094±0.701	6.228±0.322*
Combiart	3.526±0.428	199.779±8.003	48.284±1.319	7.338±0.089*
Amatem forte	2.132±0.119	184.596±4.617*	41.136±1.941	7.893±0.333*
Famter	2.623±0.195	174.964±8.395*	41.630±2.388	7.462±0.089*

Values are presented as mean± SEM, n=5, * represents significance at P< 0.05 compared to control using student t- test and Least Significant Difference (LSD) multiple post hoc comparison test.

Table 2. Effects of five Different Brands of Artermether-Lumefantrine drugs on Kidney function in male albino Wistar rats

Group	Creatinine (mmol/dl)	Urea (mmol/dl)	HCO_3^- (mmol/dl)	Na^+ (mmol/dl)	K^+ (mmol/dl)	Cl^- (mmol/dl)
Control	79.10 ± 3.87	4.2 ± 2.41	58.0 ±8.51	137.8 ± 1.10	4.0 ± 0.42	106.0 ± 2.23
Coartem	76.01 ± 2.45	4.6 ± 3.71	54.4 ± 2.61	138.2 ± 2.49	4.3 ± 0.25	103.0 ± 2.92
Lumartem	76.50 ± 1.28	1.7 ± 1.71*	55.0 ± 6.08	139.8 ± 2.86	3.68 ± 0.61	100.8 ± 3.70*
Combiart	76.77 ± 3.41	3.6 ± 2.17	61.6 ± 8.53	138.4 ± 1.67	4.28 ± 0.44	101.4 ± 1.34*
Amatem forte	76.11 ± 3.70	2.8 ± 1.67*	54.8 ± 5.63	139.4 ± 2.30	3.94 ± 0.37	100.0 ± 3.74a*
Famter	77.46 ± 1.92	1.7 ± 0.44*	56.2 ± 3.70	140.2 ± 3.49	4.5 ± 0.33	101.8 ± 2.49*

Values are presented as mean± SEM, n=5, * represents significance at P< 0.05 compared to control using student t- test and Least Significant Difference (LSD) multiple post hoc comparison test.

Mean cell haemoglobin (MCH), Mean cell haemoglobin concentration (MCHC).

Statistical Analyses

Data were analysed using Microsoft SPSS statistical software package version 20.0 and results expressed as mean ± standard error of mean (SEM). Student t-test, ANOVA and Least Significant Difference (LSD) multiple post hoc comparison test were carried out on the data and Mean difference between groups were considered statistically significant ant $p<0.05$.

RESULTS

Effect on liver function

Results obtained for liver function analyses revealed that treatment with the five different brands of drugs significantly ($p<0.05$) decreased serum total bilirubin levels compared with control. There were no significant ($p>0.05$) differences in the serum ALT levels when all treatment groups were compared with control. Treatment with Lumartem showed significant ($p<0.05$) increase in the serum levels of direct bilirubin compared with control. Lumartem, Amatem forte and Famter significantly ($p<0.05$) increased serum AST levels compared with control as shown in Table 1 above.

Effects on Kidney Function

Serum concentrations of urea, creatinine and electrolytes were analysed to ascertain kidney function. Significant ($p<0.05$) decreases in serum urea levels were observed in Lumartem, Amatem forte and Famter treated groups compared with control. There were also significant ($p<0.05$) decreases in serum chloride levels in the groups treated with Lumartem, Combiart, Amatem forte and Famter compared to control as shown in Table 2 above.

Effect on haematological parameters

From the results obtained, there were no significant ($p>0.05$) differences in WBC count and MCHC across all treatment groups compared with control. Treatment with Coartem and Lumartem induced significant ($p<0.05$) decreases in RBC count, HGB concentration and haematocrit compared with control. The groups treated

Table 3. Effects of five Different Brands of Artermether-Lumefantrine drugs on Haematological Indices in male albino Wistar rats

Groups	Control	Coartem	Lumartem	Combiart	Amatem	Famter
WBC ×10³	11.58 ± 0.79	16.55 ± 1.51	17.08 ± 0.40	15.88 ± 1.28	18.13 ± 1.54	17.01 ± 1.54
RBC × 10⁶	7.83 ± 0.14	* 7.40 ±0.25	* 7.38 ±0.18	7.86 ± 0.28	7.81 ± 0.24	8.21 ± 0.14
HGB (g/dl)	13.84 ± 0.24	*12.22 ± 0.21	*12.48 ± 0.22	13.22 ± 0.34	13.32 ± 0.39	13.90 ± 0.19
HCT (%)	51.94 ± 0.52	*46.40±0.80	*46.82±0.78	49.94 ± 1.39	49.22 ± 1.58	51.96 ± 0.75
PLT × 10³	866.60 ±38.34	*775.60 ±7.70	829.80 ±35.35	917.25 ±29.45	*791.00±24.84	863.40 ± 27.63
MCV (fc)	66.42 ± 1.15	62.82 ± 1.11	63.44 ± 0.53	63.66 ± 1.29	*63.24±0.59	63.32 ± 0.65
MCH (pg)	17.72 ±0.14	*16.52 ±0.31	16.92 ±0.17	16.86 ±0.19	17.06 ±0.15	16.94 ±0.08
MCHC(g/dl)	26.62 ±0.28	26.34 ±0.12	26.66 ±0.15	26.48 ±0.28	26.98 ±0.32	26.76 ±0.28

Values are presented as mean± SEM, n=5, * represents significance at P< 0.05 compared to control using student t- test and Least Significant Difference (LSD) multiple post hoc comparison test.

with Amatem and Coartem showed significant ($p<0.05$) decreases in MCV and MCH respectively compared with control. Also, treatment with Coartem and Amatem led to significant ($p<0.05$) decreases in PLT counts compared with control as shown in Table 3 above.

DISCUSSION

Differences in the effect of different brands of artemisinin-based drugs on various biochemical parameters have been observed in albino Wistar rats (Etim et al., 2016). The result obtained revealed that the rats administered with different brands of artemisinin at different concentrations based on body weight responded differently when compared with control (subjects receiving only feed and water). This in addition to the observed situation where patients complain of specific adverse effect attributed to certain brands of the same ACT combination informed the conduct of this study. The effects of the different brands of Artemether-Lumefantrine antimalaria drugs on biomarkers of liver function, kidney function and haematological indices were therefore investigated in albino Wistar rats.

Liver enzymes are well known biomarkers for the prediction of liver toxicity (Gray and Howorth, 1982; Rahman et al., 2001). Available evidences show that damage to liver cells results in elevations of these enzymes (such as AST, ALT and ALP) in the serum (Wolf et al., 1972) and the measurement of enzyme activities is of clinical and toxicological significance in determining liver damage by toxicants or in diseased conditions (Singh et al.,2001).

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are members of transaminase family of enzymes and are also known as aminotransferases. These enzymes catalyze the transfer of amino groups between L-alanine and glutamate for biochemical purpose of transamination. ALT and AST are found in large amounts in the liver and in small amounts in the heart, kidney and muscles. When the liver is injured or inflamed due to exposure to various toxic substances, the level of ALT and AST in the blood are usually elevated. The level of these enzymes in the blood is directly related to the liver damage (Giannini et al., 2005).

In the present study, AST activity decreased significantly ($p<0.05$) in groups treated with lumartem, amatem forte and famter when compared to the control. This is not considered to be abnormal as it is within normal range. The observed increases in the activity of ALT in groups treated with lumartem and combiart were not significant when compared with the control.

Elevated level of direct bilirubin was observed in group treated with lumartem when compared to control. Increased serum direct bilirubin causes conjugated bilirubinaemia which is one of the earliest signs of impaired hepatic excretion (Thapa and Walia, 2007). In most cases of jaundice in adult, both conjugated and unconjugated fractions of bilirubin are increased in plasma but conjugated bilirubin predominates (Crook, 2012). Significant decreases were also noticed in total bilirubin in all the treated groups. In healthy people, conjugated bilirubin is virtually absent from serum mainly because of the rapid process of bile secretion (Green and Flamm, 2002). The present study has shown that the administration of the various brands of artemether-lumefantrin resulted in significantly decreased level of total bilirubin and a non-significant difference in direct bilirubin (except lumartem treated group where direct bilirubin was significantly elevated) when compared to the control.

All known higher life forms require an electrolyte balance between the intracellular and the extracellular environments. In particular, the maintenance of precise osmotic gradients of electrolytes is important. Such gradients regulate the hydration of the body as well as blood pH and are critical for nerve and muscle function. Various mechanisms (such as tubular reabsorption) exist in living species that keep the concentration of different electrolytes under tight control (Coso et al., 2008). Correction of fluid volume and electrolyte deficits is the standard of care for critically ill patient, including those of severe *falciparum* malaria patients.

In the present study, the non-significant increases and significant decreases observed for the various kidney function parameters (such as urea, creatinine and electrolytes levels) could suggest a non-impairment of

kidney function following treatments with the different brands of drugs. Serum levels of most of these parameters are known to be elevated in conditions of kidney impairment (Bishop et al., 2000).

Preclinical data suggested that repeated exposure to artemisinin results in deranged biochemical parameters (such as AST and ALT) and may lead to decreased red blood cell count and predispose patients to anaemia (Obianime et al., 2011). Our findings show that some brands of Artemether-Lumefantrine combination has significant effects on some of the haematological parameters in male albino Wistar rats. The observed significant decreases in RBC count, haemoglobin concentration and haematocrit in groups treated with Coartem and Lumartem and the significant decreases in MCV in Amatem forte group and MCH in groups treated with Coartem respectively may be due to suppression of bone marrow function which may result in anaemia. Anaemic condition which may be a consequence of iron deficiency (Palande, 2011) and unavailability (Gupta, 2014) may be as a result of coagulation of iron with Artemether-Lumefantrine there by rendering the iron insufficient. The depletion of iron reduces the synthesis of haemoglobin in the bone marrow which in turn result in decrease in these two parameters (haemoglobin concentration and RBC counts) (Etim et al., 2016).

Packed cell volume (or haematocrit) is the measure of percentage of red blood cells in whole blood (Cellmate Wellness System, 2002). The significant ($p<0.05$) decrease in HCT in Groups 2 and 3 (coartem and lumartem) compared with control (Group 1) could be as a result of decreased haemoglobin concentration which may be due to decreased storage of iron in the liver (Murray et al., 1988). This decrease in packed cell volume can also be due to inability of bone marrow to synthesize haemoglobin owing to less availability of iron and this may result to anaemia (AlNahari, 2014).

The significant decreases in platelet count as seen in Groups 2 (Coartem) and 5 (Amatem forte) may be caused by disorders in platelets production or conditions in which platelets are used up or destroyed faster than normal and this may result to thrombocytopenia, which could lead to impairment of the normal physiological activities of the system (Stiene-Martin et al., 1998). The observed significant ($P<0.05$) decrease in platelets may also inhibit the formation of platelet plugs vital for the prevention of haemorrhage at the site of injuries as well as loss of integrity of the capillaries (Hounkpatin et al., 2012).

CONCLUSION

From the present study, some of the artimether-lumafantrine brands (such as Combiart, Amatem and Famter) appeared to be safe for malaria treatment / management except Coartem and Lumartem which were linked to haematological disturbances. Thus, caution in their use is strongly advocated. If these changes are due to any flaw in the manufacturing process or the raw materials being used for manufacturing, these issues should be rectified. Regulatory agencies should enforce stringent quality control criteria to prevent such formulation from reaching the patients.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of the paper.

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