



Quantitative estimation of enzymatic activities in antimalarial treated rats

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Abstract

Some of the effects of Therapeutic, Acute, and Overdose treatment of Chloroquine, Artesunate, and Amodiaquine- Artesunate combined therapy on the Left ventricle and Ascending Aorta of Adult wistar rats were studied using biochemical estimation of activities of Lactate dehydrogenase and Glucose 6 phosphate dehydrogenase. The drugs were dissolved in distilled water and administered orally using an orogastric tube. The animals were sacrificed on days 1, 3, and 5 respectively after which the excised tissues were subjected to biochemical analysis. The levels of Lactate dehydrogenase and Glucose 6 phosphate dehydrogenase in the Left ventricle and Ascending aorta was highest in the Overdose treatment groups showing statistically significant results ($P < 0.05$) following treatment with Chloroquine, Artesunate, and Amodiaquine- Artesunate combined therapy. There was also statistically significant alteration ($P < 0.05$) in their activities in the Left ventricle and ascending aorta, of the animals treated at Acute dose of the drugs. However, there was no statistically significant alteration ($P < 0.05$) in the levels of Lactate dehydrogenase and Glucose 6 phosphate dehydrogenase in the Left ventricle and ascending aorta of the animals treated with the Therapeutic dose of the drugs. The findings in this study suggests that Chloroquine, Artesunate and Amodiaquine – Artesunate combined therapy altered the enzymatic antioxidant defense system and elevated the levels of Lactate Dehydrogenase in the Left ventricle and Ascending Aorta of the Adult wistar rats.

Keywords: Chloroquine, Artesunate, Amodiaquine- Artesunate combined therapy, Lactate dehydrogenase, Glucose 6 phosphate dehydrogenase, Left ventricle, Ascending aorta.

INTRODUCTION

Antimalarial drugs are designed to prevent or cure Malaria (Cox, 1992). However, nearly all medicines have the potential to cause harm when they are misused or abused, and the consequences can be dangerous and even fatal (Hollinger and Dabney, 2002).

People that deliberately misuse therapeutic antimalarial drugs may do so for a number of reasons. Some may want to obtain quicker results within the shortest possible time, especially as regards the intake of artesunate whose dose duration is between 5- 7 days

(Merimekwu *et al.*, 2000). Some may doubt the efficacy of the drugs. An example is the intake of Chloroquine whose efficacy is now in doubt due to its resistance (Berman, 2004). Some people fear that the doses might not produce the anticipated results; hence they prefer to increase the dose in order to compensate for this presumed inadequacy. In some cases of prophylactic treatment, high doses of antimalarial drugs are administered. An example is chloroquine (300- 600mg daily) (Adjene and Caxton-Martins, 2006).

Some other individuals may take these antimalarial drugs at higher doses for the purpose of treating other diseases. Examples are Chloroquine taken at a dose as high as 200- 400mg daily to treat discoid lupus erythematosus, hepatic amoebiasis, and rheumatoid

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arthritis (Teixeira et al., 2002). Artemisinin is also taken at doses as high as 200- 1000mg daily to treat Cancer (Singh and Lai, 2001). However, a relatively small increase in these designed doses may lead to fatal outcomes (Nwanjo and Oze, 2007).

In Malaria endemic areas such as Nigeria, self medication is quite common (Nwanjo and Oze, 2007). Hence, the possibility of administering overdose and incidences of misappropriation are quite common (Udonan, 2000).

Hence, there is a great need to investigate some of the effects of these antimalarial drugs on vital organs of the body.

MATERIALS AND METHODS

Drugs and Administration

Chloroquine (Chloroquine phosphate BP of Evans pharmaceutical Co., Lagos.

Artesunate (G- Sunate Forte of Bliss GVS Pharmaceutcal, India) and

Amodiaquine- Artesunate combined therapy (Amodiaquine- Artesunate combined therapy of Plethico Pharmaceutical Ltd, India), was purchased from Fiolu Pharmacy, Gambari, Ilorin, Kwara - State.

Lactate dehydrogenase kit was obtained from Human Lab, Germany. Glucose 6 phosphate dehydrogenase kit was obtained from Randox lab. UK.

1 tablet of each drug was dissolved in 100ml of distilled water, and administered with an oro-gastric tube.

The average weight of rats in each treatment group was determined in order to obtain the various dose of administration.

The therapeutic dose was determined by obtaining the therapeutic dose of a 70kg man for each drug, in order to determine the equivalent dose for the specific average weight of the each treatment group.

The acute dose was determined by reducing the duration of administration of the therapeutic dose to a 7- hourly period.

The dose for overdose group was determined by administration of a double therapeutic dose following the same duration of administration as the therapeutic dose.

Hence, the doses are as follows:

Group 1a (Therapeutic Chloroquine) received 0.42ml and 0.96ml for 3 days.

Group 1b (Acute Chloroquine) received 0.74ml and 1.42ml for 1 day.

Group 1c (Overdose Chloroquine) received 0.53ml and 1.07ml for 3days.

Group 2a (Therapeutic Artesunate) received 0.24ml and 0.48ml for 5days.

Group 2b (Acute Artesunate) received 0.4ml and 0.8ml for 5days

Group 2c (Overdose Artesunate) received 0.26ml and 0.52ml for 5days

Group 3a (Therapeutic Amodiaquine- Artesunate combined therapy) received 0.98ml (*3days).

Group 3b (Acute Amodiaquine- Artesunate combined therapy) received 1.34ml (*1 day).

Group 3c (Overdose Amodiaquine- Artesunate combined therapy Artesunate) received 1.14ml (*3days).

Animals and treatment

50 rats were harvested and bred for 3 months in the Animal holdings of Human Anatomy Department, University of Ilorin, Ilorin, Kwara-State. The animals were kept in Iron- meshed cages and maintained in the Animal Holdings of Human Anatomy Department of University of Ilorin, Kwara state till their weight averaged between 200-300g. Approval was obtained from the Ethical Committee, University of Ilorin prior to the onset of the experiment.

The animals were fed with Rat pellets (Bendel feeds, Ilorin, Kwara-State) and liberally supplied with water. The animals were divided into four main groups; three treatment groups and one control group. The treatment groups were subdivided into three groups. The three main groups were administered Chloroquine, Artesunate, and Amodiaquine- Artesunate combined therapy respectively. The control group received distilled water. The subgroups received overdose, acute and therapeutic doses of the drugs respectively.

Biochemical estimation

The biochemical estimation was carried out using the procedures written in the manual i.e. Human Kit manual for Lactate dehydrogenase, and Randox Kit manual for Glucose 6 phosphate dehydrogenase.

Statistical Analysis

The data was analyzed by one- tailed students' t- test. $P < 0.05$ was considered significant between control and treatment groups.

RESULTS

Biochemical Estimation

Results showed statistically significant increase ($P < 0.05$) in the levels of Lactate dehydrogenase and Glucose 6 phosphate dehydrogenase in the Left ventricle and ascending aorta of the animals in the treatment groups

Table 1. Biochemical Estimation of LDH and G6PD Activities.

Treatment Groups	Subgroups	LDH (Left Ventricle)	T- Value	G6PD (Ascending Aorta)	T- value
Group 1	Theapeutic	22.35 ± 8.17	2.74	0.17 ± 0.12	1.45
	Acute	36.65 ± 13.87	2.64	3.27 ± 0.67	3.72*
	Overdose	64.88 ± 6.08	10.66*	7.04 ± 2.11	9.41*
Group 2	Therapeutic	24.39 ± 13.84	1.76	0.5 ± 8.08	1.85
	Acute	49.24 ± 28.88	2.95*	0.38 ± 0.11	3.42*
	Overdose	70.59 ± 4.61	15.32	0.91 ± 5.53	16.37*
Group 3	Therapeutic	14.15 ± 4.51	3.15*	4.53 ± 4.58	0.99
	Acute	33.52 ± 9.88	3.39*	0.74 ± 5.70	12.99*
	Overdose	81.79 ± 12.92	6.33*	0.88 ± 3.35	26.32*

*Indicates significant (P < 0.05) difference when compared with control.

Table 2. Average Levels of G6PD and LDH in the Left Ventricle and Ascending Aorta.

Treatment Group		Left Ventricle (u/g)		Ascending Aorta (u/g)	
		LDH	G6PD	LDH	G6PD
Group 1 Chloroquine	Therapeutic	210.00	0.80	99.32	0.17
	Acute	218.54	1.01	109.4	0.30
	Overdose	281.89	1.92	137.63	0.4
	Control	179.38	0.70	72.75	0.17
Group 2 Artesunate	Therapeutic	230.64	0.80	97.13	0.17
	Acute	235.76	0.97	121.99	0.34
	Overdose	235.86	1.48	143.34	0.50
	Control	179.38	0.70	72.75	0.17
Group 3 Amodiaquine- Artesunate	Therapeutic	225.51	0.80	86.90	0.17
	Acute	256.26	1.50	106.26	0.34
	Overdose	315.20	1.65	154.54	0.34
	Control	179.38	0.70	72.75	0.55

compared to the control group. (Tables 1 and 2; Figures 1, 2, 3, and 4)

DISCUSSION

The present study demonstrates that Chloroquine, Artesunate, and Amodiaquine-Artesunate combined therapy alters the levels of Lactate dehydrogenase (LDH) and Glucose-6-phosphate dehydrogenase (G6PD) in the Left ventricle and Ascending aorta of some of the treatment animals at varied doses with the most significant alteration occurring in the Overdose treatment group.

The increase in the level of G6PD suggests the generation of oxidative stress as a result of administration of the drugs, and the levels increased in an ascending order from Therapeutic dose to Acute dose to Overdose group. G6PD is a cytoplasmic enzyme that plays a protective role during oxidative stress in Eukaryotic

animals since they provide coenzymes and substrates to the primary antioxidant enzymes (Algarwal and Abdelhaq, 2003). G6PD, by virtue of its ability to produce NADPH along with Glutathione reductase are conventionally considered as supporters of the antioxidant systems (Idietzien *et al.*, 1994). These antioxidant enzymes regulate free radical reactions by scavenging repairing, quenching, and chain-breaking reactions (Pal, 1994, Leite and Barretto, 1998).

G6PD is a cytoplasmic enzyme that affects the production of reduced form of cytosolic nicotinoadenosine dinucleotide phosphate coenzyme (NADPH) by controlling the step from Glucose-6-Phosphate to 6-phosphogluconate in the pentose phosphate pathway (Leite and Barretto, 1998). Salvemini *et al.* (1999) have demonstrated that G6PD expression is enhanced by oxidative stress induced by agents that either increase the intracellular O₂ concentration or decrease the Glutathione pool.

Oxidative stress occurs when the generation of free

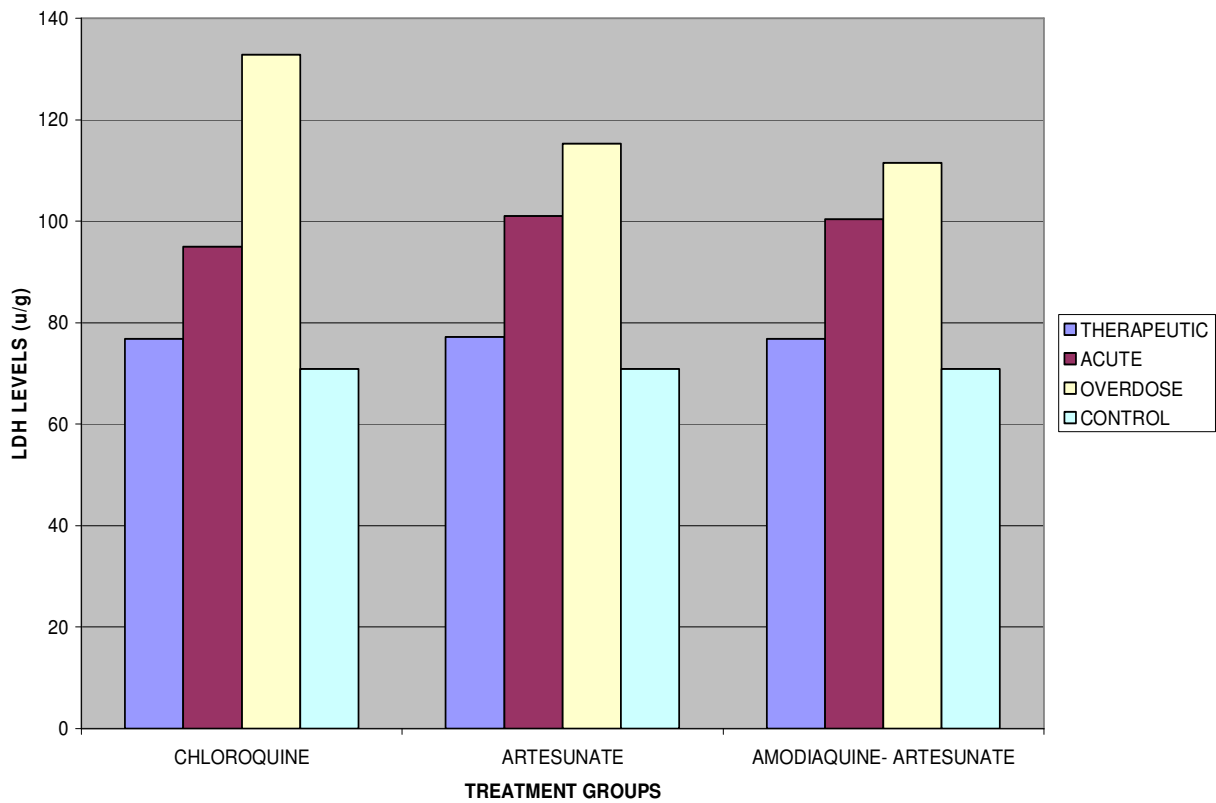


Figure 2. Lactate dehydrogenase Levels (LDH) in the ascending aorta.

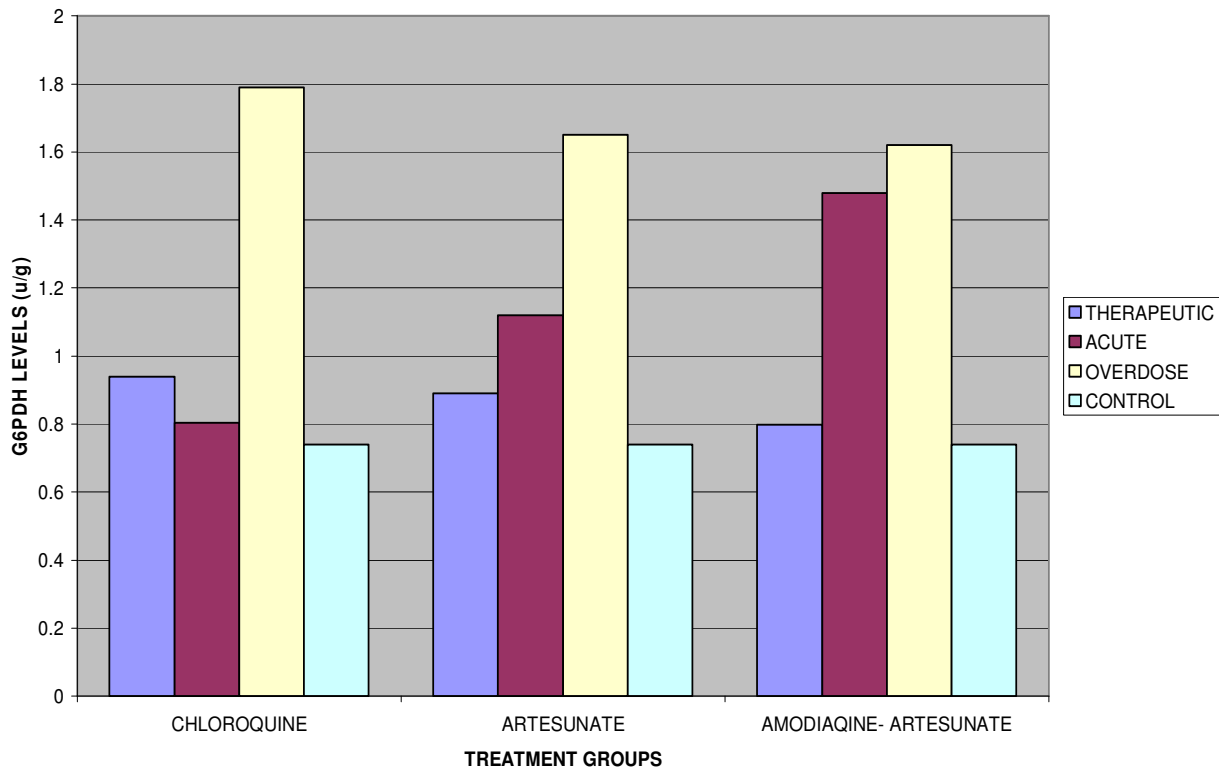


Figure 3. Average levels of glucose 6 phosphate dehydrogenase (G6PD) in the left ventricle (u/g).

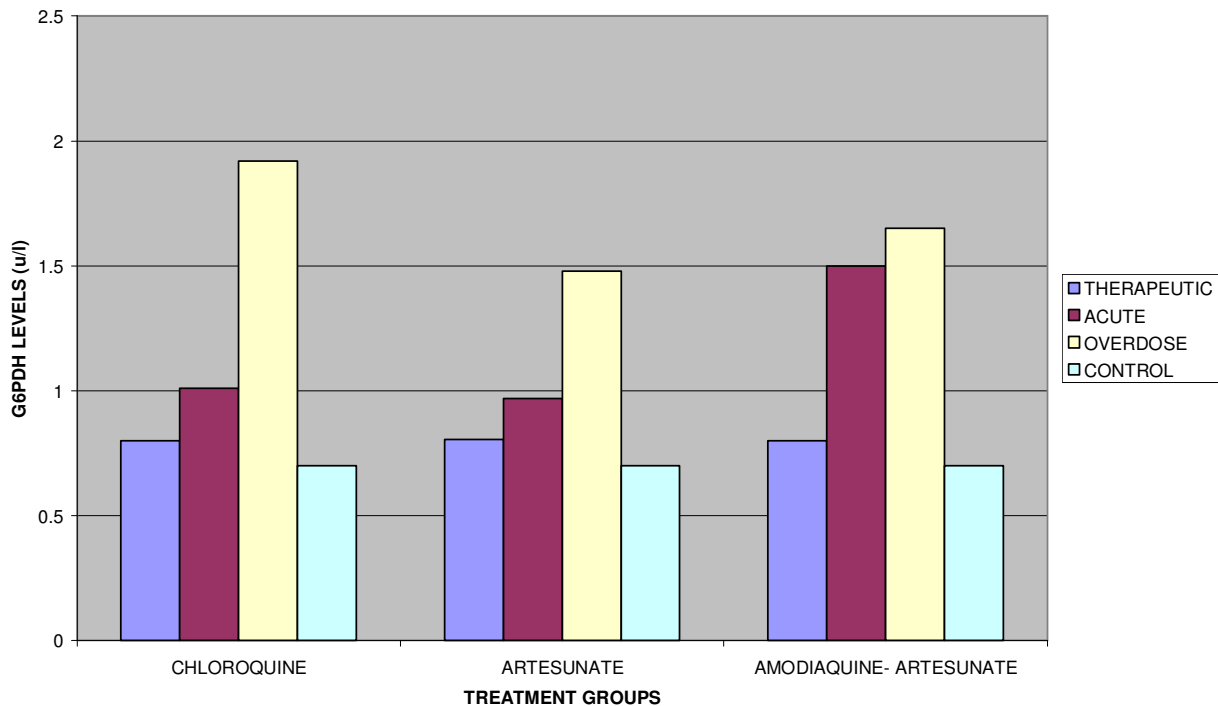


Figure 4. Average glucose 6 phosphate dehydrogenase (G6PDH) levels in the ascending aorta.

radicals increases or the capacity to scavenge free radicals and repair oxidatively modified macromolecules decreases or both (Sies, 1997). This imbalance leads to accumulation of oxidatively modified molecules predominantly end-products of superoxide (O_2^-) and hydroxyl ions (OH^\cdot) (Di Mascio *et al.*, 1991, Seif *et al.*, 1995). The reactive oxygen species (ROS) has been shown to mediate cell injury as well as contribute to the pathophysiology of the Cardiovascular system. Protection from ROS requires the maintenance of endogenous thiol pools, most importantly, reduced Glutathione (GSH), by NADPH (Jain *et al.*, 2003).

Among antioxidant enzyme system, G6PD has the ability to produce NADPH along with glutathione reductase which is a supporter of antioxidant systems (Idietzien *et al.*, 1994; De la Fuente *et al.*, 2000). The major source of NADPH is the pentose phosphate shunt provided by direct oxidation of glucose (Gohil *et al.*, 1982; Beutler *et al.*, 1996).

Artesunate contains two oxygen atoms linked together to form an endoperoxide bridge, this reacts with Iron atoms to form free radicals (Ridley and Hudson, 1998). Artesunate has been shown to induce oxidative stress (Toovey, 2006). The release of the highly reactive oxygen species from the bond kills the parasite if accumulated in the erythrocyte thereby causing lysis of the parasitic cells due to highly reactive free oxygen radicals (Ridley and Hudson, 1998).

Pathological conditions set in when the body's natural antioxidant defense system is not capable of regulating the level of free radicals in the cells (Gutteridge and Halliwell, 1990). Although oxidative stress causes increased G6PD activity (Cramer *et al.*, 1995), Free radicals propagate chain reactions that attack cellular DNA, proteins, lipids, and carbohydrates thereby causing degenerative diseases such as cancer, inflammation, apoptosis, ageing, and diabetes (Ricci *et al.*, 2003). Combination antimalarial drugs act in synergism and potentiate the actions of one another (Nwanyanwu *et al.*, 1996).

The heart muscle is one of the most vulnerable tissues to oxidative stress, because of its high rate of oxidative metabolism, intensive production of reactive oxygen metabolism, relative low antioxidant capacity and low repair mechanism (Algarwal and Abdelhaq, 2003). In Adult cardiomyocytes, G6PD has been shown to be a critical cytosolic antioxidant enzyme, essential for the maintenance of cytosolic redox status (Jain *et al.*, 2003). Elevated oxygen consumption during increased metabolic activity increases the electron leakage from the mitochondrial transport system and causes an increase in oxidative stress, lipid peroxidation and generation of free radicals such as; superoxide, hydrogen peroxide and hydroxyl radical. It also increases its vulnerability to cellular damage (Sekeroglu *et al.*, 1998).

Hence, the introduction of these antimalarial drugs fur-

ther amplifies the vulnerability of the heart to further assault through oxidative stress. In an attempt to compensate for this assault the level of G6PD was so high that there was significant difference between the levels in the treatment group compared to that in the control group.

Lactate dehydrogenase (LDH) is responsible for the conversion of pyruvate to lactate and is a key enzyme in the anaerobic breakdown of glucose to pyruvate during glycolysis. (Caxton-Martins *et al.*, 1992). LDH is present in almost all tissues and is used to detect tissue alterations in the cardiomyocytes (Zhang *et al.*, 2000; De Souza *et al.*, 2004). Because LDH is so widely distributed throughout the body, cellular damage causes an elevation in the total serum LDH, such that when there is an injury to the tissues, the cells increase in LDH thus releasing into the bloodstream, where it is identified in higher than normal values (Pagana, 1998).

LDH levels in the treatment groups were increased in the Left ventricle and Ascending aorta, when compared with the control group. The increase in the LDH in all the treatment groups suggests the fact that some amount of cellular damage has occurred in the Left ventricle as well as in the Ascending aorta. The increased level of LDH due to increased Chloroquine intake agrees with the work of Casacho *et al.* (2005) in which the LDH level was seen to have risen to 86% for 119 patients having persistent muscle enzyme disturbance due to the intake of Chloroquine in the treatment of Rheumatic diseases.

Elevated oxygen consumption during increased metabolic activity, increases electron leakage from the mitochondrial transport systems, and causes increase in oxidative stress and the generation of free radicals (Bendich, 1991). The elevated generation of free radicals can damage cellular components thus, increasing the level of Lactate dehydrogenase enzyme in the blood as well as the tissues as is reflected in the present study.

CONCLUSION

It is concluded that acute and overdose treatment of Chloroquine, Artesunate, and Amodiaquine- Artesunate combined therapy have deleterious effects on the metabolic activities (G6PDH, LDH) of the Left ventricle and Ascending aorta of adult wistar rats when assaulted with Chloroquine, Artesunate, and Amodiaquine- Artesunate combined therapy.

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