African Journal of Pharmacology and Therapeutics Vol. 2 No. 2 Pages 48-53, 2013
Open Access to full text available at http://www.uonbi.ac.ke/journals/kesobap/

Research Article

Renal impact of sub acute lamivudine-artesunate treatment in wistar rats

Temidayo O. Olurishe [a,*], Helen O. Kwanashie [a], Joseph A. Anuka [a], Haruna M. Muktar [b], and James S. Sambo [c]

[a] *Department of Pharmacology & Therapeutics, Ahmadu Bello University, Zaria-Nigeria;*
[b] *Department of Haematology & Blood Transfusion, ABUTH, Zaria-Nigeria;*
[c] *Department of Veterinary Pathology, Ahmadu Bello University, Zaria-Nigeria.*

* **Corresponding author:** Department of Pharmacology & Therapeutics, Ahmadu Bello University, PMB 1045, Zaria-Nigeria; **Tel**: +234-803-6005775; **Email**: temiolurishe@yahoo.com

Background and objectives: Lamivudine and artesunate are life saving drugs in the treatment of HIV/HBV and malaria respectively, and available data shows artesunate having anti-tumour properties. The concurrent administration of both drugs presents important safety concerns. This study investigated possible effects of lamivudine-artesunate co-administration on renal function and histology in wistar rats.

Method: Four groups of rats (n=5) were used in the study with one group as control. Two groups received lamivudine at 20 $mgkg^{-1}$, with another receiving artesunate at 10 $mgkg^{-1}$. Artesunate was added to one of the lamivudine groups. While lamivudine treatment was for three weeks, artesunate was introduced only in the last week of the study alone, or in combination with lamivudine. At termination, animals were humanely killed and kidneys harvested, weighed and subjected to H and E stain and observation. Serum urea and electrolytes were also determined.

Results: Serum biomarkers and kidney weights did not differ significantly ($p>0.05$). Various histological changes were observed in the treated groups although these didn't directly correlate the biomarkers determined.

Conclusion: The concurrent use of lamivudine and artesunate appears to be safe within the dose levels used. However caution may be needful when repeated or long term exposure is required.

Keywords: artesunate, lamivudine, HIV, HBV, malaria, concurrent drug therapy

Received: January, 2013
Published: June, 2013

1. Introduction

Renal pathology associated with malaria, ranging from electrolyte imbalance to more serious incidents with acute renal failure are part of complications that occur with *Plasmodium falciparum* infection (Naqvi et al, 2003). The kidney performs several excretory and regulatory functions including blood pressure control and maintenance of extracellular environment (Ferguson and Waikar, 2012). The high extent of delivery of materials including xenobiotics, via their passage through the tubular lumen contributes to renal injury resulting from several clinically used drugs, and experimental toxins alike (Hagos and Wolff, 2010), with the metabolic and excretory roles of the kidney making it vulnerable to various forms of renal injury (Perazella, 2009). Consequently, alteration in structural components of the kidney, which may result from disease or toxicological effects of drugs/xenobiotics may result in deleterious effects that often affect optimal function. Alterations in renal function leading to increase or decrease renal clearance may result in changes in blood concentrations and pharmacological responses (Doogue and Polasek, 2011).

Artesunate is an antimalarial drug used for the treatment of falciparum malaria in regions of malaria endemicity. It has been used in combination with other

 ISSN 2303-9841

antimalarial drugs due to evidence of enhancement of efficacy and delay in development of resistance (Taylor et al, 2003). However it is still used as monotherapy in many countries including some regions in Asia for the treatment of uncomplicated malaria (Saunders et al, 2012). In addition to its anti malarial action, artesunate is also being considered a candidate anti tumour drug due to evidence of its effect against several tumour cell lines including pancreatic (Du et al, 2010), hepatic carcinoma (Wang et al, 2002), with recent clinical trials showing its effects against breast and colon cancers (Liu et al, 2010). This increases its propensity for use with existing drugs in comorbidities. However its current use along with lamivudine, an antiretroviral agent used in the treatment of Hepatitis B infection (HBV) in Africa due to the rising prevalence of the infection, requires important safety assessment.

Artesunate has been established to partly owe its antimalarial action to the formation of free radicals. Much data in the literature shows that reactive oxygen species (ROS) and oxidative stress are implicated in nephropathy (Haugen and Nath, 1999; Manucha, 2007; Ghosh et al, 2010). Lamivudine is used in HIV therapy and also included in prophylaxis for both HIV and HBV infection. It is also safely used in HBV even in early pregnancy (Yi et al, 2012). With the geographic overlap of malaria, HIV and HBV, concurrent therapy with these life saving drugs is often expedient. We thus hypothesize that the concurrent use of lamivudine and artesunate may result in nephrotoxic consequences that may lead to structural and functional changes in the kidney. Hence this study investigated the renal consequences of lamivudine-artesunate co-administration in healthy adult wistar rats.

2. Materials and Methods

2.1 Animals and Animal Care

Animals were obtained from the animal house of the Department of Pharmacology and Therapeutics Ahmadu Bello University, Zaria, Nigeria. Adult wistar rats of either sexes (n=5) weighing on the average 220-228 g were used for this study. Animals were treated according to the NIH animal care protocol and the CPCSEA 1986 guidelines; and also in accordance with approved institutional conventions. The animals were acclimatized in the experimental room for about two weeks before commencement of the study. Animals were placed on standard animal diet (Vital) and water *ad libitum* for the duration of the experiment. Wood shavings were used as beddings and these were regularly changed.

2.2 Experimental Design

The experimental design for the study was based on four groups of rats receiving treatment as follows. Group I served as vehicle control (saline control) while group II received lamivudine at a dose of 20 mg/kg. Group III received lamivudine at 20 mg/kg and also artesunate 10 mg/kg, while group IV received only artesunate at a dose of 10 mg/kg. The experimental duration was twenty one days, and all animals on lamivudine received treatment for the entire experimental duration. Animals that received artesunate either alone, or in combination with lamivudine were treated similar to the control animals for the first 14 days, and received artesunate only in the last seven days of the study. Artesunate was administered only for seven days because most artesunate containing antimalaria therapies don't exceed seven days. All drugs were administered intraperitoneally. Lamivudine was obtained from Evans Nigeria, while artesunate was obtained from Tuyil, Ilorin Nigeria.

2.3 Biochemical Analysis

At the end of the twenty-one days of drug administration, animals were fasted overnight, weighed and euthanized with chloroform as described (Saxena et al, 2009). Blood was obtained via exsanguination through the jugular vein. The blood was collected into non heparinised vacutainers and centrifuged, and serum obtained was used for the determination of renal biomarkers. Determination of serum urea was based on the diacetyl monoxime method using thiosemicarbazide (Natelson 1951, Marsh et al., 1965). Potassium and sodium were determined using flame photometric methods, while chloride levels were determined using titrimetric methods.

2.4 Determination of Relative Kidney Weight and Histology

Immediately following blood collection, the kidneys of the animals were carefully removed and weighed using a digital denver weighing balance. Relative kidney weights were thereafter determined. The kidneys were immediately preserved in 10% formalin solution before the tissues were later processed for Hematoxylin and Eosin stained sections. The sections were observed and photomicrographs made are presented (**Figure 3A - 3C)**.

2.5 Statistical Analysis and Presentation of data

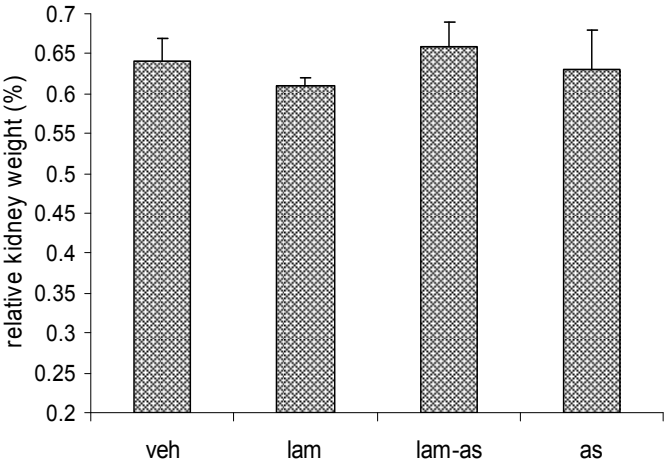
The data from the studies were subjected to One Way Analysis of Variance followed by Dunnett's post hoc test, with p values less than 0.05 being considered statistically significant. Data from the study are presented in charts as mean ± standard error of the mean. Photomicrographs are also shown to depict the observed histological changes in the kidney.

3. Results

Data from the study didn't show any significant difference in the relative kidney weights in comparison with the controls (**Figure 1**). There was also no statistically significant difference in the renal biomarkers that were determined when compared with the saline group (**Figure 2A – 2D**). Although there were slight differences observed in some of the groups in comparison with the control, this was not statistically significant. Various degrees of histological changes were observed in the renal architecture as observed from histological examination (**Figure 3A – 3D**). Some areas of necrosis and hypertrophized glomeruli were seen in sections of rat treated with lamivudine and artesunate. Rats that received artesunate alone showed regions of entire necrosis with collapsing glomeruli and some hypertophized glumeruli.

 ISSN 2303-9841

The kidney from lamivudine treated rats also showed some areas of inflammatory cell infiltration and abnormal glomeruli structures.



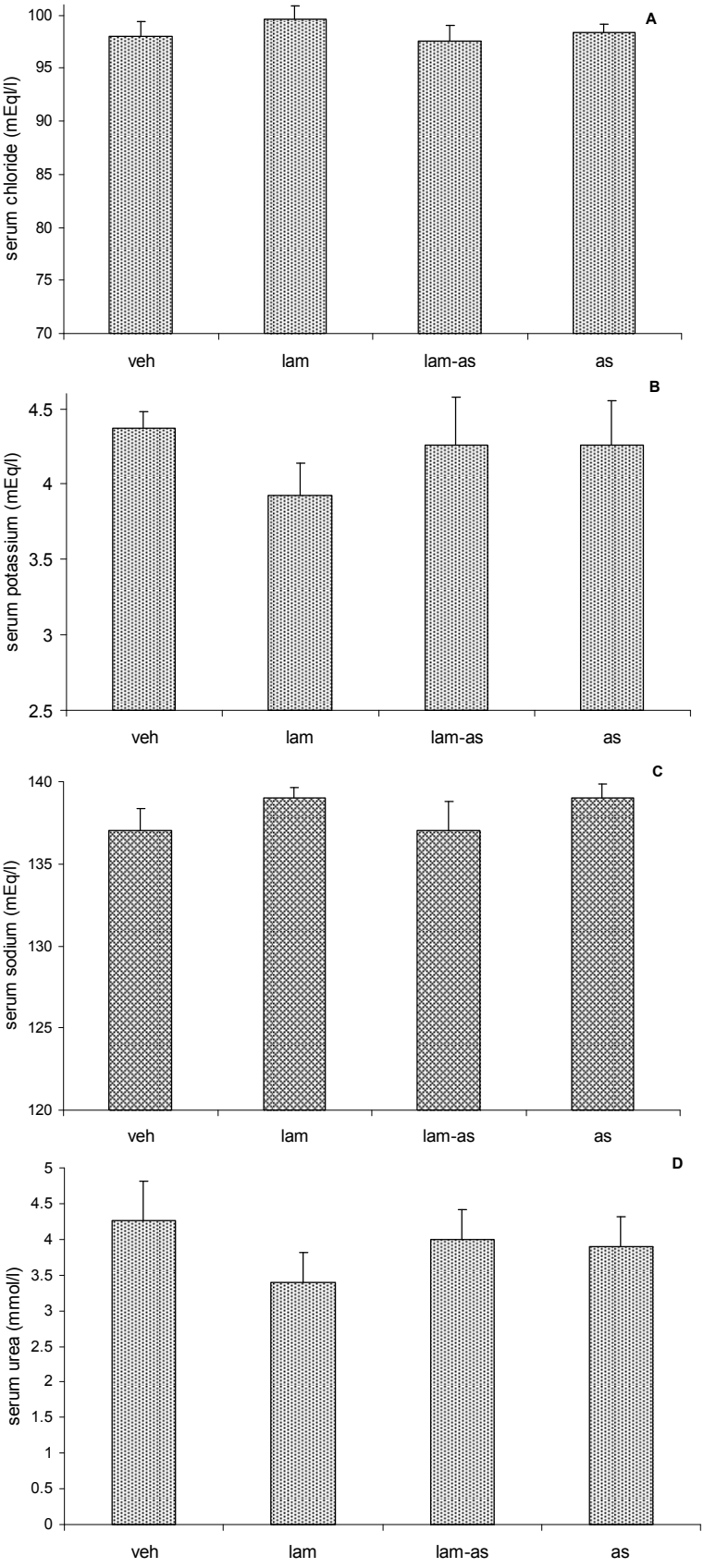
Data shown are mean ± SEM of (n=5).
There was no statistically significant difference following ANOVA and Dunnett's multiple comparison post-hoc test.
***Veh**=saline; **lam**=lamivudine; **lam-as**=lamivudine and artesunate; **as**=artesunate.*

Figure 1: Effect of lamivudine-artesunate treatment on relative kidney weights in rats

4. Discussion

Organ weights are an essential part of toxicological and risk assessment of drugs, chemicals and other biologicals (Michael et al, 2007). These results did not show any statistically significant differences in relative kidney weights in the rats. This is further corroborated by the absence of statistically significant differences in the values of serum electrolytes and urea in comparison with the control group. This thus indicates the relative safety of the drug combination on the renal system at the doses and route used in the current study. Previous data at a dose of 12 mg/kg, showed that intravenous sodium artesunate resulted in diuresis and increased renal excretion of sodium and chloride in rats (Campos et al, 2001). Campos and his co-workers also reported a similar alteration of electrolytes in a man who received intravenous artesunate which however returned to normal after therapy. In the current study in which artesunate was administered intraperitoneally, the absence of similar effects may suggest that in routine therapy of uncomplicated malaria where intravenous administration may not be required, electrolyte disturbances and physiological dysfunction of the kidney may not occur. With the combination group on lamivudine and artesunate showing similar results, the effect of the combination is unlikely to be deleterious to the kidney.

Treatment with artesunate resulted in varying degrees of histological changes which were also observed in the group that received both lamivudine and artesunate. The extent of observed changes however differs from some reports in which both functional and histological changes were corroborated.



Panel A, B, C and D are serum chloride, potassium, sodium and urea levels.
Data shown are mean SEM (n=5). There were no statistically significant difference following ANOVA and Dunnett's post-hoc multiple comparison test.
***Veh**=saline; **lam**=lamivudine; **lam-as**=lamivudine and artesunate; **as**=artesunate.*

Figure 2: Effect of lamivudine-artesunate co-administration on renal biomarkers

ISSN 2303-9841

The study of Li et al, (2007) reported renal tubular damage following intravenous administration of 240 mg/kg resulting in diuretic effect that was reversed after therapy both in malaria and non malaria infected rats (Li et al, 2007). The work of Ejiofor and co-workers (2009) also reported some glomerular renal tubular necrosis as well as focal fibrosis at 15 mg/kg artemether. As dose effect relationship is an important aspect of efficacy and toxicity, the lower doses may thus be devoid of some of these reported adverse events. In both the individual drug treatment groups and the combination group, renal biomarkers did not suggest significant physiological dysfunction. However the presence of changes seen in the architecture of the kidneys is an indication that some level of damage may occur and this may be exaggerated at higher doses or with repeated exposures. With artesunate being considered a candidate drug in tumors, the nature of its use may thus require monitoring due to the apparent propensity of artesunate to cause nephrotoxicity.

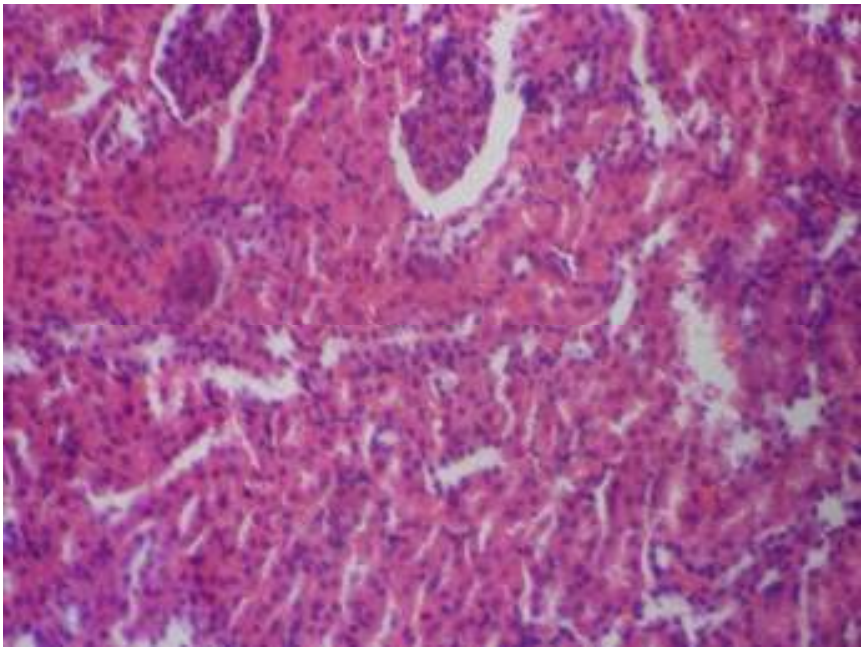


Figure 3A: Photomicrograph of the kidney of a control rat

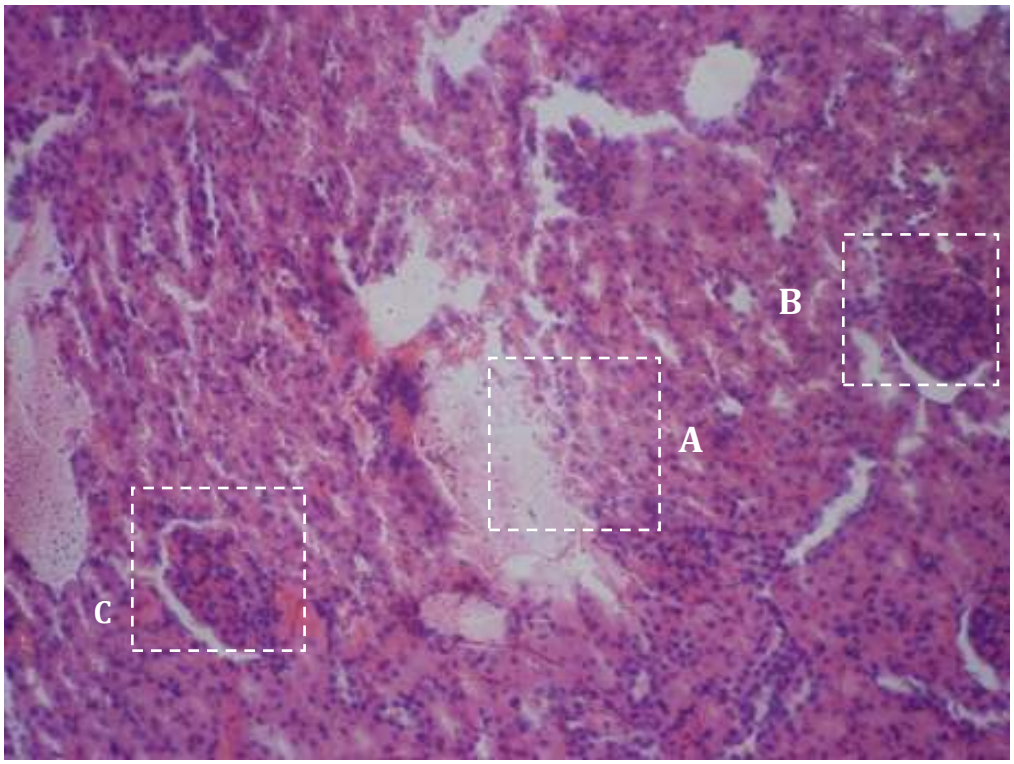


Figure 3B: Photomicrograph of H and E stained section of the kidney of a Lamivudine-artesunate treated rat (x400) showing some areas of necrosis (**A**), hypertrophied glomeruli (**C**) and infiltration of inflammatory cells (**B**)

 ISSN 2303-9841

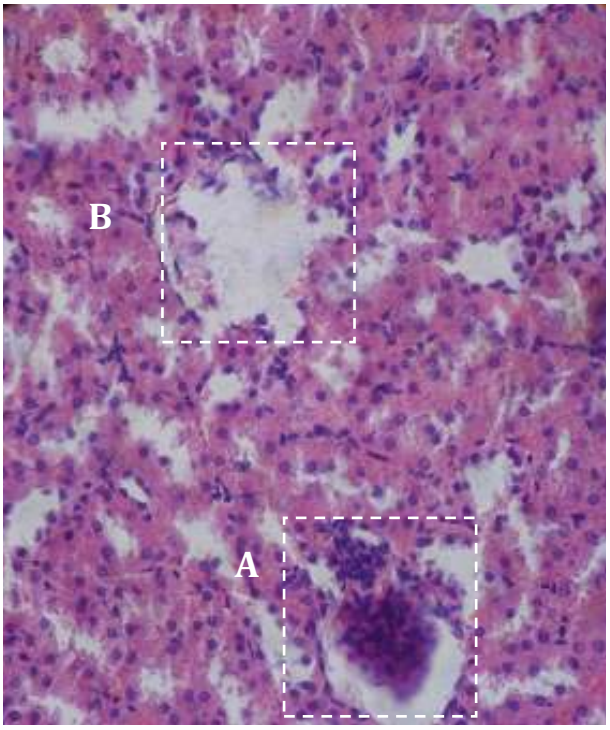


Figure 3C: Photomicrograph of H and E stained section of the kidney of an artesunate treated rat (x400) showing some atropic glomeruli (**A**)and some entirely necrotized areas (**B**)

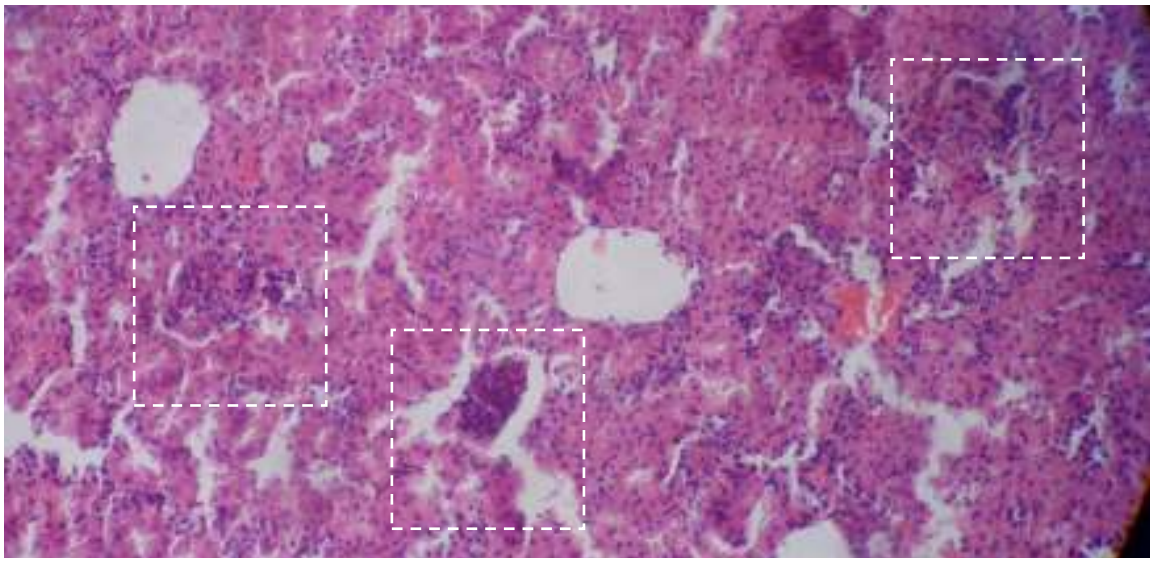


Figure 3D: Photomicrograph of an H and E stained section from the kidney of a lamivudine treated rat (x400), showing atropic as well as hypertophic glomeruli. Some regions of infiltration with inflammatory cells are also seen

5. Conclusion

The co administration of lamivudine and artesunate did not result in any significant adverse effects that produce observable functional changes in the kidney despite histological changes that were observed. However with increased dose and with multiple exposures as is the case very often requiring multiple malaria treatment courses over short intervals, there may be more evident nephropathy taking into consideration the observed histological changes seen.

Conflict of Interest declaration

The authors declare no conflict of interest

Acknowledgements

The authors wish to acknowledge the technical assistance of Mr. Olu Ayegbusi of the Ahmadu Bello University Teaching Hospital, Shika, Nigeria

ISSN 2303-9841

References

Campos SB, Rouch LHK and Seguro HC (2001). Effects of Sodium Artesunate, A New Antimalarial Drug on Renal Function. *Kidney Int.* **59**:1044-1051.

Doogue MP and Polasek TM (2011). Drug Dosing in Renal Disease. *Clin. Biochem. Rev.* **32**:69-73.

Du JH, Zhang HD, Ma, ZJ and Ji, KM (2010). Artesunate induces oncosis-like cell death in vitro and has antitumor activity against pancreatic cancer xenografts in vivo. *Cancer. Chemother. Pharmacol.* **65**:895-902

Efferth T, Giaisi M, Merling A, Krammer PH, Li-Weber M, et al (2007) Artesunate Induces ROS-Mediated Apoptosis in Doxorubicin-Resistant T Leukemia Cells. *PLoS ONE* **2**(8): e693. doi:10.1371/journal.pone.0000693

Ejiofor JI, Kwanashie HO, Anuka JA and Ibrahim NDG (2009). Histopathological Effects of Artemether on Selected Organs in Rat. *Toxicol. Environ. Chem.* **91**(6):1183-1190.

Ferguson MA and Waikar SS (2012). Established and Emerging Markers of Renal Function. *Clin. Chem.* **58**(4):680-689.

Ghosh J, Das J, Manna P and Sil PC (2010). Acetaminophen induced renal injury via oxidative stress and TNF-α production:Therapeutic potential of arjunolic acid. *Toxicology* **268**(1-2):8-18

Hagos Y and Wolff NA (2010). Assessment of the Role of Renal Organic Anion Transporters in Drug-Induced Nephrotoxicity. *Toxins*, **2**:2055-2082.

Haugen E and Nath KA (1999). The Involvement of Oxidative Stress in the Progression of Renal Injury. *Blood. Purif*. **17**:58-65

Li Q, Xiea LH, Johnson TO, Si Y, Haeberle AS and Weina PJ (2007). Toxicity Evaluation of Artesunate and Artelinate in *Plasmodium berghei*-Infected and Uninfected Rats. *Trans. R. Soc. Trop. Med*. **101**(2):104-112.

Liu WM, Gravett AM Dalgleish AG (2011). The antimalarial agent artesunate possesses anticancer properties that can be enhanced by combination strategies. *Int. J. Cancer*, **128**:1471-1480

Manucha W. (2007). Biochemical-molecular markers in unilateral ureteral obstruction. *Biocell.* **31**(1):1-12

Marsh W H, Fingerhurt B and Miller H. (1965). Automated and manual direct methods for the determination of blood urea. *Clin. Chem.* **2**(6): 624-627

Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, Johnson JK and Schafer K (2007). Evaluation of Organ Weights for Rodent and Non-Rodent Toxicity Studies: A Review of Regulatory Guidelines and a Survey of Current Practices. *Toxicol. Pathol.* **35**(5):742-750. DOI: 10.1080/01926230701595292.

Naqvi R, Ahmad, E, Akhtar F, Naqvi A and Rizvi A (2003). Outcome in severe acute renal failure associated with malaria. *Nephrol. Dial. Transplant.* **18**:1820-1823.

Natelson S, Scott, ML and Beffa CA (1951). Rapid method for the estimation of urea in biologic fluids. *Am. J. Clin. Path.,* **21**: 275-281.

Perazella MA (2009). Renal Vulnerability to Drug Toxicity. *Clin. J. Am. Soc. Nephrol.* **4**:1275-1283.

Saunders D, Khemawoot P, Vanachayangkul P, Siripokasupkul R, Bethell D, Tyner S, Se Y, Rutvisuttinunt W, Sriwichai S, Chanthap L, Lin J, Timmermans A, Socheat D, Ringwald P, Noedl H, Smith B, Fukuda M, Teja-Isavadharm P. (2012). Pharmacokinetics and pharmacodynamics of oral artesunate monotherapy in patients with uncomplicated Plasmodium falciparum malaria in western cambodia. *Antimicrob. Agents. Chemother*. **56**(11):5484-5893.

Saxena PN, Anand S, Saxena N and Bajaj P (2009). Effect of arsenic trioxide on renal functions and its modulation by *Curcuma aromatica* leaf extract in albino rat. *J. Environ. Biol.* **30**(4):527-531.

Taylor WRJ, Rigal J and Olliaro PL (2003). Drug resistant falciparum malaria and the use of artesunate-based combinations: focus on clinical trials sponsored by TDR. *J. Vect. Borne. Dis.* **40**:65-72

Yi W, Liu M and Cai HD (2012). Safety of lamivudine treatment for chronic hepatitis B in early pregnancy. *World. J. Gastroenterol*. **18**(45):6645-6650

 ISSN 2303-9841