

**Effects of Artesunate Alone and In Combination with Folic Acid on the Liver and Serum Iron Level of Male Wistar Rats**

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**ABSTRACT**

The effects of oral administration of artesunate alone and with folic acid on the liver and on the serum levels of iron were assessed in eighty-one male wistar rats with a mean weight of 180 g (172-188 g). Thirty-six rats received artesunate of graded doses ; another thirty-six rats received a combination of artesunate and folic acid; while normal saline was administered to the remaining nine rats which served as the negative control group. Comparison of means of results was done with the student's t-test at a 95% level of significance. The results showed that in rats treated with 6.00 mg/kg of artesunate alone, there was a significant decrease in liver weight from 3.25±0.55 to 2.64±0.12g. Necrosis of the hepatocytes as revealed by liver histology also occurred. The serum iron level rose significantly from 784±11.49 µmol/L to 1773±11.32 µmol/L. It was also found that folic acid reversed metabolic and tissue disorder associated with lower doses of artesunate but offered partial relief to the same disorders associated with higher doses of the drug. This is evident by the decrease in serum iron and the the healthy cytoarchitecture of the liver .

**KEYWORDS:** Artesunate, folic acid, iron, serum, liver

## INTRODUCTION

In Nigeria, 80% of the human population are exposed to malaria. At least 60 million people have repeated malaria in a year. The mortality rate has been put at 100,000 persons per annum (Jeremiah *et.al*,2007). In sub-Saharan Africa about 300,000 children die yearly of malaria. In the world, the World Health Organization estimates that 300 to 500 million clinical cases and 1.5 to 2.7 million deaths due to malaria occur each year world wide (Ogunbonna *et.al*,1990).

Artesunate is the synthetic derivative of Artemisinin, the antimalarial principle isolated from the plant *Artemisia annua* (Woedenbag *et al*,1994). Artesunate is considered an effective alternative drug for *P. falciparum* because clinical findings to date have not revealed any pattern of resistance to it (Kabwang *et al* 1994, Baradell and Fitton *et al*,1995). Artesunate is commonly administered with folic acid. The objective of this study is to evaluate the effect of artesunate and its concomitant administration with folic acid on the liver and also on the serum iron levels. This study is of clinical significance because artesunate breaks the megaloblast, attacks the cell membrane of the parasite in it and consequently kills the parasite. The Iron in the megaloblast is liberated into the peripheral blood circulation from where they shunt into the serum and plasma resulting in megaloblastic anemia which in turn inhibits DNA synthesis in red blood cell production especially when there is folic acid deficiency. In humans folic acid is reduced to tetrahydro folic acid (THFA) by an enzyme dihydrofolate reductase (DHFR). THFA (the

active form of folic acid) is required for the de-novo synthesis of nucleic acids and certain amino acids. This is why artesunate should be administered in conjunction with folic acid. The findings in this study when extrapolated to human will bring succour to malaria patients who takes solace artesunate as drug of therapy. (Reich and Deykin 1978, Udobre *et al* 2009).

Artesunate ( NEROS Pharmaceuticals Ltd Lagos), Folic acid (Vitabiotics England) were purchased in Uyo.

## MATERIALS AND METHOD

Drug administration and collection of blood  
Eighty one male wistar rats were obtained from the National Veterinary Institute, VOM in Jos, Plateau State. The rats were kept in the animal House under standard Laboratory condition in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo and fed on Growers Marsh (Top feeds, Sapele, in Nigeria) and water *ad libitum*. The rats were weighted after three weeks and had a mean weight of 170 g.

The eighty-one (81) male wistar rats were divided into nine groups of 9 rats each and then treated as shown in table 1. Following daily oral administration of the drugs the rats were sacrificed on the 6<sup>th</sup> day, and blood was collected by passing a needle on syringe through the ventricle of the heart ( Reich and Deykin 1978) .

The serum was prepared from the clotted blood by gently decanting the serum from the cells into iron-free centrifuge tubes. The tubes were then spun at 10,000 revolutions per

second for 3 min in a centrifuge machine (MSE, England). The serum was then decanted into another set of clean tubes and stored at  $-15^{\circ}\text{C}$  for 24 h.

### **Analysis of serum**

The serum samples obtained from the rats treated with artesunate alone, artesunate and folic acid and then physiological saline (control) respectively were analyzed in an atomic absorption spectrophotometer, (Perkin Elmer 2280) for iron levels at wavelength of 248nm. The results obtained were compared with the control values.

### **Histological Analysis**

The liver tissue samples removed from the experimental rats were fixed in 10% neutral solution for 42 h, and then passed through 70%, 95% and absolute alcohols for  $1\frac{1}{2}$  h each. The tissues were then infiltrated through molten paraffin wax in the oven maintained at  $60^{\circ}$  for 1 h and then sectioned at five microns ( $5\ \mu$ ) thick for light microscopy.

The haematoxyline and Eosin method of staining was applied. Sections were dewaxed in xylene, cleared in absolute, 95%, 70% alcohol and then in water. The sections were stained in haematoxyline for 15 minutes, differentiated in 1% acidified alcohol by rinsing twice and then counter stained with eosin for 3 minutes and dehydrated in 70%, 95% and absolute alcohol. The sections were cleared in xylene and mounted with DPX mountant for microscopy.

### **Statistical Analysis.**

The student's t-test was used to test whether there existed any significant differences between the means of the treatment groups

and those of the corresponding control groups. The data obtained are expressed as mean  $\pm$  S.D student's t-test was used for the analysis and  $P \leq 0.05$  was taken to be statistically significant.

### **RESULTS AND DISCUSSION**

Tables 2 and 3 show that the concentrations of iron in the serum increases significantly ( $P \leq 0.05$ ) with increase in the dose of artesunate. In rats treated with a mixture of artesunate and folic acid, the folic acid reduced the serum iron concentration. For example, the serum iron concentration was  $1068 \pm 14.28$  when 1.50 mg/kg artesunate was administered but reduced to  $803 \pm 13.47$  when the same dose was coadministered with 1.50 mg/kg folic acid. In the presence of high iron concentrations artesunate releases free radicals which kill the schizonts of malaria parasites. These results indicate that equivalent doses of folic acid can reverse metabolic disorders occasioned by the administration of low doses of artesunate but might interfere with the activity of artesunate against the schizonts of plasmodia ( Udobre *et al*, 2009).

At a dose level of 6.00 mg/kg artesunate, the 1.50 mg/kg folic acid administered could not bring down the serum iron concentration. The high dose of the drug might have destroyed the megaloblasts and the iron in them liberated into the peripheral blood circulation from where the iron shunt back into the serum and plasma thus increasing the iron level at the expense of tissues and organs, a condition referred to as megaloblastic anemia (Liy and Wu, 1998)

The results in tables 4 and 5 show that at the dose level of 6.00 mg/kg artesunate the percentage weight of the liver decreased significantly from the control value of  $3.25 \pm 0.12$  to  $2.64 \pm 0.12$  ( $P \leq 0.05$ ). At the dose level of 6.00 mg/kg artesunate co-administered with 1.50mg/kg folic acid, the percentage liver weight increased from  $2.64 \pm 0.12$  to  $2.92 \pm 0.20$  ( $P \leq 0.05$ ), but not up to the value for control rats ( $3.25 \pm 0.55$ ). These results support the earlier observation that co-administration of folic acid with artesunate can reverse the metabolic disorders associated with artesunate administration at lower doses of artesunate. This reversal effect may be achieved at higher doses of artesunate if higher doses of folic acid are employed (Edoho *et al* 2006)

Nuclei stained blue-black in all the sections and cytoplasm shades of pink. In the group that received normal saline liver cells radiate from central vein. The liver cells have a distinct cell outline. The nuclei stain deeply basophilia. The cells cytoplasm stain deeply eosinophilia. Hepatic sinusoids run between two sheets of liver cells. These imply that the hepatocytes of the control group are normal (as seen in fig 1a). In the group which received high dose of artesunate of 6.00 mg/kg body weight, necrotic areas are seen. The cell outline is not prominent; the liver cell nuclei are pyknotic. The hepatic sinusoids are wider. There is venodilatation with blood clot inside it. These imply that the hepatocytes are destroyed at a dose level of 6.00 mg/kg body weight artesunate (see fig 1b).

In the group which received 6.00 mg/kg body weight artesunate and 1.50 mg/kg body weight folic acid, there is less distortion of the cytoarchitecture; the cells appear less sequestered; the cell outline is not prominent and the cell nuclei appear pyknotic. The hepatic sinusoids are wider than with artesunate alone and run between the hepatic cells (fig 1c). These results imply that folic acid help to relieve liver disorder associated with artesunate administration.

### **CONCLUSION**

This study shows that administration of a high dose of artesunate up to 6.00 mg/kg to male wistar rats caused necrosis of the hepatocytes, significant decrease in liver weight and significant increase in serum iron level. The doses of folic acid offered total relief to metabolic disorder associated with lower doses of artesunate but partial relief to the same disorder associated with higher doses of the drug. In this study, it is observed that the combination therapy yielded good result but that the folic acid should not be administered in very high doses to prevent the loss of efficacy of artesunate and not in very low doses so as to preserve its clinical relevance.

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**Table 1: table of dosage pattern of drug administration to the rats.**

Group	Dose of Drugs/Chemicals in mg/kg body weight
A	0.75 mg/kg artesunate
B	0.75 mg/kg artesunate + 0.75 mg/kg folic acid
C	1.50 mg/kg artesunate
D	1.50 mg/kg artesunate + 1.50 mg/kg folic acid
E	3.00 mg/kg artesunate
F	3.00 mg/kg artesunate + 1.50 mg/kg folic acid
G	6.00 mg/kg artesunate
H	6.00 mg/kg artesunate + 1.50 mg/kg folic acid
I	Physiological Saline (Control)

**Table 2: Effect Of Artesunate Alone And With Folic Acid Administration On Serum Iron Level**

Group	Treatment Group	Mean Serum Iron ( $\mu$ M)
A	0.75mg/kg artesunate	804 $\pm$ 15.45
B	0.75 mg/kg artesunate + 0.75 mg/kg folic acid	790 $\pm$ 14.18
C	1.50 mg/kg artesunate only	1068 $\pm$ 14.28*
D	1.50 mg/kg artesunate + 1.50 mg/kg folic acid	803 $\pm$ 13.47*
E	3.00 mg/kg artesunate	1130 $\pm$ 15.33*
F	3.00 mg/kg artesunate + 1.50 mg/kg folic acid	978 $\pm$ 13.08*
G	6.00 mg/kg artesunate only	1773 $\pm$ 11.32*
H	6.00 mg/kg artesunate + 1.50mg/kg folic acid	1661 $\pm$ 14.23*
I	Physiological Saline (Control)	784 $\pm$ 11.49

The results are expressed as mean  $\pm$  standard deviation \* significant ( $p < 0.05$ )



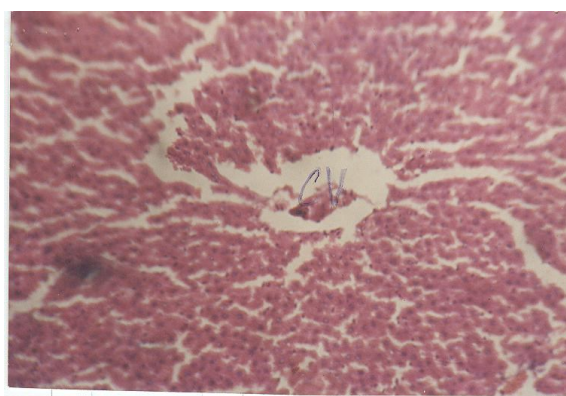
**Table 3: Liver Weight Results**

Group	Treatment Group	Liver weight (g)
A	0.75 mg/kg artesunate	3.10±0.24
B	0.75 mg/kg artesunate + 0.75 mg folic acid	3.17±0.21
C	1.50 mg/kg artesunate	3.24±0.39
D	1.50 mg/kg artesunate + 1.50 mg folic acid	3.27±0.58
E	3.00 mg/kg artesunate	2.99 ± 0.46
F	3.00 mg/kg artesunate + 1.50 mg folic acid	3.06 ± 0.50
G	6.00 mg/kg artesunate	2.64 ± 0.12*
H	6.00 mg/kg artesunate + 1.50 mg folic acid	2.92 ± 0.20*
I	Physiological Saline (Control)	3.25 ± 0.55

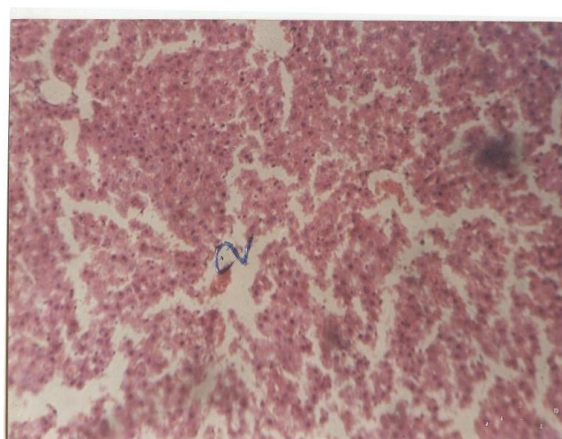
The results are expressed as percentage liver weight ratio, which is liver weight/body weight x100

(a) Magnification × 2500

(b) Magnification × 2500



(c) Magnification × 2500



**Fig. 1: Liver sections from rats treated with (a) physiologic saline (b) Artesunate alone (c) Artesunate and folic acid.**

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