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Research article

TESTOSTERONE LEVELS IN COARTEM, CHLOROQUINE FANSIDAR AND LONART REGULATED BY CHOLESTEROL

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ABSTRACT

Periodic evaluation of the effects of lonart, coartem, fansidar and chloroquine on cholesterol and testosterone using ELISA were investigated in male albino arts for the period of 28 days. Coartem (8mg/kg) was administered for 3 days, lonart (16mg/kg) was administered for 3 days, also chloroquine (14.3mg/kg) was administered for 3 days while fansidar, (22.5mg/kg) was administered in a single dosage, all through oral routes. The results showed that cholesterol levels were significantly high in lonart ($p<0.05$), whereas it was significantly reduced in chloroquine, fansidar and coartem, ($p<0.05$). However, the testosterone levels were significantly low ($p<0.05$) in both lonart and chloroquine. But in fansidar and coartem the testosterone levels increased significantly, ($p<0.05$). It is shown that the low or high levels of testosterone with some antimalaria drugs in the study are drug dependent and also due to cholesterol levels, such effects which may affect male fertility.

Key words: Antimalarials, cholesterol, testosterone, fertility.

INTRODUCTION

Antimalaria drugs are meant to cure malaria disease which is one of the devastating and debilitating diseases in Africa. The death tolls from the disease is on the increase annually particularly in children and pregnant women, [1], [2]. The increase in death rates is amidst the intake of the antimalarials which means the rather inefficacies of the drugs based on the effects of the falciparum resistant strains, [3]. Importantly, the issue of the adverse effects of most antimalarials seems to overwhelmed the curative outcome. Some of the drugs have been found to affect body organs and systems. Adverse effects of some antimalaria drugs have been reported in renal system, liver, the spleen[4]. Some antimalaria drugs are associated with defective reproductive functions[5], decrease sperm count, including motility and viability, [6], [7],[8]. Effects of some antimalarials on cholesterol level have also been reported,[9]. Quinine is found to exert increase perioxidative effects on the liver micromal cells, [10], also oxidative stress on renal tissues and decrease non enzyme and enzyme antioxidant have been reported, [11]. Several studies have been carried out on chloroquine and its anti fertility potentials, [12], [13],[14], [15], [7]. However, comparative studies on some old antimalarials e.g. chloroquine, fansidar with the new ones; lonart and coartem and their effects on testosterone and cholesterol, levels have not been fully documented. And specifically, the assessment of the direct effects of cholesterol on the testosterone levels are not also fully documented. Though, there are contradictory reports in favour of no chloroquine effects on testosterone,[16], [17], [18]. But there are cumulative and very assertive and confirmative reports that tally with previous ones on the adverse effects of chloroquine on testosterone, [12], [14]. These controversies stimulated our interest in the study with much emphasis on cholesterol which synthesizes testosterone, [19], [20]. The antimalarials effects on the reproductive indices is based on the effects of three radicals general by them which is a vital mode of destroying P.falciparum. Testosterone is a male sex hormone which is responsible for making males though the women also have some trace of the hormone. It is also known as androgen, produced by the testes; the ledge cells present in certain parts of the testes. It is a steroid hormones synthesized by cholesterol. Men are infertile without testosterone since during the process of spermatogenesis germ cells are unable to progress beyond meiosis i.e. the reproductive cell division in the absence of testosterone. Testosterone also regulate sex drive, bone mass, fat distribution, muscle size and strength and red blood cells production. Testosterone levels also decrease with increase in age. Cholesterol is a fat-like compound. It is very essential for the formation of sex hormones, progesterone, testosterone, estrogen, [21]. There are three types of cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high density lipoprotein HDL.

Cholesterol contributes to the structure of cell walls, make up the digestive bile acids, enable the production of vitamin D, maintains membranes, [22]. Hypercholesterolemia results in artherosilerosis which is the principal causes of coronary heart disease, myocardiac infarction etc. But it serves as anti oxidant,[23]. Interestingly, our study had also reconfirmed the potentially high risk associated antimalarials with artheroslerosis i.e antimalarials that have elevated cholesterol levels. This report is consistent with our recent studies, [9]. It is therefore pertinent to review periodically the effects of current antimalaria drugs on the body physiology to avoid a more devastating and debilitating effects than the malaria disease which the drugs are meant to cure.

MATERIALS AND METHODS

(A) **Experimental Animals:** A total of twenty five (25), adult male albino rats weighing from the range of 54-165g were used for the study. The animals were maintained with water and pellets foods in a well ventilated, Faculty of Pharmacy animal house, University of Uyo.

(i) **Grouping of animals and administration of drugs:** The drugs were purchased in a licensed pharmaceutical shop.

The animals were divided into five (5) groups as follows: Group A served as control and were administered with distilled water. Group B rats were administered with coartem, (18mg/kg) for 3 days. Group C rats were administered with lonart (16mg/kg) for 3 days, while Group D, animals were administered with chloroquine (14.3mg/kg) for 3 days. Then Group E had fansidar as single dosage (22.5mg/kg). The drugs were given based on the body weight of the animals and orally using canula by-passing the esophagus and delivered into the stomach,[24],[25].

(2) Blood collection and assay of plasma testosterone and cholesterol.

Blood Collection Plasma: The rats were anesthetized with chloroform and 6ml of blood was collected by cardiac puncture, it was spun for 10 min for the plasma collection, ([26].

Assay of Plasma Testosterone and cholesterol: This was done using (ELISA) Enzyme linked immunosorbent assay technique and according to the manufacturer's procedures, fortress diagnostic 150, 13485, 09107, U.K. as follows.

(1) 10µl of plasma/serum reference, control and sample were dispensed in different wells.
(2) 50µl of working testosterone working enzyme was added to each well.
(3) The micro plate was swirled gently to mix for 20-30 seconds.
(4) 50µl testosterone biotic reagent was then added to each well in the plate.
(5) The micro plate was swirled or agitated once again.
(6) The micro plate was covered with foil paper and incubates for 60 minutes at room temperature.
(7) The contents of the micro plate were descanted and the plate blotted with absorbent paper.
(8) 300µl of wash buffer was added to the wells and the contents decanted three times.
(9) 100µl of the working substrate solution was added to all wells.
(10) The micro plate was incubated at room temperature for 15 minutes.
(11) 50µl of the stop solution (Hcl) was added to each well and mix for 15-20 seconds
(12) The absorbance in each well was read at 450nm within 30 minutes of adding the stop solution and the results recorded.

Principles of enzyme linked immunosorbent assay (ELISA) and cholesterol assay:

(i) **ELISA:** The reaction involves antibody–enzyme-antigen and native antigen. By mixing biotinylated antibody, enzyme-antigen conjugate and plasma or serum containing native antigen will result in a competitive reaction between the native antigen and the enzyme. Thus, the enzyme activity in the antibody bound fraction will be inversely proportional to the native antigen concentration i.e. in the test sample.

(ii) **Plasma Colorimetry Cholesterol Assay:** The cholesterol estimation was done by the colorimetric method according to manufacturing instruction. 10µl of the plasma samples were added to 100µl of cholesterol reagent in a curettes and incubated for 10 minutes, at room temperature and read at 540nm using water as blank and the results recorded.

The principle of the assay is that the plasma sample is reacted upon by cholesterol oxidase in the presence of water and molecular oxygen to give cholesterol it and hydrogen peroxide 4 amino antipyrine and P-hydroxyl benzoic acids catalyzed by peroxidase to coloured quinonoic derivative and water. The coloured complex derivative absorbs light maximally at 540nm wavelength and is indirectly measured to give the cholesterol concentration using colorimeter.

RESULTS

The results showed that the mean testosterone in the control (12.40 $\pm$1.80) was less than that of the group treated with coartem, (17.08 $\pm$ 3.10) ($P < 0.05$). The mean value of testosterone in the group treated with lonart (9.90 $\pm$ 1.77) was less than that of the control. Also choroquine value (11.26 $\pm$ 1.36) was less than that of control table 1, but fansidar mean testosterone level was higher than that of control, (13.00 $\pm$ 4.21). The mean cholesterol levels in lonart (4.13 $\pm$ 0.14) was higher than that of control; (3.88 $\pm$ 0.15). But that of coartem, chloroquine and fansidar, 3.69 $\pm$ 0.14, 3.84 $\pm$ 0.04, 3.61 $\pm$ 0.05 respectively were less than that of control, Table 2.

Table 1: Testosterone levels in rats treated with coartem, lonart, chloroquine and fansidar and observed for 28 days

S/N	Groups	Testosterone ng/ml
1.	Control	12.40 $\pm$ 1.80
2.	Coartem	17.08 $\pm$ 3.10
3.	Lonart	9.90 $\pm$ 1.77
4.	Chloroquine	11.26 $\pm$ 1.36
5.	Fansidar	13.00 $\pm$ 4.21

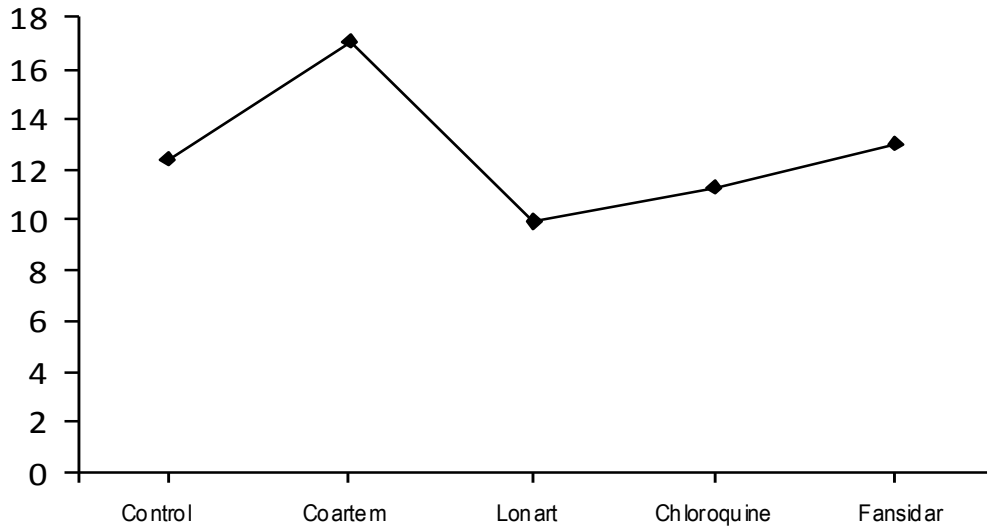

Fig. 1: Effect of coartem, lonart, chloroquine and fansidar on Testosterone levels

Table 2: Cholesterol levels in rats treated with coartem, lonart, chloroquine and fansidar and observed for 28 days

S/N	Groups	Cholesterol (mmol/L)
1.	Control	3.88 ± 0.15
2.	Coartem	3.69 ± 0.14
3.	Lonart	4.13 ± 0.14
4.	Chloroquine	3.84 ± 0.04
5.	Fansidar	3.61 ± 0.05

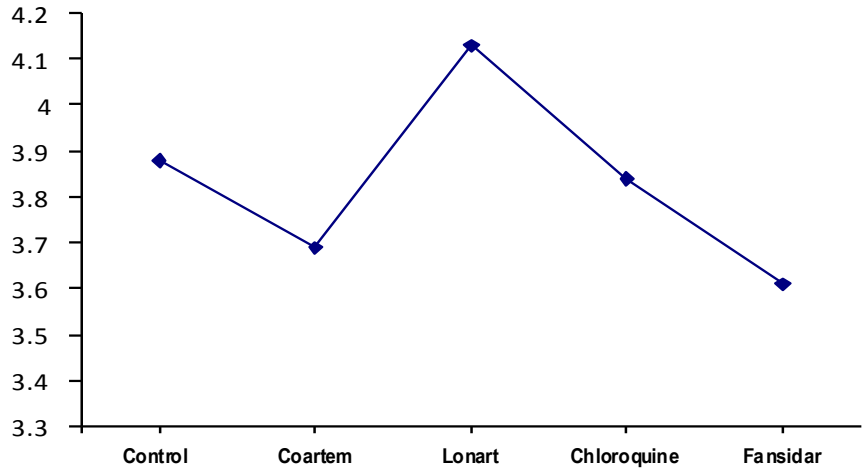

Fig. 2: Effect of coartem, lonart, chloroquine and fansidar on Cholesterol levels

DISCUSSION

The study has shown the effects of lonart, coartem, chloroquine and fansidar on the cholesterol and testosterone levels. In the study chloroquine had clearly demonstrated its negative relationship with testosterone as it lowered the testosterone levels. Our observation of the chloroquine effect, on testosterone is in line with previous studies,[12] [14] [15], [7]. These findings also confirm the likely effects of the reduced testosterone which include the reduction in leydic cells and the effects on semiferons tubules. This may eventually lead to male infertility. This findings contradicts that of,[17] who reported that chronic administration of chloroquine caused insignificant increase in testosterone levels which according to the author might be due to stimulation of leydic cells and also insensitive and resistant against the chloroquine, other studies;[16] [17] have reported the non effects of chloroquine on testosterone. These variations or contradictions may be as a result of the methods/ assay, and or the duration of administration of antimalaria drugs,[18]. This employed enzyme immune assay, but the study was for 4 days only which was too short for any adverse effects to be observed,[13]. Our study also employed a very sensitive Enzyme linked immunosorbent technique (ELISA) in assaying the testosterone levels in chloroquine and is very reproducibly and the study observed for 28 days. Chloroquine is still enjoying a good position in malaria therapy as it is the cheapest antimalaria drug and highly available; particularly in the rural setting, [27]. The outcome of the deleterious effects of chloroquine on the reproductive status is quite worrisome. This is because malaria is very endemic in tropical Africa and with this there is the increase demand of this drug. It means that the population at risk of chloroquine intake in reproductive potentials will also increase. Specifically, the male reproductive potential will decrease as without testosterone, the spermatogenesis is impossible. The malaria death tolls is very high in Africa and its means apart from elimination by the malaria disease the drugs on the other hand will be maiming the malaria patients still adding in the reduction of the population. The study had also revealed the relationship between the testosterone and cholesterol particularly in chloroquine and lonart. The levels of cholesterol were found to be reduced in chloroquine and this also affects the levels of testosterone which has been observed to be reduced too. This point to the testosterone levels cholesterol dependency. Cholesterol synthesize steroid hormones which testosterone belongs, [20]. However, low cholesterol levels have been reported in chloroquine treated rats, [9]. This means that the low cholesterol in the study may be drug dependent, low cholesterol affects cell membrane development, [28]. This study also relates such effect of the old drugs; like chloroquine with the new antimalaria drug, lonart and coartem. Lonart showed a negative and positive relationship with both testosterone and cholesterol. Testosterone levels were reduced in lonart (Lumenfantrine and artemether) but cholesterol levels were raised. Our findings are in contrast with previous reports,[29] who reported a rather anticholesteremic action of lonart. However,[30] had earlier reported none effect of lonart on testosterone level. The variations in the report may be due to methods applied, drugs dosage and the duration of studies. Lonart is now the first line treatment drug due to drug resistance P.falciparum strain which affects chloroquine, fansidar etc. The raised cholesterol level indicates, artherosclerosis, the principal causes of coronary heart disease. It means this may be associated with lonart. Also, the reduced testosterone level associated with lonart may lead to impotency in males. And with malaria endemicity in Africa, the population attributable risk of lonart related impotency may also increase. However, coartem showed increase testosterone levels in its administration indicating its therapeutic potentials in the treatment of impotency so

also with fansidar an old antimalaria drug. The increase in testosterone levels may be the results of antagonizing effects of the antimalaria drugs on the negative feed back control of gonadotropin secretions by androgen subsequent to gonadotropin increase. Both fansidar and coartem have low cholesterol, high testosterone levels, but reduced cholesterol matched a reduced testosterone levels in chloroquine administration. This is a direct negative effects of the drug on both compounds. However, lonart high cholesterol levels result in low testosterone levels, a positive, negative relationship.

The study concludes from these observations that cholesterol has influence on the testosterone levels and such effects are drugs dependent. However, counter reports[31] showed no effects of coartem on the testosterone levels. Our findings have confirmed that longer durational use of antimalaria drugs have effects on testosterone level a rather dangerous signal on reproductive health.

RECOMMENDATION

Antimalaria drugs must be taken under physicians prescriptions. Coartem and fansidar drugs are suggestive for men with low sperm count but caution is to be taken on the related effects of low cholesterol level which may affect body cells membrane development particularly red blood cells which may result in haemolytic anaemia.

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