# The effect of some antimalarial drugs on human serum lactate dehydrogenase (LDH) activity

Edeh Imomotimi E[1], Uwakwe AA[2] and Chinaka NC[3*]

[1&2] *Department of Biochemistry, University of Port Harcourt, PMB* -5323*, PHC, R/S, Nigeria.*
[3] *Department of Biochemistry, Madonna University, PMB*-48*, Elele, R/S, Nigeria.*
cn_chinaka@yahoo.com*

## Abstract

The *invitro* study on the effect of the antimalarial drugs; Chloroquine, Lariam, Camoquine, Quinine, Halfan and Fansidar on human serum lactate dehydrogenase activity was investigated at pH 7.5., results obtained revealed that chloroquine had little or no significant effect on the enzyme lactate dehydrogenase activity while others (lariam, camoquine, quinine, halfan, and fansidar) activated the enzyme in a concentration dependent manner.

**Keywords:** Drugs, anti-malarial, activity, concentration, enzyme, inhibition, diagnosis.

## 1. Introduction

### 1.1 Drugs

Drugs can be defined as chemical substances, which by interaction with biological systems change the biological system. Drugs may cause contraction of muscles, secretion of glands, change the metabolism of tissue and cause or prevent growth or division of cells. Drugs can act on central nervous system to alter the pattern of behavior to relieve pains or to affect some neuronal disorder (Burgen & Mitchell, 1979). Drugs can be specific.

### 1.2 Lactate dehydrogenase (LDH)

Lactate dehydrogenase is a tetrameric enzyme (isoenzyme). Its use as a diagnostic enzyme has been amply documented (Varley *et al.*, 1980; Dioguardi, *et al.*, 1966). Lactate dehydrogenase catalyses the reaction:

$$CH_3CHOHCOO^- + NAD^+ \leftrightarrow CH_3COCOO^- + H^+$$

Lactate Pyruvate

Two distinct subunits of LDH have been identified: those designated H for Heart (myocardium) and M for Muscles (Cahn *et al.*, 1962). These two subunits are combined in five different ways. The lactate locations are as follows;

| Type | Composition | Location |
|---|---|---|
| LDH1 (Most anodic) | H4 | Myocardium, RBC and renal cortex |
| LDH2 | H3M | Myocardium, RBC and Renal cortex |
| LDH3 | H2M2 | Lungs, lymphoid tissue, brain and kidney |
| LDH4 | HM3 | Liver and skeletal muscles |
| LDH5 (Most cathodic) | M4 | Liver and skeletal muscles |

From the table above, the major sources of enzyme have been listed. All tissues are minor sources. Very high blood levels of LDH activity suggest malignancy or megaloblastic anaemia (Norbert, 1986).

Several chemotherapeutic drugs (anti-malarial) and other actions have been looked into by several investigators (Lawrence *et al.*, 1999), but this present research is aimed at the effect of Chloroquine, Lariam (Mefloquine), Camoquine (Amodiaquine), Quinine, Halfan (Halofantrine), and Fansidar on human serum lactate dehydrogenase (LDH) activity. It is hoped that the findings would help understand the possible effect of these drugs on carbohydrate metabolism.

## 2. Materials and method

Materials used for analysis include;

- Centrifuge

- Spectrophotometer
- Weighing balance
- Sample bottles, measuring cylinder, beakers, test tubes/racks, Pasteur and micro pipettes
- Standard laboratory reagent and chemicals
- Anti-malarial drugs (Chloroquine, Lariam, Camoquine, Quinine, Halfan and Fansidar).

### 2.1 Preparation of drugs

0.01g of each drug was weighed and dissolved in 100ml of distilled water. The solution was properly homogenized by vigorously shaking it and this served as drug stock.

*Drug dilution*

| Concentration (mg) | Stock (ml) | Water (ml) |
|---|---|---|
| 10 | 1.0 | - |
| 8 | 0.8 | 0.2 |
| 6 | 0.6 | 0.4 |
| 4 | 0.4 | 0.6 |
| 2 | 0.2 | 0.8 |
| 1 | 0.1 | 0.9 |

### 2.2 Blood collection

Blood samples were obtained from healthy students via venous puncture using a syringe with needle and transferred into a plain sterile container, after which it was spun in a centrifuge at a speed of 5000rpm for 10mins. The serum is carefully siphoned out using a Pasteur pipette and transferred into a clean sample container for further analysis.

### 2.3 Experimental methods

The following experimental methods were carried out:

a) Effect of anti-malaria drugs on human serum lactate dehydrogenase (LDH) activity.

b) Effect of varying substrate concentrations of the drug(s) on the enzyme LDH.

c) Enzyme activity on drug substance.

### 2.4 Determination of lactate dehydrogenase (LDH) activity

The method used in this analysis is known as the optimized kinetic method, DGKC technique. The method of Engelhardt and Notges (1970); Berge Meyer, (1972); Weishaar and Col, (1975) were used for analysis. The principle underlying the analysis is that pyruvate reacts with NADH in the presence of the enzyme LDH to yield L-lactate and $NAD^+$ as shown by the equation below;

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L-lactate} + \text{NAD}^+$$

#### 2.4.1 Micro technique

Procedure: 30ml of the working reagent (Randox LDH kit) and 0.1ml of distilled water was added and allowed to stand for 2-3mins at 30$^o$C into a test tube, after which 0.1ml of sample was then added as well as 20ml of normal saline and 20µl of the specimen. After thorough mixing, the mixture was allowed to stand for some minutes and absorbance read at 1$^{st}$, 2$^{nd}$, 3$^{rd}$ and 4$^{th}$ minutes at a wavelength of 340nm in a spectrophotometer. This served as control.The change in extinction per minute (-ΔE/min) for every reading was determined and mean value recorded.

### 2.5 Determination of the effect of the anti-malaria drugs on human serum LDH activity

Procedure is as described in 2.2.2 was repeated in the presence of 0.1ml of various concentrations of the test compounds in place of 0.1ml of water. The concentration determined for each of the drugs is thus:1.0 (mg/l), 0.8 (mg/l), 0.6 (mg/l), 0.4 (mg/l), 0.2 (mg/l), and 0.1 (mg/l).

## 3. Results

The results of the effects of anti-malarial drugs; chloroquine, lariam (mefloquine), camoquine (amodiaquine), quinine, halfan (halofantrine) and fansidar on human serum lactate dehydrogenase activity are presented in tables and the graphs illustrated in the

appendix. From the table, the effects of the anti-malarial drugs are as follows:

### 3.1 Effect of Chloroquine on human serum LDH activity

From the experiment, it was observed that human serum lactate dehydrogenase is not activated by the anti-malarial drug chloroquine. The result obtained shows that the drug has little or no effect on the enzyme. The result is shown in fig. 1. At concentration of 0.8 the activity is very minimal.

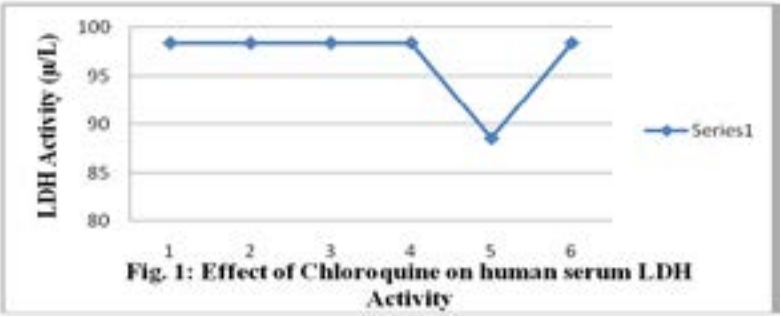


**Fig. 1: Effect of Chloroquine on human serum LDH Activity**

### *3.2 Effect of Lariam on human serum LDH activity*

Lariam like some anti-malarial drugs was found to increase the activity of LDH in a concentration dependent manner. From the result illustrated in fig. 2, it shows that lariam is the least active drug among the drugs studied.

### *3.3 Effect of Camoquine on human serum LDH activity*

Investigations under this study reveal that camoquine increased the activity of LDH in a concentration dependent manner as illustrated in fig. 3.

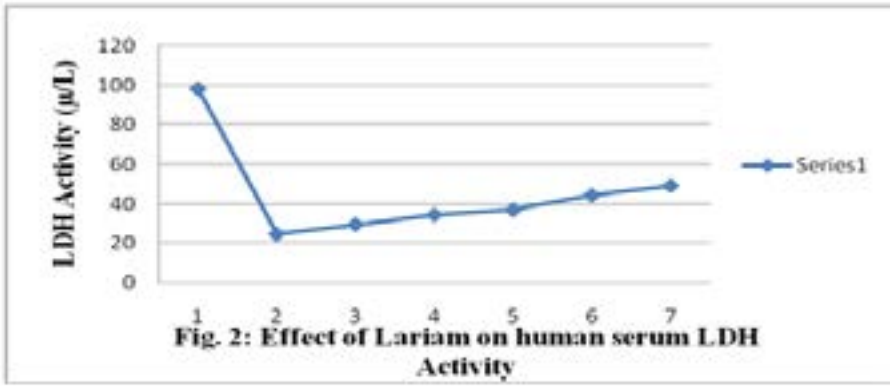


**Fig. 2: Effect of Lariam on human serum LDH Activity**

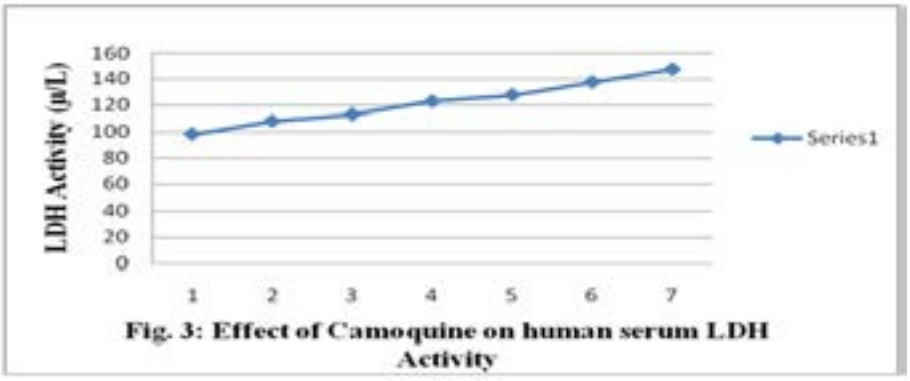


**Fig. 3: Effect of Camoquine on human serum LDH Activity**

### *3.4 Effect of Quinine on human serum LDH activity*

The result obtained indicates that human serum LDH is activated by quinine in a concentration dependent manner, showing a more similar pattern as was observed using camoquine. This is illustrated in fig. 4.

### *3.5 Effect of Halfan on human serum LDH activity*

Halfan was also found to activate human serum LDH activity in a concentration dependent fashion. From the result, it shows that halfan is more active than chloroquine, lariam, camoquine and quinine and almost the same with fansidar as illustrated in fig. 5.

### *3.6 Effect of Fansidar on human serum LDH activity*

As was the case with halfan and quinine, human serum LDH was activated by fansidar in a concentration dependent manner. The result in fig. 6 shows that fansidar is one of the most active drugs under this study.

## 4. Discussion and conclusion

Concentration dependency is an important aspect of drug administration particularly in relation to toxicity control. However, drug therapy is based on the concept of enzyme inhibition and is designed with a view towards inhibiting a specific enzyme in a specific metabolic pathway (Delvin, 1986).

This finding could hold some relevance in clinical diagnosis involving the enzymes. In routine laboratory diagnosis, elevated activity of an enzyme could either point to or confirm hepatobiliary disease (Whitby *et al.*, 1980; Cheesbrough, 1987).

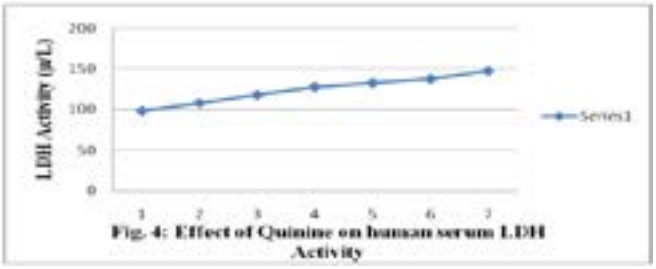

Fig. 4: Effect of Quinine on human serum LDH Activity

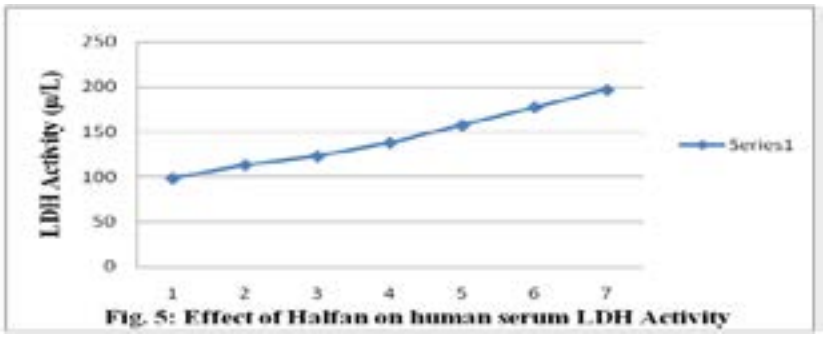

Fig. 5: Effect of Halfan on human serum LDH Activity

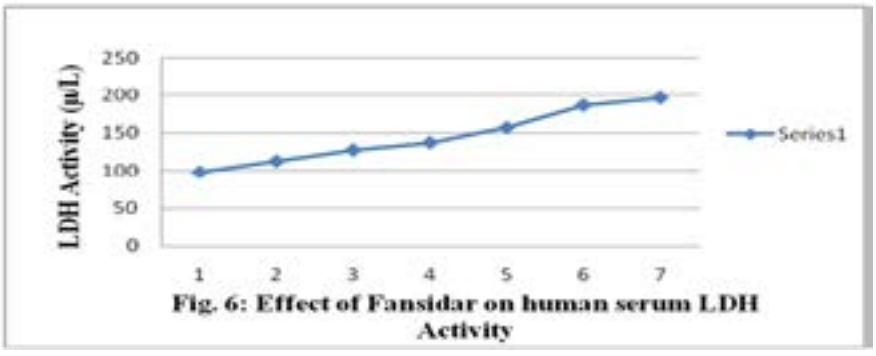

Fig. 6: Effect of Fansidar on human serum LDH Activity

This study has revealed very notable effect of the anti-malarial drugs lariam, camoquine, quinine, halfan and fansidar on human serum lactate dehydrogenase activity *invitro*. From the experiment, it was observed that chloroquine had no significant effect on the enzyme LDH activity unlike other anti-malarial drugs which were observed to have activatory effects that are concentration depend-

ent.

The activatory effects of the other anti-malarial drugs could be resulting from interplay of electrostatic and hydrogen bonding interactions between the drug molecules and the enzyme's active site. Human LDH is one of the key enzymes involved in carbohydrate metabolism. The enzyme catalyses the conversion of pyruvate to lactate with concomitant generation of NADH, a vital reducing equivalent (Varley *et al.*, 1980).

The LDH reaction is particularly important during conditions of hypoxia or such condition involving stress and is of prime importance in the muscular tissues as well as the erythrocytes where it is relied upon for the generation of the much needed energy to meet up the increased demand. An activation of this enzyme would therefore be of advantage to such systems requiring its function while its inhibition could hamper the functional integrity of such systems.

That the anti-malarial drugs (lariam, camoquine, quinine, halfan and fansidar) activated the enzyme therefore translate that these compounds in addition to their reported anti-malarial properties also possibly improve the energy balance of the organisms by their effects on LDH.

Considering this findings, it is imperative to note that patients on these drugs, undergoing liver function test may be susceptible to wrong diagnosis especially in cases where other confirmatory tests are not performed. It is also hoped that these findings would be useful in the choice/design of anti-malarial drugs for such individuals as athletes requiring the proper functioning of the LDH systems for normal activity.

## 5. References

**1•** Burgen ASV and Mitchell JF (1979) Guddum's Pharmacology, Oxford University Press.London. 8th Ed. pp: 214-217.

**2•** Cahn RD, Kaplan ND, Lavin L and Zwilling LE (1962) Mature and development of LDH.Sci. 136, 962-963.

**3•** Cheesbrough M (1989) Medical laboratory manual for tropical countries. 1, 2nd Ed. ButterworthHeinemann ltd, Oxford. pp: 518

**4•** Devlin MT (1986) Text book of biochemistry with clinical correlations. John Wiley and Sons Inc., 2nd Ed.

**5•** Lawrence DR., Bennett PN and Brown MJ (1946) Clinical Pharmacology (18th Ed.) Churchillliving stone (publish Edinburgh). pp: 238-245.

**6•** Norbert WT (1986) A text book of Clinical Chemistry.

**7•** Varley H, Gowenlock AH and Bell M (1984) Practical clinical biochemistry, (5th Ed.)Heinemann London. pp: 891-900.

**8•** Whitby LG, Percy-Robb IW and Smith AF (1980) Lecture notes on clinical chemistry (2nd Ed.)Blackwell scientific republications, London.