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Plasma Bilirubinaemia; A Physiologic Index for Monitoring Cellular Effects of Amalar, Chloroquine, Cotecxin and Fansidar

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Abstract: Problem statement: Antimalaria drugs are highly consumed in Africa due to increase prevalent of malaria attacks. Though the disease is often cured with some antimalarials the physiologic effects of such drugs may not be considered in pharmacologic preparations and in prescriptions. Such non consideration is likely to result in serious health problems which may outweigh the effects of malaria disease or become double jeopardy in the event of management of the disease.**Objective**;. To determine the negative effects of amalar, chloroquine, cotecxin and fansidar on bilirubin concentration. **Approach:** Thirty male and female albino rats weighing 80-141 g were used for the study. The animals were grouped into four drugs with six animals in each drug group including control. The drugs (powdered) were administered orally per weights of the animals and based on the curative and preventive dosages using canula by-passing the esophagus and delivered into the stomach. The effect of the antimalaria drugs was monitored for 28 days by colorimetric measurement of bilirubin concentration in the blood plasma collected by cardiac punture. **Results:** Fansidar decreased the concentration of bilirubin significantly as compared with that of control (8.64±0.33), control, 16.65±1.00) $p<0.05$. Chloroquine also deplete the plasma bilirubin concentration significantly as compared with control, (11.99±2.82) 16.65±1.00), $p<0.05$. Amalar also reduce the plasma bilirubin concentration significantly as compared with control, 12.91±1.00, 16.65±1.00, $p<0.05$. However, cotecxin slightly reduce the bilirubin concentration and was not significant when compared with control, 14.50±3.8, 16.65±1.00 ($p>0.05$). **Conclusion:** The study had unveiled the possibility of erythropoietic porphyria, ineffective haemoglobination and anaemia in the administration of amalar, chloroquine, and fansidar and the need to encourage potent and cells friendly malaria therapies to avoid cellular damages.

Key words: Bilirubinaemia, physiologic index, antimalarials

INTRODUCTION

Bilirubin is a major end product of haemoglobin degradation. It is formed from the conversion of porphyrin component of haemoglobin by macrophages and kupffer cells when the erythrocytes have lived up to 120 days in the blood circulation and had become too tragile to continue to exist in the system and perform the oxygen uptake and distribution function[1]. The amount of bilirubin presence in the plasma depends, on the number of aged red blood cells that has been destroyed and the activities of macrophages including its releases and that of the kupffer cells. Bilirubin exists in two forms; the free bilirubin is the type released by macrophages while the conjugated one is the one conjugated by the liver. However, the free one is still conjugated when bound to albumin and transported across the hepatic cell membrane[2]. It could be conjugated with glucuronic acid at 80% to form bilirubin and with sulfate at 10% to form bilirubin sulfate and 10% with other substances.

The conjugation of bilirubin is the pathway for its excretion by the liver into the gastrointestinal system[3] Bilirubin is converted to urobilinogen and excreted in feces in oxidized form as stercobilin. Bilirubin is also excreted by the kidney into the urine as urobilin but of low percentage.

The importance of this study dwells on the clinical application of bilirubin in diagnosis in relation to physiologic implication in blood and liver

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functions. Excess amount of bilirubin in the body (above 0.5 mg dL^{-1}) could lead to jaundice, a yellowish tint in the body tissue leading to skin yellowing and that of the deep tissue. This may occur in infection of the liver e.g., hepatitis B or in obstruction of the bile duct and in rhesus incompatible blood transfusion[3].

Excess bilirubin in the plasma could be the result of increase haemolysis of erythrocytes which may be associated with disorders e.g., enzyme deficiency (G6PD), unphysiologic stimulation of immune system(s), complement antibodies to the erythrocytes, splenic poling in splenomegaly e.g., malaria.

However, bilirubinaemia is a pathologic condition where the bilirubin presence in the plasma is in trace quantity below 0.5 mg dL^{-1}. Chloroquine inhibits the total production of porphyrin. This negative metabolism affects the normal quantity of bilirubin in circulation this formed the basis of our investigation. Inhibition of porphyrin means non incorporation of this appendage of protoporphyrin moiety for the formation of haemoglobin which will eventually affects the oxygen carrying capacity of erythrocytes subsequently resulting in anaemia, hypoxia, cardiac increase output, arrest and possibility of death.

Chloroquine was therefore compared with other antimalarials particularly the new ones, amalar, cotecxin to induce physiologic relevance in their usages. This is because of the half life of these drugs which may exert deleterious effects as weighty as those of radioactive elements as malaria is also a life disease.

There is therefore need to review routinely physiologic implications of antimalaria drugs to be able to index realistically of epidemiologic morbidity and mortality rates from malaria disease.

MATERIALS AND METHODS

Animals: Thirty animals weighing averagely between 80-141 g were used for the study. The animals were obtained from the Faculty of Pharmacy animal house and were of good health. Pellet food and water were used in maintaining the animals. Animal right consent was not sought before the use of the animals but the animals were not tortured in the course of the study. The animals were grouped into four drugs with six animals in each drug group including control.

Drugs administration: Four drugs; amalar® chloroquine® cotecxin® and fansidar® were used for the study. The drugs were purchased from a registered pharmacy shop where the study was done.

Using the methods of[4,5], the drugs were administered based on the weights of the animals deriving from the average weight of man; (70 kg) as standard. The drugs were also administered orally based on the curative and preventive dosages using canula by passing the esophagus and delivered into the stomach.

The bilirubin concentration per drugs were monitored for 28days adopting the WHO[6] model for monitoring parasite clearance and antimalarials efficacy but malaria parasites were not given to animals in our study.

Blood and plasma collection: Blood was collected from the animals by cardiac puncture applying anaesthesia (chloroform). The blood was immediately spun at 1,200 rpm for 5 mins to collect the plasma.[7] the plasma were then used for colorimetric assay of bilirubin.

Colorimetric assay:
Reagents:

- Sulphalinic acid, 29 mmol L^{-1}, Hcl, 0.17N
- Sodium Nitrate; 25 mmol L^{-1}
- Caffeine Nitrate; 0.26 mol L^{-1}
- Tartrate, 9.93 mol L^{-1}, Sodium hydroxide; 1.9N

In this assay total bilirubin was measured as standard plasma estimation.

Cuvette concentration:

	Sample blank	Sample (mL)
Reagent 1	0.2	0.2
Reagent 2	-	0.05 mL
Reagent 3	1.00	1.00
Sample	0.20	0.20

The contents in the cuvettes were mixed and allowed to stand for 10 min at 20-25°C after adding, reagent 1-3 and before adding reagent 4 and the contents were further mixed and allowed to stand for 5-30 min at 20-25°C. The absorbance of the sample which equaled the concentration was read at 578 nm wavelength in µmol L^{-1}.

RESULTS

The results showed that fansidar decrease the concentration of bilirubin significantly against that of control (8.64±0.33), control, 16.65±1.00) ($p<0.05$), Table 1. Also chloroquine affect the Plasma concentration of bilirubin by decreasing it significantly; (11.99±2.82) as compared with the control; 16.65±1.00 ($p<0.05$).

Table 1: Effects of amalar, chloroquine, cotecxin and fansidar on plasma bilirubin concentration (average values in 30 animals in 4 drug groups and control)

Drugs	Bilirubin concentration µmol L^{-1}	p-value
Amalar	12.91±3.48	p<0.05
Chloroquine	11.99±2.82	p<0.05
Cotecxin	14.50±1.38	p>0.05
Fansidar	8.640±0.33	p<0.05
Control	16.65±1.00	p<0.05

Amalar also has low plasma bilirubin concentration 12.91±3.48, when compared with control (16.65±1.00) p<0.05. Table 1. But cotecxin slightly reduce plasma concentration of bilirubin, 14.50±1.38 and was not significant when compared with control (16.65±1.00) Table 1.

DISCUSSION

The assay of bilirubin have shown different cumulative effects as per the various drugs and regimen pattern; preventive and curative dosages. Fansidar as preventive drug drastically affected the bilirubin concentration in the body below the normal values more than any other drug in the study. The drug is highly consumed as alternative to cholorquine both as preventive and curative. It's consumption as curative have led to increase drug pressure as the action is slow when the malaria parasites have already been established in the blood stream. Since the parasite clearance is low at this period of administration patients usually moved for further treatment drug[8]. Despite its inefficacy due to parasite resistant strain, *Plasmodium falciparum*[9] it is still consumed as protection against malaria due to the fact that chloroquine itches and is bitter. But chloroquine is found to be safer than fansidar.

Fansidar is reported to cause fetal malformation, skin rash, hair lost, erythema, lyell's syndrome, leucopenia and polyneutritis [10] . The leucopenia effects of fansidar is partly complimented in this study in relation to the haemopoietic system and the erythrocytes which the pigment, haemogblobin is observed to be indirectly affected in our observation. However, the effects of fansidar on bilirubin has not been established else where, hence the relevance of this study in blood physiology and pharmacologic preparations.

The low bilirubin caused by the administration of fansidar means that fansidar has the tendency of inhibiting the incorporation of protoporphyrin which porphyrin is derived into the formation of haemoglobin molecule and such will lead to low or no haemoglobin formation in the erythrocytes. Such will lead to deserythropoiesis and anaemia in people taking fansidar and in malaria attacks. The same applies to amalar also curative drug. This is a serious matter because protective drugs are meant to reduce the frequency of malaria disease which will also reduce the drug intake and the effects. With these observations it means there will still be increase tendency of drug effects on cells and organs with curative antimalaria and malaria prevalence. This is why it is very celebrative for the action of cotecxin that has no serious depleting effect on the Plasma bilirubin the only drug in this investigation. Perhaps with this development cotecxin may be given as preventive regimen or as combined therapy with any antimalaria drugs that is physiologic friendly. Our study has further confirmed the effects of chloroquine on porphyrin[11]; but has further highlighted the need for continuous comparative studies on different forms of antimalaria drugs to actually evaluate continuous physiologic relevance and not only pharmacologic implications as major index in deciding the safer therapy in rolling back malaria in Africa.

CONCLUSION

The study has shown the effects of certain antimalaria drugs on bilirubin which has unveiled the physiologic implications in Pharmacologic preparations particularly the tendency of anaemia and erythropoictic porphyria. It is advisable for health seekers to avoid endangering their health. But Government, Health organizations including Non Governmental ones, health research institutes, grant awarding bodies need to pay more attention to physiologic implications of antimalarials in circulation to be able to prone down the effects of such drugs which would be of jeopardy in malaria infection.

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